Studies in Physiology

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Introduction

This thesis presentation consists of published work, abstracts of work in progress and manuscripts either submitted or about to be submitted. It can be seen that the work embraces a number of areas and therefore this narrative is included to draw the work together.

The early studies relate to urine flow and uterine activity, not because either of these activities was considered the primary point of interest, but because they were used as an end point to study neurohypophyseal action and this at the time was regarded as a good end-point signal of brain activity. Work of this kind was characteristic at the end of an era in which the question of chemical transmission within the brain was still in doubt. The techniques that were in use then were often indirect and a long way removed from the direct question that was being asked. It is for the same reason that studies on 5-hydroxytryptamine appear. The question had arisen in the literature as to whether 5-hydroxytryptamine was in fact another renal hormone and the purpose of that work was to explore that possibility.

Some more direct experiments on chemical transmission in the brain then follow. These include some early histochemical enzyme studies on specific nuclear and cell groupings in the hypothalamus. The preoccupation with hypothalamic physiology which arose from the previous studies dominates the next few years of work. Now, instead of being primarily concerned with the role of the hypothalamus in the regulation of endocrine systems the emphasis shifts to the role of the hypothalamus in the control of circulatory change. Interest in neurochemical problems did not entirely wane and that work was extended by one study on the cholinesterase enzyme histochemistry of the brain stem of the cat.

It was at about this time that it became possible to begin the electrophysiological studies which have constituted the major methodology of most of the later work. Earlier electrophysiological experiments reflect some of the technical difficulties of the day so that more emphasis was placed on evoked potentials than unit potentials and some excursion was made into the origin of evoked potentials.

In the course of the work of the hypothalamic regulation of the circulation, one problem kept surfacing. The cardiovascular changes that we were interested in resulted from the sudden and unexpected stimuli. This invariably produced an orientation reflex. The question that now surfaced was how the brain organizes the orientation reflex. The orientation reflex as a new or unexpected stimulus consists of a turning of the head, eye and sometimes the whole body to bring the object of interest into view. At the time when work on the orientation reflex commenced the multiplicity of sensory pathways within the brain was becoming obvious but the hypotheses which was being developed to explain those sensory pathways were even then seen as naive. The orientation reflex was a fundamental behavioural reflex, with the overriding characteristic that it was used to identify a stimulus. Thus the reflex should be served by a sensory system which was influenced by all modalities but conveyed only very imprecise information about the stimulation. For many reasons the superior colliculus seemed to be the likely candidate for organizing such a reflex and it was for this reason that work was started on the analysis of sensory inputs to the superior colliculus.

This work has now shown that the superior colliculus indeed receives a great deal of information, much of it of proprioceptive origin and it has also been possible to show that within the superior colliculus a variety of sensory modalities impinge on cells that can influence head movement. This has in turn led to an analysis of inputs to the superior colliculus from extraocular and neck muscle afferents. As this work progressed it became more and more obvious that a large amount of fundamental information concerning the head movement system was not available, and without this fundamental information, the brain system analysis could be completely understood. This led to the initiation of studies not only on the superior colliculus but on the muscles of the neck, their sensory receptors, and the physiology of the upper cervical cord and trigeminal system. Some of this work which has occupied the last few years appears in this thesis in full, some in draft form and some in abstract prior to writing up in full. Of the names that appear in the publications of the last few years, P.K. Rose is a student who completed his Ph.D. with me in 1974 and F.J.R. Richmond is a student who will complete her Ph.D. this year.

Towards the end of the submission is a short review which was solicited and will shortly appear in the Canadian Journal of Physiology and Pharmacology. It is hoped that this review might provide a useful summary of the work of the last few years.

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Simultaneous observations on urine flow and uterine activity in the conscious bitch. By V. C. Abrahams and Mary Pickford.
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Our aim was to discover whether the oxytocic and vasopressor factors of the posterior lobe of the pituitary were simultaneously released from the gland under various physiological conditions which would appear, functionally, to require only the release of one of them. Harris (1947) has shown that electrical stimulation of the hypothalamus in the conscious rabbit causes the release of both factors. Verney (1947) has shown that, amongst other things, a small increase in the tonicity of the blood passing to the central nervous system, and emotion, cause antidiuresis. Our observations were made on ovariectomized bitches in which one carotid had been exteriorized, and in which the upper end of one uterine horn had been brought out to the flank. Activity of the uterus was recorded by inserting in it a balloon filled with normal saline at a pressure of about 30 mm Hg.

It was found that emotional stimuli, and also intracarotid or intravenous injections of concentrated NaCl solution, caused not only an inhibition of water diuresis, but a simultaneous and coterminous increase in uterine activity. The antidiuretic effect could be matched by the intravenous injection of, for example, 3 mU Pitressin, but an equivalent increase in uterine activity needed 80–100 mU Pitocin. The simultaneous injection of 3 mU Pitressin and 5 mU Pitocin did not increase uterine activity observably more than 5 mU Pitocin alone. One bitch was subjected at operation to section of the supraoptico-hypophysial tracts. The day before operation the uterus showed its usual activity. The day after operation the activity was greatly reduced, and during the remaining days of the preliminary polyuria it showed scarcely any spontaneous contractions, though it responded to an intravenous injection of Pitocin. During the normal interphase the uterus showed almost normal contractions. With the onset of the permanent polyuria it again became quiescent. For a time after operation both oral and intravascular NaCl induced uterine contractions, without altering the rate of urine flow.

So far, it appears that in the bitch, the oxytocic and vasopressor factors of the posterior lobe of the pituitary are released simultaneously from the gland, and that the effect produced depends on the sensitivity of the peripheral organ.

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SIMULTANEOUS OBSERVATIONS ON THE RATE OF URINE FLOW AND SPONTANEOUS UTERINE MOVEMENTS IN THE DOG, AND THEIR RELATIONSHIP TO POSTERIOR LOBE ACTIVITY

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For some 20 years there has been controversy regarding the active principles of the posterior lobe of the pituitary, whether the naturally released substance has multiple actions or whether there are two or more distinct hormones (Abel & Nagayama, 1920; Kamm, Aldrich, Grote, Rowe & Bugbee, 1928; Rosenfeld, 1940; van Dyke, Chow, Greep & Rothen, 1942; Zetler, 1953). Whatever the final decision, account must be taken of the considerable body of work showing the pituitary substance(s) as secreted has multiple simultaneous actions, though the relative intensities of the different effects vary. Haterius & Ferguson (1938) and Haterius (1940), using rabbits, and Ferguson (1941), using cats and rabbits, showed that electrical stimulation of the pituitary stalk or its immediate neighbourhood caused increased uterine activity and antidiuresis. However, these workers did not make simultaneous observations on the two organs and their animals were anaesthetized. By means of remote-control stimulation Harris (1947) was the first to make observations on conscious animals (rabbits) and to note that when the supraoptico-hypophysial tract or the neurophypophysis was stimulated, both inhibition of diuresis and increased uterine activity occurred at the same time. This important work established that electrical stimulation at a given site induced simultaneous activity in different organs sensitive to different fractions of the product of the pars nervosa. Stimuli of a more physiological nature have led to the same conclusion. For instance, it has been shown that suckling, which involves liberation of the oxytocic fraction, also causes antidiuresis in rabbits, dogs, cows and the human species (Peeters, Coussens, Bouckaert & Oyaert, 1949; Peeters & Coussens, 1950; Cross, 1951; Kalliala & Karvonen, 1951; Kalliala, Karvonen & Leppänen, 1952). Since the antidiuretic action of the oxytocic fraction itself is very low this means that there must have been

a simultaneous release of the pressor fraction with which the antidiuretic hormone is closely linked.

This paper records the results of experiments on conscious animals made to determine whether procedures known to cause antidiuresis by liberation of the antidiuretic hormone (vasopressin) would at the same time increase uterine activity by the liberation of oxytocin.

METHODS

The experiments were made on five bitches in which the rate of urine flow and uterine activity were simultaneously observed. The urine was collected by means of a catheter inserted through the urethra into the bladder at the time of observation. All animals were given a hydrating dose of water at least 2½ hr before observation, which was always made during the course of water diuresis, unless the contrary is stated. Water diuresis was induced by giving 250–400 ml. water by stomach tube, the volume given depending on the size of the dog.

Operations. In all animals the perineum was slit dorsally to make the urethra easily accessible.

Owing to its pelvic anatomy it is not possible in the bitch, as in the rabbit, to fistulate the vagina and insert recording apparatus into the uterus through the cervix. Therefore, in five dogs the upper end of one uterine horn was divided and the top of the distal stump brought through the antero-lateral abdominal wall and stitched to the skin. Healing was rapid and providing the fistula was used frequently, little difficulty was found in inserting the balloon at will. Once or twice subcutaneous false passages appeared, and twice, due to contraction of the ring of fibrous tissue at the orifice, it was necessary to make a small incision under local anaesthesia to enlarge the opening.

Three of the dogs, Angela, Topsy and Lady, were ovariectomized at the time of fistulation of the uterus; of the other two, Darkie was ovariectomized only after a number of observations had been made on her, and Colleen was observed with ovaries intact. Because of uncertainty whether after ovariectomy the uteri would develop spontaneous activity without oestrogen administration, two 2 mg tablets of stilboestrol were implanted subcutaneously in Angela and Topsy when ovariectomy was performed. The tablets were of the variety used for oral administration and were absorbed in less than 5 days. Lady was given a total of 5 mg stilboestrol in oil subcutaneously in divided doses from the second to the tenth postoperative days. Finding that spontaneous activity persisted without further treatment Darkie received no stilboestrol. The uterus became active just as in the other animals.

Angela, Lady and Topsy were provided with left-sided carotid loops in which the sinus was denervated.

Operations for section of the supraoptico-hypophysial tracts were performed transorally.

Recording of uterine contractions. A short piece of steel wire with a knob at one end was bound to the end of fine polythene tubing so that it projected for about an inch. A small rubber balloon was slipped over the wire and, in turn, bound to the tube. Tubing and balloon were filled with 0·9% NaCl solution, inserted into the uterine horn and joined to the mercury manometer designed by Condon (1953) for recording rat blood pressure. The pressure in the manometer was raised to between 25 and 30 mm Hg. Providing the rubber joints were reduced to a minimum the record of uterine contractions showed a pressure change of 2 cm Hg for a change in volume of the system of 0·1 ml.

Miscellaneous. The animals were each given the same daily volume of milk and gravy. Additional fluid taken voluntarily in the form of water was used as a measure of the presence or otherwise of polyuria. This method of assessing the degree of polyuria was used rather than putting the animal in a metabolism cage, first because the animal with diabetes insipidus drinks so much more water than when it is normal that any error is insignificant unless one is interested in diabetes insipidus *per se*, and secondly, because a metabolism cage involves long periods of restricted activity with

no possibility of exercise unless someone is available to catheterize the animal before it is allowed free.

After death serial sections of hypothalamus and pituitary were cut at 10μ and five sections put on each slide. The slides were then stained in rotation with (*a*) toluidine blue, (*b*) protargol or Roger's $AgNO_3$ stain, (*c*) combined chrome-alum-haematoxylin and phloxin to show Gomori substance. Some sections of the anterior lobe were stained with Schiff's reagent or with Mallory's trichrome stain. Sections of uteri and kidneys were stained with haematoxylin and eosin, and suprarenals with Sudan IV and Sudan black B for total lipids.

The preparations of posterior lobe extract used were the 'Pituitrin' (whole extract), 'Pitressin' (vasopressor fraction) and 'Pitocin' (oxytocic fraction) of Parke Davis and Co.

RESULTS

It is probably as well to describe at once the condition of the uteri as found at post-mortem. So far only Lady, Darkie, Colleen and Topsy have been killed. Histological examination showed no sign of infection. The exteriorized horns looked bigger and had slightly thicker mucous and muscular coats than the other horns. This is probably owing to the distension suffered by repeated insertion of the balloons. In Darkie the surface epithelium on the side of the fistula was absent in places, and there were a few red blood cells in the lumen. In all other respects the uteri had the appearance to be expected after ovariectomy.

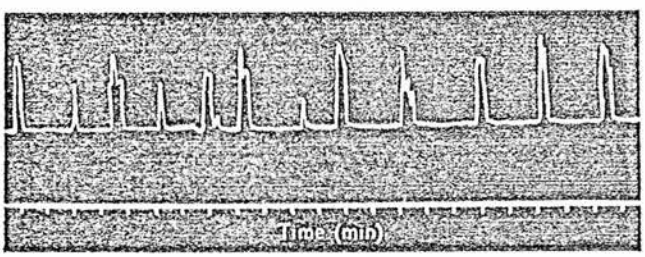

Fig. 1. Topsy, 5 Feb. 1954. Spontaneous contractions of uterus, $\frac{2}{3}$ actual size. Ovariectomized 19 Jan. 1954. Time in 1 min.

General observations

After inserting the balloon in the uterus the raised pressure in the manometer always fell slowly, presumably due to a decrease in tone of the uterine muscle. A steady base-line was reached in 25–35 min. Superimposed on the base-line, whether falling or steady, were the individual contractions. They might be large, 30–40 mm, or small, 3–4 mm. Large to moderate-sized contractions were the more common. Except in Lady once, and in Angela (Fig. 5), for a few days, each contraction was of short duration and was not a maintained alteration in tone (Fig. 1). The type of spontaneous contraction and its size could not be correlated with any known factors. However, under standard conditions of hydration, recumbency and lack of stimulation, contractions

were of regular pattern on any one day. Thus any change in uterine activity was measured with reference to the pattern on that particular day. In all animals on occasion, but by no means regularly, following insertion and distension of the balloon uterine contractions were fairly large for 10–15 min and then settled to a smaller size.

In all instances this spontaneous activity developed within 3–6 days of ovariectomy and persisted without any oestrogen treatment save that given at the time of operation. In Lady and Topsy contractions were present for 43 days when the animals were subjected to operation for section of the supraoptico-hypophysial tracts, and in Angela for 50 days when she fell ill of an undiagnosed complaint and lost weight rapidly. She was treated with sulphathiazole tablets and injections of penicillin and made a complete recovery in 3 weeks, except that the uterine contractions were small. Despite courses of treatment with stilboestrol the contractions did not increase in size, and she was no longer used for this series of experiments. In Darkie the uterus was active from the fifth day after ovariectomy until the twelfth when she was subjected to supraoptico-hypophysial tract section. The behaviour of the uterus after operations on the hypothalamus will be described later.

Effect of giving fluids by mouth

Giving fluid by mouth had no constant observable effect on size or frequency of uterine contractions (fifty observations). The few occasions when a change in activity was seen can, on the whole, be reasonably explained. For instance, infrequently, immediately after giving water there was increased rate of contraction for 15–20 min, which can be attributed to a reflex arising from passage of the stomach tube and rapid distension of the stomach with water. Then, in Lady on two consecutive days, but in none of the other animals, giving water resulted in increased size of contraction for the next 2 hr. On the third day the administration of the same volume of 0·9% NaCl solution had the same effect, so that the reponse was not specific for water. These responses of Lady were isolated and no explanation for them was apparent.

Effect of intravascular injections of NaCl

Between them Angela, Lady and Topsy received eleven intracarotid injections of 1·7 M-NaCl during the course of water diuresis. The volume injected varied from 1·2 to 11 ml., and the time taken to make the injection from 7 to 60 sec. In every instance there followed the inhibition of urine flow and later recovery as described by Verney (1947) and shown by him to be mediated by the pars nervosa. In every instance, too, during urinary inhibition, and contemporaneous in onset and disappearance with it, there was an increase in size or frequency, or both, of uterine contraction. Fig. 2 shows that in Lady the inhibition of urine flow resulting from intracarotid injection of 2·4 ml.

1·7 M-NaCl was marked and was accompanied by an increase in frequency of uterine contraction. Urine flow and uterine contractions showed a parallel return to pre-injection levels. Figs. 3 and 4 record what happened in Angela

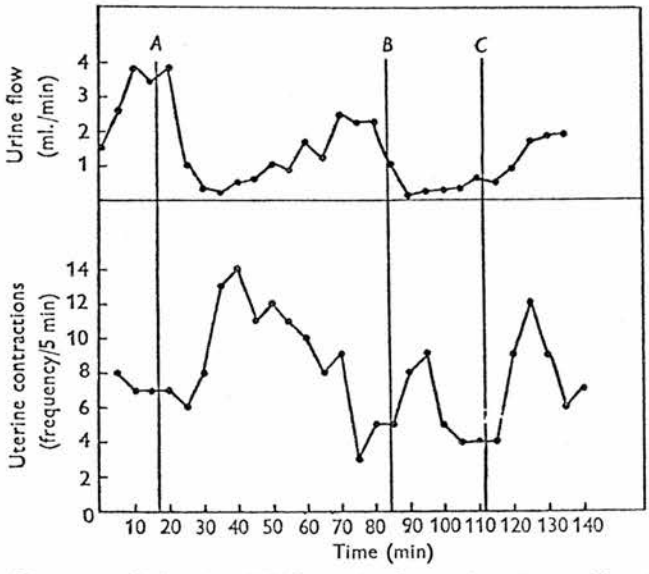

Fig. 2. Effect of intracarotid injection of NaCl on urine flow and uterine motility. Lady, 23 Apr. 1953. Upper record, urine flow. Lower record, frequency of uterine contraction. At zero time 350 ml. water by mouth. At *A*, 2·4 ml. 1·7 M-NaCl solution by intracarotid injection in 5 sec. At *B*, 5 mU Pitressin intravenously. At *C*, 5 mU Pitocin intravenously.

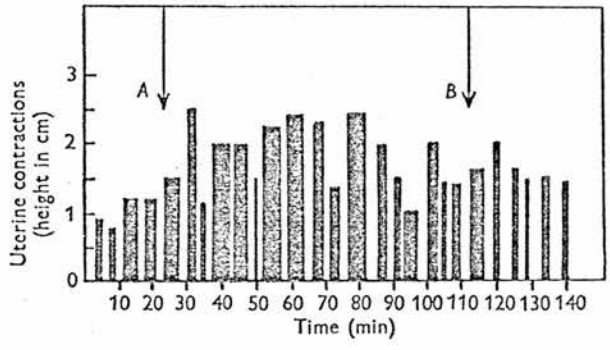

Fig. 3. Effect of intracarotid and intravenous NaCl on frequency, duration and size of uterine contraction. Angela, 10 Apr. 1953. Ovariectomized 24 Feb. 1953. At *A*, 4 ml. 1·7 M-NaCl by intracarotid injection in 40 sec. At *B*, 4 ml. 1·7 M-NaCl intravenously in 40 sec. See also Fig. 4.

on a day when she showed prolonged tonic contractions of the uterus, and Fig. 5 on another day when the contractions were short-lasting. Topsy's uterus reacted in the same way as that of Angela and Lady to intracarotid injection

of 1·2 ml. 1·7 M-NaCl on a day when she had not been hydrated nor given water at the time of observation. The urine flow rate was initially low, 0·7 ml./min, nevertheless it decreased to 0·2 ml./min.

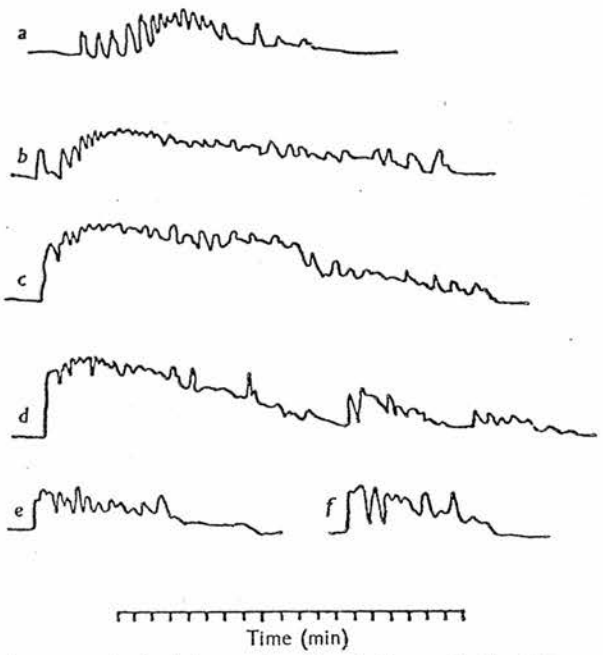

Fig. 4. Tracings from record of uterine contractions before and after intracarotid injection of 4 ml. 1·7 M-NaCl. Angela, 10 Apr. 1953. (*a*) and (*b*) before injection, (*c*), (*d*), (*e*), and (*f*) 20 min, 55 min, 80 min and 100 min after injection respectively. All tracings $\frac{4}{5}$ actual size. See also Fig. 3.

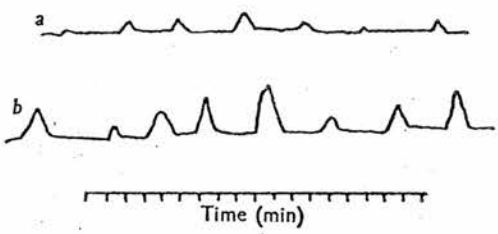

Fig. 5. Tracings from record of uterine contractions before and after intracarotid injection o 4 ml. 1·7 M-NaCl in 35 sec. Angela, 13 Mar. 1953. (*a*) Before injection; (*b*) 40 min after injection. Tracings $\frac{4}{5}$ actual size.

Amounts of 1·7 M-NaCl effective by intracarotid injection had no effect on either urine flow or uterine activity when given intravenously. This is shown in Angela in Fig. 3, *B*. If, however, enough 1·7 M-NaCl was given intravenously to cause antidiuresis (15–20 ml.), then also there was an increase in uterine motility. Sometimes, after either intravenous or intracarotid injection of NaCl

solutions, uterine contractions did not return completely to the pre-injection condition. This, however, occurred on those occasions when the urine flow also made only a partial recovery from inhibition for the reason that almost all the water given had by that time been excreted. Fig. 7 shows this in Lady after she had received an injection of posterior lobe extracts. It is probable, therefore, that at this late time in the observation the animal's own posterior lobe was excreting more actively than just before the injection was given.

Effect of intravenous injections of sucrose

Verney (1947) has shown that both intra-arterial and intravenous injections of hypertonic sucrose solution result in antidiuresis due to release of vasopressin.

Two observations only were made on Topsy. In one 23 ml. 0·8M-sucrose was injected in 14 min and was without effect on the rate of urine flow, whilst the rate of uterine contractions was reduced from 4 to 3 in 5 min for 25 min. On the other occasion 10 ml 1·35M-sucrose was injected in 6 min and caused both an inhibition of urine flow and increased uterine activity which began and ended together.

Effect of acetylcholine

This was tested in Topsy and in four dogs used in another series of experiments. As this work will be fully reported in another paper, it will only be stated here that on intracarotid injection of 200 μg acetylcholine there followed contemporaneously with inhibition and recovery of urine flow (Pickford & Watt, 1951) an increase and then decrease in frequency of uterine contraction.

Effect of emotional stimuli

Following a series of intracarotid injections of acetylcholine and other solutions Angela developed a dislike of the procedure. This first became apparent on one occasion when there was some difficulty with the injection which was finally not made. Nevertheless, antidiuresis resulted. (Control observations made by Verney (1947) and by us have shown that normally intracarotid injections do not of themselves cause antidiuresis.) This seemed an opportunity to test whether an emotional type of stimulus also caused an increase in uterine activity. The stimulus used was to boil the syringe in sight of the dog and make mock preparations for injection by holding the carotid loop, though the skin was not even pricked. This manoeuvre was performed on two occasions and was followed by urinary inhibition and increased uterine activity (Fig. 6). In Topsy the emotional stimulus applied was that used by Verney, namely, a just resented faradic stimulation of the lumbar muscles. Here, too, the resulting urinary inhibition was accompanied by a marked increase in size and frequency of uterine contraction, the time relationships of the urinary and uterine responses being similar.

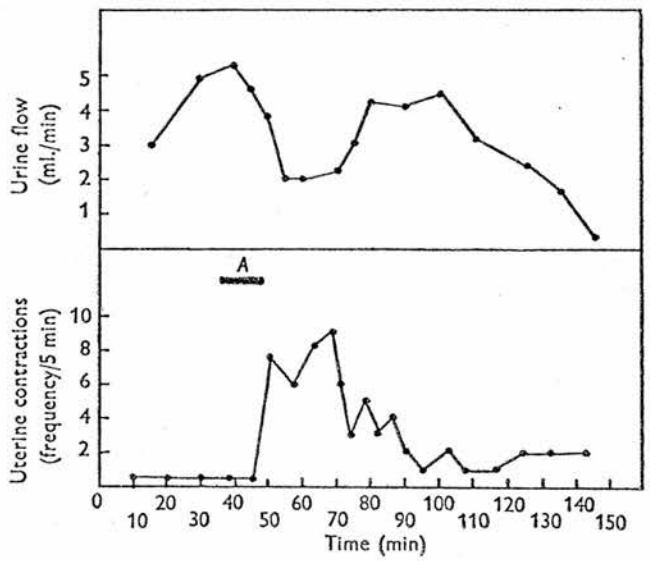

Fig. 6. Effect of emotion on urine flow and uterine motility. Angela, 16 Apr. 1953. Upper record, urine flow. Lower record, frequency of uterine contraction. At zero time 300 ml. water by mouth. During period *A* preparations were made for an intracarotid injection which was not given.

Attempted assay of the amount of active substances released

In Fig. 2 it can be seen that 5 mU Pitressin injected intravenously at *B* was just more than enough to match the inhibition of urine flow induced by the intracarotid injection of 1·7 M-NaCl given at *A*. The effect of Pitressin on the uterus though definite, was short-lived and not as great as that due to injection of hypertonic NaCl solution. At *C* (Fig. 2) 5 mU Pitocin was injected intravenously. This caused a transitory delay in recovery of the urine flow and again only a brief increase in frequency of uterine contraction. It was possible that full uterine activity required the presence of both posterior lobe factors, though there is evidence that the opposite is the case in the rabbit when large doses of pituitary extracts are used (Morgan, 1937; Harris, 1947, 1948), but 5 mU Pituitrin had no greater effect than the same amount of Pitocin. A mixture of 5 mU each of Pitocin and Pitressin was no more successful in matching the action of injected hypertonic NaCl solution. It was found that in Lady (15 kg) and Angela (15 kg) the effect of intracarotid hypertonic NaCl solution was equated by intravenous administration of <5 mU Pitressin and 80–100 mU Pitocin, and in Topsy (7 kg) by <5 mU Pitressin and 50–70 mU Pitocin. Fig. 7 shows the effect of 100 mU Pitocin on Lady. Since commercially prepared Pitocin is contaminated with about 5% pressor fraction some antidiuresis was to be expected.

Observations on the anoestrous animal

As might be expected, in the anoestrous bitch the uterus showed little spontaneous activity. On some days no response at all could be elicited to any stimulus tried. On other days, however, a little spontaneous activity was present, and at such times an intravenous injection of 80–100 mU Pitocin increased the frequency of contraction, as did also 15 ml. 1·7 M-NaCl given intravenously, that is, the uteri of Darkie and Colleen showed exactly the same response to these stimuli as did those of the ovariectomized animals, though on a minute scale (contraction height 1–2 mm). The urinary responses were alike in both anoestrous and ovariectomized animals.

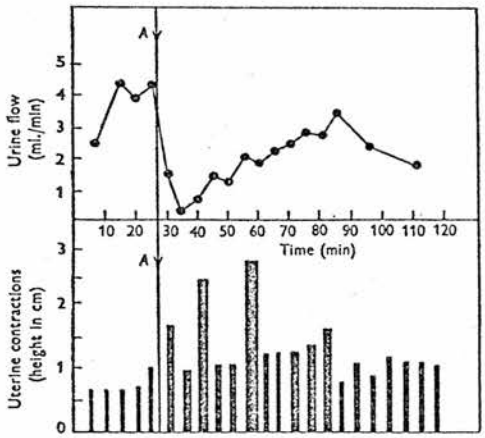

Fig. 7. Effect of intravenous Pitocin on urine flow and uterine motility. Lady, 12 May 1953. Upper record, urine flow. Lower record, frequency of uterine contraction. At zero time 350 ml. water by mouth. At *A*, 100 mU Pitocin intravenously.

RESPONSES OF ANIMALS AFTER OPERATIONS ON THE HYPOTHALAMUS

In Lady, the supraoptico-hypophysial tracts were successfully sectioned so that she showed marked polydipsia (Fig. 8). At post-mortem examination the posterior lobe was atrophied. In the hypothalamus there were hardly any supraoptic or paraventricular cells on the right side and a considerable reduction in their number on the left. Of the cells remaining most were empty of Nissl granules, though nucleus and nucleolus were well-defined and eccentric. In sections stained to show Gomori substance all cells seen were loaded with darkly staining material. Short fragments of axons in the sections were also deeply stained in a beaded manner similar to that described by Bargmann (1949) and Bargmann & Hild (1949). Topsy had two operations. In the first the lesion was too anterior and postoperative polydipsia was small and fleeting (1000 and 700 ml. on the 2 days immediately following operation). The second operation 4 weeks later was more successful and gave rise to moderate polydipsia (Fig. 8). At autopsy the posterior lobe was seen to be shrunken and

highly cellular. In the hypothalamus there were scarcely any supraoptic or paraventricular cells on the left and on the right few paraventricular but a moderate number of the rostral supraoptic cells. The median eminence was much thinner than normal. In Darkie, the lesion was in the mid- and posterior hypothalamus and, functionally, she had what may be termed a latent diabetes insipidus following a fairly long interphrase (Fig. 8). Histological examination showed a somewhat shrunken posterior lobe and a considerable reduction in the number of both supraoptic and paraventricular cells. Fig. 8 shows the water intake of these three animals so that the behaviour of the uterus on any day may be related to the fluid demand.

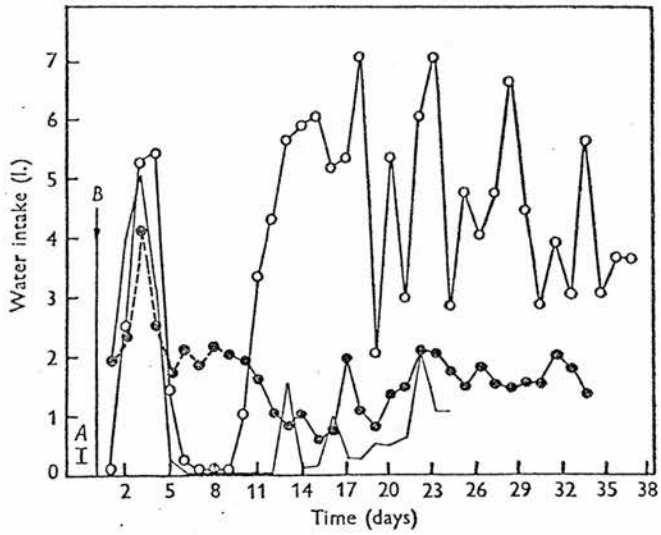

Fig. 8. Record of water intake of three dogs following lesions made in the hypothalamus at operation. *A* shows pre-operative range of water intake. *B*, day of operation. O—O, Lady (15 kg); —, Darkie (13·5 kg); ❶—❶, Topsy (7 kg).

Spontaneous uterine activity

Fig. 9 is taken from records of uterine movements in Lady for the last day before and the first few days after operation. It will be seen from Fig. 8 that on the first postoperative day the water intake had not yet increased, and from Fig. 9 that uterine movements were present and of fair size. During the preliminary polyuria uterine movements almost disappeared, though the uterus was capable of contraction as shown by its response to 100 mU Pitocin given intravenously (Fig. 9, *F*). During the normal interphase when water intake was nil uterine contractions reappeared and were of moderate size, though irregular in timing. When the permanent polyuria set in, spontaneous uterine activity became minimal and irregular, and remained so for the next 30 days. In these records it seems as though changes in uterine behaviour slightly

preceded changes in water intake. This may be more apparent than real since on any day when uterine records were obtained the water intake for the 24 hr including that day was measured only the next morning.

In Topsy similar though less striking results were obtained. For 4 days after the first unsuccessful operation uterine movement though infrequent and irregular (one contraction in 5–12 min) was of normal size. Thereafter the contractions returned to pre-operative frequency and regularity. After the

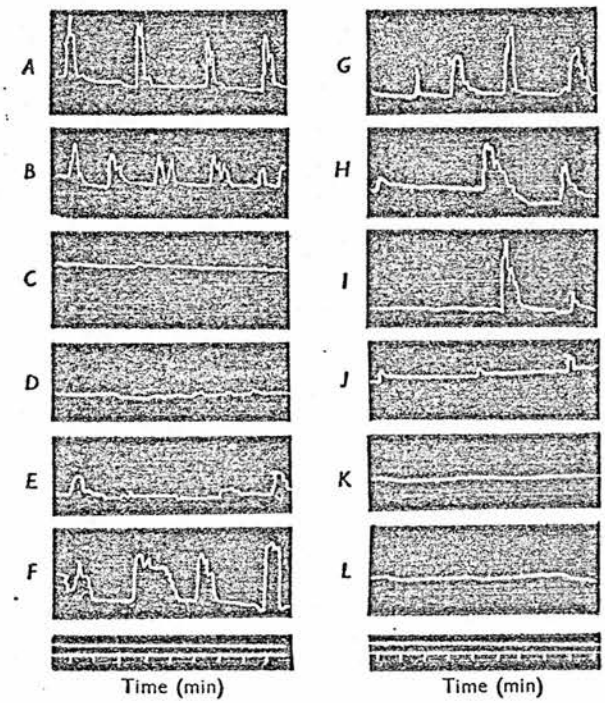

Fig. 9. Records of spontaneous uterine contractions before and after induction of experimental diabetes insipidus. Lady, ovariectomized 15 Apr. 1953. *A*, normal, 27 May 1953. Section of supraoptico-hypophysial tracts 28 May. *B*, 29 May; *C*, 30 May; *D*, 31 May; *E*, 1 June; *F*, 1 June, 25 min after intravenous injection of 100 mU Pitocin; *G*, 3 June; *H*, 4 June; *I*, 5 June; *J*, 6 June; *K*, 7 June; *L*, 12 June. For water intake on these days see Fig. 8. Records $\frac{4}{5}$ actual size.

second operation spontaneous activity was just present, with a contraction occurring about once in 5–10 min for 4 days. For the next 7 days the contractions were larger, regular and infrequent. From that time on they steadily declined in size and frequency. This last may well have been due to the length of time since ovariectomy. During the preliminary polyuria in Darkie uterine contractions were small and irregular. On the fifth postoperative day, when water intake had declined, they suddenly became large, regular and frequent. It was noteworthy that if she was given water at this period the rate of urine

flow never rose above 0·2 ml./min and she always became restless and apparently slightly distressed, as though she were suffering from water intoxication. The uterine contractions during the interphase were larger than at any other time. This phase lasted until the twelfth day when uterine contractions became small, infrequent and irregular, and so remained.

Effect of oral water

Once the interphase period had passed Lady, Topsy and Darkie reacted to water given by mouth in a way that had not been seen before operation, namely, that in about 30 min the infrequent slow small uterine contractions decreased still further until such time as most of the water had been excreted, when the rate of contraction again increased. A possible explanation of this is that with a reduction in amount of the posterior lobe system there was just enough oxytocin circulating under ordinary conditions to maintain a little uterine activity, but that on giving water the final stimulus to uterine movement was withdrawn.

The effect of intravascular NaCl *etc.*

After the hypothalamic operation on Lady and Topsy neither intracarotid injection of 1·7 M-NaCl nor of acetylcholine caused either antidiuresis or increased uterine activity. The effect of intravenous injection of 1·7 M-NaCl was studied 7 times on Lady. The greater volume (15–20 ml.) of NaCl injected by this route as compared with the intracarotid, usually led to a fleeting diuresis. The uterus responded by an increase in size of contraction which developed slowly over 20–30 min and persisted for at least 2 hr, the longest time of observation before the dog became restless. The uterine response to injected Pitocin was the same as before operation. Thus the uterus reacted to NaCl given intravenously, but in a manner differing from the normal in its time relationships and unlike its response to Pitocin, either before or after operation.

DISCUSSION

In this series of animals before operation on the hypothalamus any stimulus which caused antidiuresis also caused increased uterine activity. The stimuli used were emotion, intracarotid and intravenous injections of hypertonic NaCl and of acetylcholine, and intravenous injection of sucrose. The results were the same in kind, though not in degree, in both the ovariectomized bitch and in the anoestrous one with intact ovaries. In the former during the period of our observations, spontaneous uterine activity was considerable and the increase due to any form of stimulation marked. In the anoestrous animal spontaneous activity was minimal or lacking, and that due to stimulation correspondingly small, though unmistakable.

In the present experiments we may safely assume that both intracarotid and intravenous injection of 1·7 M-NaCl solutions caused antidiuresis in the

normal dog by means of a release of antidiuretic hormone. This is clear from the work of Verney (1947). It seems probable that the oxytocic effect also depends on posterior lobe hormone release. First, the change in uterine motility began and ended at the same time as changes in urine flow. Secondly, after diabetes insipidus was induced intracarotid injections of 1·7 M-NaCl caused neither antidiuresis nor the characteristic increase in uterine activity. On a number of occasions in Lady when she was diabetic intravenous injection of hypertonic NaCl solutions did have an action on the uterus, but it was relatively slow in onset and lasted for more than 2 hr as against quick development and duration of 40–60 min when she was normal. It may be argued that with only a small part of the posterior lobe system intact and its blood supply perhaps disturbed as a result of operation, there might be delay in release of any hormone formed, but it is difficult to see why its production or presence should persist for so much longer than normal. Further, in the diabetic animal intravenous injection of 1·7 M-NaCl induced no antidiuresis. Thus, it seems likely that the uterine effect was directly related to NaCl, or else it would have to be concluded that the remaining posterior lobe hormone-secreting tissue in the hypothalamus contained only oxytocic material. This is contrary to the findings of Dicker & Tyler (1953*a*) and of Vogt (1953) that extracts of dog ventral hypothalamus have considerable pressor but minimal oxytocic activity, though Zetler (1953) found both substances to be present, oxytocin being in smaller amounts than vasopressin. The special postoperative conditions excepted, the weight of evidence favours the idea that intravenous, like intracarotid, injections of hypertonic NaCl solutions affects the uterus of the non-diabetic animal by virtue of the release of posterior pituitary hormones.

In the non-diabetic animals emotion had effects parallel in time on both urine flow and uterine movement. In the two experiments on the intravenous administration of sucrose, this substance only increased uterine activity on that occasion when it was given in concentration sufficient to cause antidiuresis. There is evidence (Pickford & Watt, 1951) that both intravenous and intracarotid acetylcholine injections cause the release of the antidiuretic hormone. In the present observations it induced increased uterine activity parallel with antidiuresis. The action of acetylcholine will be discussed more fully in another paper.

So far, these findings all lend weight to Abel's unitary hypothesis (Abel & Nagayama, 1920) and are in agreement with more recent work on the simultaneous onset of the various actions ascribed to different fractions of posterior lobe extracts. Thus Harris (1947, 1948) found on remote control stimulation of the stalk or pars nervosa of conscious rabbits that both antidiuresis and increase in uterine motility developed, and also that peristaltic movements became apparent. More strikingly, because the stimulus was a physiological one, Cross (1951) showed clearly that suckling caused antidiuresis in rabbits,

and gave reasons for the belief that both vasopressin and oxytocin were secreted. Others have found the same to be true in cows (Peeters & Coussens, 1950) women (Kalliala & Karvonen, 1951), and bitches (Kalliala *et al.*, 1952). Andersson (1951) found that an antidiuresis-inducing measure, namely the injection of hypertonic NaCl into the carotid of goats caused milk ejection, but he did not at the same time note what happened to urine flow.

In animals with experimental diabetes insipidus we found that when polydipsia, temporary or permanent, was present uterine contractions were small or absent, whilst during the normal interphase activity was present and marked. In one dog (Lady) during the interphase movements were less regular than before diabetes was induced. In Topsy and Darkie they were regular. The misplaced first operation in Topsy was interesting in that mild and transient polydipsia was associated with normal-sized, though somewhat irregular, uterine contractions. In Darkie, again, spontaneous uterine movement varied inversely with the urine volume, being small and irregular in the polydipsic phase and larger than at any other time during the prolonged oligodipsia.

Thus, throughout all our observations, any measure leading to antidiuresis at the same time caused increased uterine activity in both normal and ovariectomized bitches, and in ovariectomized animals with diabetes insipidus the greater the 24 hr water intake the less the spontaneous uterine activity, and vice versa. Under ordinary conditions, i.e. ovaries intact and not in late pregnancy, the muscular elements of mammary glands and uterus are unlikely to be thrown into vigorous activity every time moderate water retention occurs because it is only in certain circumstances when sensitized that the contractile cells react energetically to posterior lobe hormones. If contractions do occur in the uterus they are probably too small to be noticeable. Even in our ovariectomized bitches there were very few occasions when the uterine contractions were great enough to make the animal perceptibly aware that something was happening.

When attempts were made to assay the quantities of active substances secreted, then difficulties arose in accepting Abel's hypothesis, at least without modification. In all animals it appeared that the stimuli applied caused the release of 15–20 times as much oxytocic as antidiuretic material. Cross (1951), comparing antidiuresis with milk ejection following suckling in rabbits, found a vasopressor/oxytocic ratio of 1/100. Peeters (1950) also found a 1/100 ratio in cows. Using electrical stimulation in rabbits Ferguson (1941) and Harris (1947, 1948) observed the secretion of a substance resembling Pitocin more than Pitressin in its effects; the ratio Harris found being vasopressin/oxytocin as 1/4 to 1/10. Thus the disproportion between the two fractions secreted is not peculiar to one species nor to one mode of stimulation. Nor does it seem that the discrepancy can depend on using as standard commercially prepared beef

extracts. Dicker & Tyler (1953*a*, *b*) assayed rat, cat, dog and guinea-pig pituitaries in the same terms and found the vasopressor/oxytocic ratio in the dog was 1·0 and in the other species varied from 1·0 to 0·4. They also found in the same animals that the posterior lobe was depleted of oxytocin relative to vasopressin after parturition and during lactation. These last results are not unexpected if the natural secretion always has the composition suggested by the work of others and ourselves.

On teleological grounds it could be argued that because the regulation of urine volume, even in pregnant and lactating animals, is demanded more often than either uterine contraction or milk excretion, the kidney has developed a greater sensitivity to posterior lobe hormones than the other two organs. This, however, does not explain how the gland containing these substances in a 1/1 ratio as far as we can tell with the methods used, releases them simultaneously in anything from a 1/4 to 1/100 ratio. One possibility is that although the stored material in the gland appears microscopically uniform yet, actually the active substances are largely separate from each other, occurring either in different parts of the gland or different parts of the same cell, or that their release is controlled by separate nerve fibres. The two substances cannot be completely independent since the production of one is always accompanied by production of the other, with oxytocin predominating. In several species the ratio of vasopressor to oxytocic activity is greater in hypothalamic than in posterior lobe extracts (Dicker & Tyler, 1953*a*; Vogt, 1953; Zetler, 1953). Does this mean that in these species more or less of the vasopressin present in the gland is converted to oxytocin at the moment of stimulation? It is difficult to see how this could be, but if no such conversion occurs and unless there are but few fibres controlling vasopressin as compared with oxytocin output, it might be expected in the experiments of Ferguson (1941) and Harris (1947, 1948) that electrical stimulation, by affecting all nerve fibres, would cause release of equal amounts of both hormones.

That the disproportion between vasopressin and oxytocin, though always present, can vary so greatly may depend on the species of animal or on the type of stimulus. Harris (1948) used electrical stimulation and found a vasopressor/oxytocic ratio of 1/4 to 1/10. Cross (1951) designed his experiments specifically to cause the release of oxytocin, noted that antidiuresis also occurred, and found a ratio of 1/100. We designed our experiments to cause the release of vasopressin, noted that oxytocic effects were seen at the same time, and found a vasopressor/oxytocic ratio of 1/15 to 1/20.

So far no results give decisive evidence for or against Abel's unitary hypothesis (Abel & Nagayama, 1920).

The observation that spontaneous uterine activity is markedly decreased when polydipsia is present after operation for the production of diabetes insipidus raises questions as to the part played by oxytocin in maintaining the

uterus in a reactive condition. That operation in the hypothalamic area caused the observed decrease in uterine activity is negatived by the misplaced operation in Topsy, which had no depressing effect, and the fact that the uterus, like the urine output, had a phase of apparent normality between the two polydipsic phases. There seem to be four possibilities to account for the findings. First, that the posterior lobe is directly responsible for maintaining spontaneous activity in the uterus; secondly, that some interplay between oxytocin and gonadotrophins is essential; thirdly, that the importance of the posterior lobe lies in its control over electrolyte balance; and fourthly, that operation for the induction of diabetes insipidus at the same time interferes with anterior lobe function, with all the sequelae that that implies. Concerning the first possibility, it is unknown whether oxytocin sensitizes uterine muscle to the action of gonadotrophins, or the reverse, or whether these substances are equal partners. Further, it is even questioned whether oxytocin is necessary for normal parturition. References on this subject may be found in Reynolds (1949). Some information may be obtained from work now in progress to test whether uterine activity appears after ovariectomy in animals first made diabetic. Regarding the third alternative, it is known that uterine activity is altered by a changed electrolyte balance (Reynolds, 1949, 1951). In one set of observations on Lady in the diabetic state intravenous injection of 1·7 M-NaCl increased uterine activity, though in a manner different from that seen when she was normal. This experiment is being repeated, but results are not yet available. The effect of NaCl may be a direct one, or a general electrolyte disturbance may act through the adrenal cortex. In a recent paper Jones & Wright (1954) describe changes they noted in the histological pattern of rat cortices after diabetes insipidus of 4–6 months' duration. Deane (1950) also found adrenal cortical changes, but not until diabetes had been present for more than 2 months. These late effects make it difficult, though not impossible, to assign to the adrenal cortex the cause of the immediate postoperative changes in uterine behaviour. Concerning the last possibility, that operation may affect the anterior lobe, Jones & Wright (1954) state that at the time when the adrenal cortical changes were present the body and organ weights of their animals were normal and that the females had normal oestrous cycles. We noted no abnormality in the adrenals of our dogs, either in breadth of zones, or in lipoid distribution. Further, the anterior pituitary appeared histologically normal. Thus, evidence is lacking that anterior lobe dysfunction is responsible for the rapid changes in uterine behaviour. The problem of the role of the posterior lobe hormone in maintaining the uterus active needs further analysis.

No attempt was made to study the function of the uterine nerves in relation to the results.

SUMMARY

1. Simultaneous observations on urine flow and uterine movements were made on conscious dogs provided with a fistula of one uterine horn.

2. Stimuli such as emotion, the intracarotid or intravenous injection of hypertonic NaCl solutions or of acetylcholine, and intravenous sucrose solutions, all of which inhibit water diuresis by liberation of posterior lobe antidiuretic hormone, also caused increased uterine activity which was co-terminous with the antidiuresis.

3. The results of stimulation were the same in both normal anoestrous and ovariectomized bitches. In the latter uterine activity was far greater than in the former providing observations were made in the few weeks following ovariectomy.

4. Reasons are given for the belief that the effects on the uterus are owing to liberation of the oxytocic factor of the posterior pituitary.

5. In order to simulate the effects of stimulation on the uterus and urine flow it was necessary to inject intravenously <5 mU commercial pressor extract and 15–20 times more than this of oxytocic extract.

6. When experimental diabetes insipidus was induced uterine activity was nil or minimal during the polyuric phases and well marked during the interphase.

7. When diabetes insipidus was present intravenous injection of hypertonic NaCl solutions caused no antidiuresis, but did cause an increase in uterine activity which differed in its time course from that seen in normal animals.

8. The results are discussed with particular reference to the disproportion between the amounts of vasopressor and oxytocic factors liberated relative to those found in the gland, and to the dependence of uterine motility on the presence of oxytocin.

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Journal of Physiology, **128**, 82*P*.

Observations on the flow of fluid through the ureter of the dog.

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Observations were made on the anaesthetized dog of the pressure-flow relationships and the pattern of flow through the ureter perfused *in situ* with its circulation intact, and on the action of physiological and pharmacological agents administered intravenously. As has been noted by previous workers the outflow from the lower end of the ureter is intermittent. The pattern of flow varied with the perfusion pressure. There was no outflow if the perfusion pressure was less than 15–20 cm water. The pressure in the renal pelvis was found to be less than 1 cm water. The outflow pattern was unrelated to the direction of perfusion. 5-Hydroxytryptamine decreased flow through the ureter contemporaneously with changes in blood pressure, there being no flow at the peak of the blood pressure rise unless the perfusion pressure was increased above its previous level. Adrenaline and noradrenaline reduced flow through the ureter, the action of noradrenaline being the more prolonged. Vasopressin in doses sufficient to cause antidiuresis was without effect on the flow through the ureter.

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PART I. THE BALANCE OF ENERGY

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Introduction

THE intake of energy by human beings is estimated by measuring the intake of food. When the weight of the body shows no material change, this intake of energy must also represent the expenditure. Measurement of intake, however, gives no indication of the time-course of the expenditure of energy, and without this information we cannot form a true picture of the various metabolic demands which the individual meets in the course of his working day; neither do we know the amount of energy he uses during his leisure time.

Estimation of the expenditure of energy under actual working conditions can be accomplished only by indirect calorimetry, by measuring the rate of oxygen consumption. If measurements are made often enough it is possible to follow the course of energy expenditure over a period of time. There have been some notable studies on men at work in which the expired air was collected in Douglas bags with subsequent measurement of its volume and oxygen content. Moss (1935) used a similar technique with coalminers in England.

Many of the difficulties which had to be faced in the past in studies of this kind have been removed by the introduction of the Kofranyi-Michaelis (K.M.) respirometer (Kofranyi and Michaelis, 1940; Müller and Franz, 1952) which is made by the Max Planck-Institut für Arbeitsphysiologie in Dortmund. The respirometer is a dry gas meter which is worn like a haversack. It records the volume of the expired air and simultaneously ejects a sample of this air into an attached rubber bag (Fig. 1); the sample then has to be transferred to a gas sampling tube for analysis. The respirometer is light, weighing just over 6½ lb., (3 kg.), and has little or no hampering effect on muscular work. It is shown in action under rather awkward conditions in Plate 1, and details of its use are given in Appendix B (p. 52).

We used the K.M. respirometer to find, in terms of Calories per minute, the metabolic cost to each of our subjects of the majority of everyday activities. In all cases, whether the men were underground or in the colliery offices on the surface, at home or at out-of-doors recreation, the expenditure of energy was estimated under conditions as near as possible to the normal. Thus the 'Calorie value' of many human activities was measured for each of our subjects, and these values, together with the record of the duration of each of the man's activities, enabled us to calculate his energy expenditure over twenty-four hours; moreover we obtained a picture of the fluctuations in his energy expenditure during the twenty-four hours. Each man was studied for seven days.

Running parallel with the determination of the output of energy was the dietary survey which gave the energy intake for each man. We were then in a position to strike a balance between the two.

Most textbooks of physiology include a table which lists the energy requirements of the different types of workers in our society. Many of these tables, compiled at a time when methods of determining energy expenditure and classifying the severity of work were less efficient than they have since become, are of doubtful accuracy. Moreover, with the increasing mechanization of industry and the introduction of shorter working hours, it is probable that, in many cases, gross differences no longer exist between the energy requirements

of those employed in heavy industry and those in sedentary work. In order as far as possible to gauge the extremes existing today, we chose for this survey two sharply contrasting groups of men—on the one hand, underground miners who undeniably carry out hard manual work, and, on the other, colliery clerks who work sitting or standing in offices.

Very detailed tables giving the Calorie values of various types of food have long been in general use. But Orr and Leitch in 1938 were able to find relatively few Calorie values for the multifarious activities of man. It is hoped that our figures will fill some of the gaps that remain in this field.

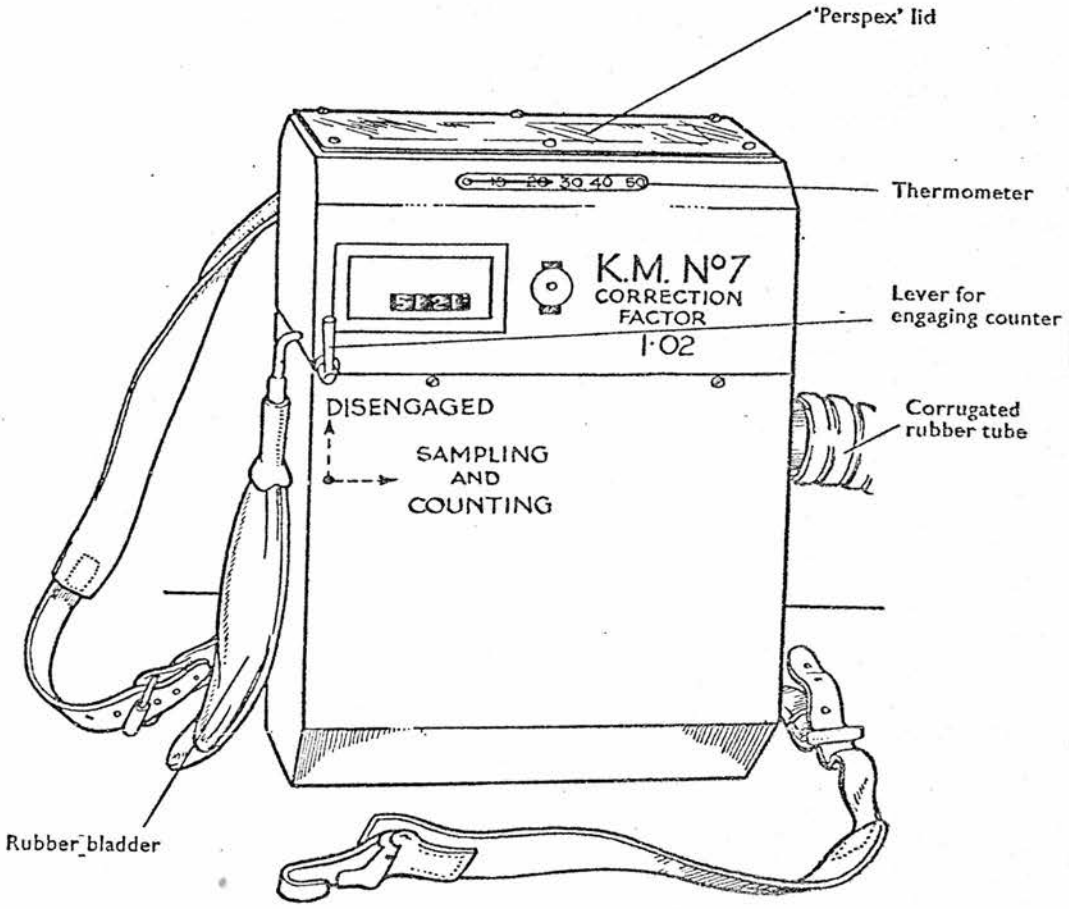

FIG. 1. The Kofranyi-Michaelis respirometer.

The Social and Physical Environment

'THE KINGDOM OF FIFE'

The 'Kingdom of Fife', bounded on three sides by the sea, has a character all its own. In the Middle Ages the fishing villages and sea towns drove a thriving trade with Europe and the wealth of the coastal settlements has always contrasted sharply with the rather bleak townships inland. Coal has been mined

in Fife since the thirteenth century, and by the early part of the seventeenth century coal workings were being driven under the Firth of Forth. The Fife coalfield promises to be the most important in Scotland in the near future.

THE MINE

The Wellesley Colliery, where we carried out our survey, is at Buckhaven on the north shore of the Firth of Forth. A shaft was sunk as long ago as 1886 and coal was brought up that shaft in 1887. The main shaft, the one we went down, was sunk in 1907 and completed in 1911 and the workings now extend far beneath the sea. Mechanization is making progress but the men have still to expend a great deal of energy walking long distances to and from the coal-face (Fig. 2). Technical details of the mine are given in Appendix A (p. 49).

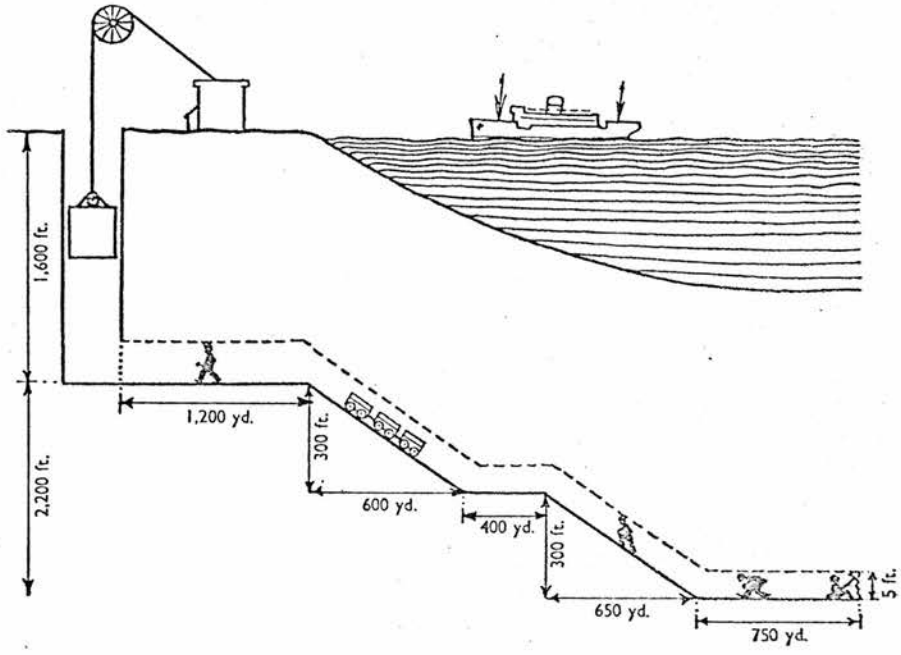

FIG. 2. Diagram of Wellesley Colliery, Buckhaven, Fife. The drawing is not to scale.

THE MINING COMMUNITY

A mining community is held together by many bonds. The shift system imposes a common load and discipline on all, not least upon the wives; and even surface workers, such as clerks, do not escape its responsibilities. Although the mine in which we worked was large, employing 1,500 men, it was still small enough for everyone to know everyone else, at least by reputation. Death or injury in the colliery has a personal significance, and a miner is inevitably conscious of danger throughout most of his working day. The communal response to danger is a great stimulus to friendliness, and helps to build between the community and the outside world the shell which is so noticeable after even a short stay in a mining town. We were fortunate in being accepted from the beginning as members of the community and we received nothing but friendly help and guidance throughout our stay.

THE SELECTION OF VOLUNTEERS

Through the good offices of representatives of the National Coal Board and of the National Union of Mineworkers we met a number of miners and clerks, together with their womenfolk, some three weeks before the survey was due to start. We told them our plans and asked for volunteers. The response was more than adequate.

TABLE 1

The subjects of the survey: particulars of physique

Name	Age	Height		Weight		Surface area	Theoretical basal rate (Robertson and Reid, 1952)	Values adopted for energy expenditure during sleep†
	(years)	(in.)	(cm.)	(lb.)	(kg.)	(sq. m.)	(Calories per minute)	
				Miners				
James D.	20	63	160	120	54·5	1·55	0·99	1·07
Willie B.	41	65	165	121	55	1·60	0·92	0·75
Benny L.	30	68	173	121	55	1·65	1·00	0·87
Tom A.	27	64	163	126	57	1·60	0·98	0·84
James C.	41	63	160	134	61	1·63	0·94	0·97
Pat C.	32	67	170	137	62	1·72	1·04	0·88
Dave S.	30	66	168	137	62	1·70	1·01	1·10
George M.	40	65	165	139	63	1·69	1·00	1·10
James H.	40	66	168	140	63·5	1·72	1·02	1·02
Willie K.	24	68	173	142	64·5	1·77	1·09	1·14
Jock N.	30	67	170	145	66	1·75	1·06	0·93
John H.	32	69	175	147	66·5	1·80	1·09	1·05
James W.	30	68	173	149	67·5	1·80	1·09	—
Alex W.	33	71	180	151	68·5	1·86	1·12	1·05
George L.*	41	63	160	153	69·5	1·73	1·00	0·89
Jock E.	27	70	178	160	72·5	1·90	1·13	1·06
Crawford C.	45	65	165	164	74·5	1·82	1·03	1·19
Will J.	32	65	165	169	76·5	1·84	1·10	1·09
Jock F.	43	65	165	173	78·5	1·85	1·06	1·05
Dave W.	42	71	180	181	82	2·02	1·17	1·20
				Clerks				
Jimmie R.	32	67	170	121	55	1·63	0·99	0·86
David H.	23	69	175	132	60	1·73	1·08	1·12
John A.	22	67	170	138	62·5	1·73	1·09	—
Joe K.	29	67	170	138	62·5	1·73	1·05	1·04
George B.	46	68	173	142	64·5	1·76	1·00	1·10
Willie C.	20	71	180	144	65·5	1·83	1·17	1·12
Ian C.	29	66	168	145	66	1·74	1·06	1·13
Bob T.	28	68	173	149	67·5	1·80	1·10	1·05
George C.	32	65	165	155	70·5	1·78	1·07	1·07
James L.	22	70	178	159	72	1·83	1·15	1·17

* Failed to complete the experiment.
† Refer to text (p. 10) for method of arriving at these figures.

In the selection of suitable men, attention was paid to the following points:

1. For general convenience the men were chosen from those living within easy reach of the colliery.

2. The men had to be working on the day-shift or be prepared to change to this shift.

3. It was desirable to have miners working at all eight coal-producing faces in the pit in order to give a complete picture of the conditions underground.

4. We tried to study pairs of men working side by side.

Twenty miners started; one failed to complete the experiment due to a domestic crisis. Ten clerks started and all finished. Heights, weights and ages of the men are given in Table 1. All the men had been living in Fife for at least two years. Most were Fifers born and bred, but a few had come from the West of Scotland and one from England. Only one was unmarried. Many were fortunate enough to live in modern houses built since 1945 on housing estates, but about half were in older houses with less adequate accommodation and amenities.

THE NATURE OF THE WORK

Miners

Eighteen of the men were strippers, working the coal on the long-wall system. The coal was undercut beforehand by machinery and the strippers had to hew it away from the face with a pick and then to shovel it on to mechanically-operated pans or conveyor belts. Two of the men were brushers preparing a new section of the pit for coal-getting. All these men had to carry out hard muscular work. At most of the faces the climate was warm but comfortable, wet-bulb readings being 70° to 72° F. (21–22° C.). However, in one section known as the Dip mine it was hot, humid and uncomfortable, the wet-bulb temperature varying from 81° to 84° F. (27–29° C.). The dry-bulb readings were usually 2° F. higher. Ventilation was good, and temperatures never rose more than 2° F. during the course of a shift. Fuller details of the nature of the work and the conditions underground are given in Appendix A (p. 49).

During the week of observation each of our subjects completed five day-shifts, being wound down the pit between 5.45 and 6 a.m. and wound up between 1.30 and 2 p.m. In addition all but one of the men worked a short overtime shift (6 a.m. to 12 noon) on the Saturday, and two also worked a shift on the Sunday. One managed to work eight shifts, 60 hours, in a single week. There are strong financial inducements to work such hours.

Clerks

Six of the clerks worked in check offices recording the attendance of the miners. The other four worked in dispatch offices and were responsible for the weighing, labelling and dispatch of coal wagons. Much of the work was done at high desks where the man could either sit on a high stool or stand, and so 'standing' is a frequent entry in our analysis of the working activities of the clerks. The dispatch clerks had some walking to do while they labelled and checked the wagons in the railway yards. Although the clerks were working in the offices of a colliery, their duties, which were largely completion of forms and keeping of ledgers, were characteristic of office work anywhere. The clerks, like the miners, worked on a shift system for six days of the week with an average weekly attendance of just under 51 hours. One clerk had a subsidiary week-end job as a barman.

ACTIVITIES OUTSIDE WORKING HOURS

Miners

Even men who lived close to the pit had to be out of bed by about 5 a.m. in order to be wound down at the proper time, and breakfast was usually hurried. After the shift the men took a shower in the pit-head baths and changed their

clothes. They usually went home to a large meal prepared especially for them and then slept or dozed for two to three hours in an armchair or on a bed. Thereafter they took things comparatively quietly, reading, playing with their children and sometimes going for a short stroll in the evening. Bedtime was usually between 11 p.m. and midnight. Most spent Saturday afternoons watching soccer, and on Saturday evenings many were to be found in local inns and public houses. A mid-week visit to the cinema was common.

None of the miners who were our subjects had energetic recreations, and none played outdoor games. In this respect they were possibly not quite representative of the mining community. Some miners in the colliery regularly played golf on two excellent courses in the neighbourhood.

Clerks

One clerk was an energetic cyclist, another a keen bowler and a third was an enthusiastic golfer. It would not be fair, however, to assume that the clerks, spending less energy in their employment than the miners, therefore tended to have more vigorous recreations. The pattern of the clerks' lives in their leisure time was very similar to that of the miners. Our results showed that the energy expended by a clerk and by a miner when not at work was, on the average, almost identical.

Work in the Field and the Laboratory

THE EXPENDITURE OF ENERGY

The research team was divided into two main groups separated by some nineteen miles. The field workers were lodged in a miners' hostel at Buckhaven, close to the colliery, while the laboratory workers lived in a students' residence in St. Andrews.

Field Workers

These made a continuous record of the nature and duration of every activity on the part of the subjects and from time to time took measurements with the K.M. respirometer. When the respirometer was used the quantity of air breathed out, the exact duration of the sampling, and an exact description of the subject's activity during sampling were at once entered on a special analysis sheet, Fig. 3. The sample of the expired air collected in the rubber bladder attached to the respirometer was transferred without delay to a numbered glass sampling-tube and the number of the tube was entered on the analysis sheet. Whenever the respirometer was used underground a sample of the mine air was taken at the same time, since the oxygen and carbon dioxide content of the mine air differed somewhat from that of the air on the surface and varied from time to time.

Subjects at work. During the working shifts an observer was present with each man and recorded his subject's activities during every minute of the time. For this purpose a notebook was used (Fig. 4) each page of which contained 120 small squares, one square representing one minute. By using code letters, for example 'S' for 'sitting', 'W' for 'walking', 'H' for 'hewing', and so on, it was possible to record the duration of any particular activity to the nearest minute.

Subjects off-duty. For the remainder of the twenty-four hours, each man was asked to record how his time was spent as accurately as possible in a similar notebook. Each man was visited every day at his home by one of the observers

NAME..... Sample Tube No. .....

Date / / Age..... Ht..... Wt.....

Time of taking sample Hr.

ACTIVITY PLACE

Air Temp..... Barometer.....

Gas Meter:		Time sampled	m. sec.
Final reading	litres	K.M. No.	K.M. Factor
Initial reading	,,	Temp. expired air	
Difference	,,		

Observer Leader of team

Atmospheric corr. factor		Gas Analysis:	Inspired air	Expired air
Ventilation rate	l/m			
		CO_2 %		
		O_2 %		
O_2 consumption	l/m	Calories per min.		
R.Q.				

Analyst

Calorie value expired air $\frac{\qquad}{20} = 0\cdot$ Calories per min.

FIG. 3. Analysis sheet. The first part was filled in by the observer immediately after taking the sample from the subject; the second part was completed in the laboratory by the analyst and the computer.

who went over the entries in the notebook and cleared up any obscurities. The observer then added up the times spent on the separate activities and transferred the essential facts to a 24-hour sheet, Fig. 5.

Laboratory Workers

These used one of the excellent laboratories in the Physiology Department of the United College of the University of St. Andrews. Analysis of the samples of expired air was carried out using Haldane's gas analysis apparatus, five sets of which were available. Gas analysis was always done in duplicate and the results were entered on the analysis sheets. The sheets were then passed to a computer who worked out the energy expenditure for the activity in question. Forty gas samples could comfortably be analysed in one working day.

Additional details of the methods used in the field and in the laboratory are given in Appendix B (p. 52).

THE INTAKE OF ENERGY

A full description of the technique used and of the results obtained in this part of the investigation is given in Part II of the Report.

Subject ..... JOCK C.
Observer ..... DURNIN
Date ..... 29·8·52

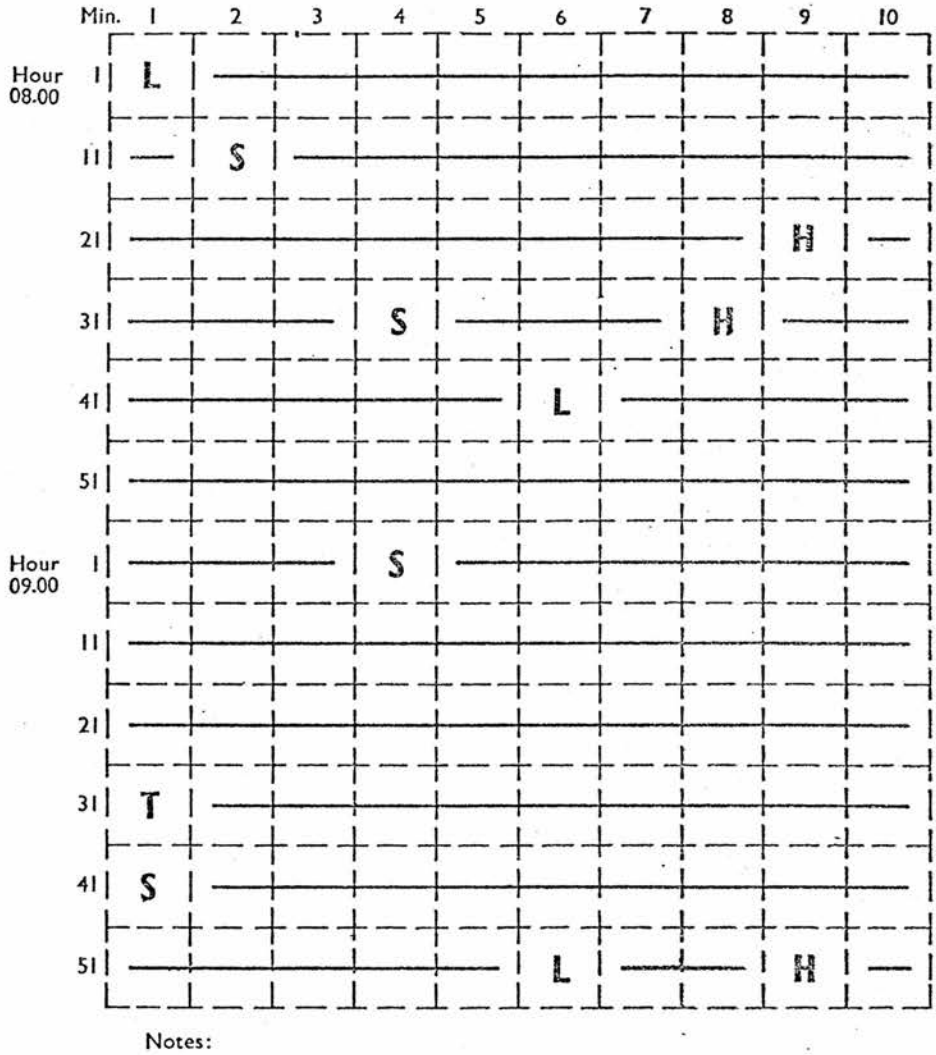

FIG. 4. Sample page of notebook. During the period of 2 hours covered by this page, the subject has spent 32 minutes loading (L), 15 minutes hewing (H), 10 minutes timbering (T), and 63 minutes sitting (S).

The dietary survey was carried out by the individual inventory method. All the food eaten by each man was weighed or measured throughout the period of seven consecutive days during which he was under observation. The task of the dietitians was made easier by the fact that the men normally had certain of their meals at a different time from the other members of the family and often had special dishes prepared for them. This was their usual practice and quite

Name..... Date.....

Age..... Height..... Weight.....

Occupation.....

Activity	Time spent		Cal./min.	Total Calories
	hr.	min.		
A In bed				
B Recreational and off work:—				
(1) Light sedentary				
(2) Washing, shaving, dressing				
(3) Light domestic work				
(4) Walking at m.p.h.				
(5)				
(6)				
(7)				
(8)				
(9)				
(10)				
(11)				
(12)				
Total recreational and off work				
Observer:—				
C Working:—				
(1) Walking				
(2) Standing				
(3) Sitting				
(4) Loading				
(5) Hewing				
(6) Timbering				
(7)				
(8)				
(9)				
(10)				
Total working				
Observer:—				

Grand total for day:

FIG. 5. The form used to summarize the activities and energy expenditure of one man over a period of 24 hours.

independent of our activities. The intake of energy in the form of food was calculated by reference to tables of food values. The intake of protein, of fat, of carbohydrate, of minerals and of vitamins was found in a similar way.

Results

THE EXPENDITURE OF ENERGY

Tables 31 to 35 (Appendix C, p. 55) give all the Calorie values obtained for all the activities of each of our subjects. These figures give the gross observed rates of energy expenditure in Calories per minute; we could see no purpose in trying to find the net rate of energy expenditure by deducting a figure supposed to represent the basal metabolic rate.

We attempted to obtain several readings on each man for every common activity, and the tables give the results of every observation where the subject was breathing easily and where the respirometer, to the best of our knowledge, was in order. Not unexpectedly, very considerable variation was found in the metabolic cost of apparently the same activity carried out by the same person at different times. In the total of 752 results, however, there were 9 so out of line that the probability of an error was great. These 9 results have been omitted when finding the means, and are indicated by asterisks in the tables.

The mean of each series of results for any one subject was taken as the metabolic cost of that particular activity to that subject. In using the mean we bore in mind the possible effect of training and practice. Many persons do not take kindly at first to breathing through a mouthpiece, and most research workers have laid stress on the importance of training and of experience in the use of metabolic equipment. This, no doubt, is reasonable when the basal metabolic rate is being found in an anxious patient in hospital. We, however, found that training had no effect on the rate of energy expenditure, either when the subject was lying quietly or when he was engaged in heavy muscular work. This not unimportant finding may have been due partly to the wholehearted co-operation of our subjects and their confidence in the observers, and partly to the fact that we normally used a light plastic valve-assembly and a respirometer which did not greatly obtrude on the wearer's consciousness. The effect of practice is discussed further in Part III (p. 41).

Because of practical difficulties, no routine measurements of energy expenditure during sleep were possible. We derived values for our subjects' expenditure of energy during sleep in the following way. The mean of all the figures for each subject when lying, was divided into the predicted basal metabolic rate (B.M.R.) for that subject, using Robertson and Reid's 1952 standards. We then took the mean of these values in their turn, keeping the values for the miners separate from those for the clerks. In the case of the miners the mean was 0·717 and in that of the clerks, 0·823. Implicit in this manoeuvre is the assumption that the energy expenditure actually observed when the men were lying at rest is related to the true basal rate. The expenditure of energy by each individual during sleep was then calculated by multiplying the mean of the observed values for energy expenditure by that individual when lying at rest, by 0·717 in the case of a miner and by 0·823 in the case of a clerk. The relevant figures are given in Table 1 (p. 4).

Many observers have recorded metabolic rates during sleep below basal levels. On the other hand, the specific dynamic effect of the day's food probably

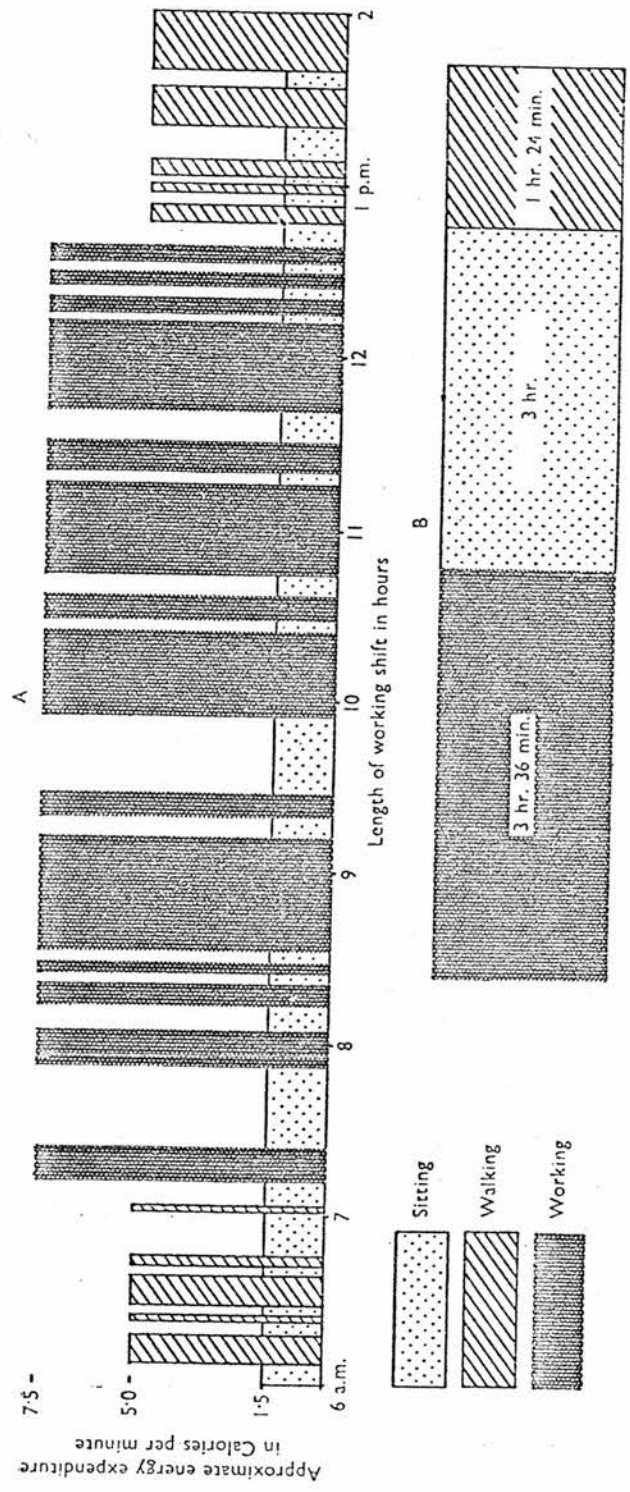

FIG. 6. Diagram of a typical working shift underground.
A. Shows a typical pattern of activity and the corresponding rates of energy expenditure.
B. Shows the relative amounts of time spent in each of the three main activities.

persists during the early hours of sleep and raises the metabolism above the basal level. When the whole period of sleep is taken into account these two factors may well cancel each other out in many instances. It must be recognized that assessments of the expenditure of energy during a period of sleep rest on unsure foundations; however, an error of as much as 10 per cent in this computation for any individual amounts to only some 50 Calories in the estimate of the total daily output of energy.

No measurements were taken for the cost of dressing, washing and shaving. In a pilot study (Passmore, Thomson and Warnock, 1952) in which careful measurements of such activities were made on five young men, the Calorie value of washing, dressing and shaving was found to be 1·9 times the cost of standing, with only minor variations. Accordingly, in the case of our subjects, these activities have been costed by multiplying by the factor of 1·9 the values observed for each man while standing.

In Table 36 (Appendix C, p. 62) a summary is given of the total expenditure of energy by each miner during an entire week. This table also contains an analysis of the time spent on each activity, together with its Calorie value. Table 37 gives the same information for the clerks. Fig. 6 (p. 11) shows graphically the pattern of energy expenditure by a miner during a working shift underground.

Table 2 below summarizes the findings on the expenditure of energy. The miners on the average each spent nearly 900 Calories per day more than the clerks. There is no doubt, then, that those of our subjects who were engaged in heavy physical work used more energy each day than did those engaged in sedentary work. These findings may not apply to all modern types of heavy industry but they certainly hold for mining under the conditions we experienced. Table 2 shows, moreover, that it was while engaged in their daily work that the miners made their extra demand on energy, requiring during their working hours roughly twice as many Calories as the clerks during theirs. When not at work, miners and clerks made closely similar demands on energy; in spite of the smaller amount of energy used by the clerks at work, they gave no evidence of spending more energy than the miners in active physical pursuits in their leisure time.

TABLE 2

Mean daily expenditure of energy of the 10 *clerks and the* 19 *miners during a 7-day week*

Activity	Mean daily expenditure of energy per head during one week	
	Clerks (Calories)	Miners (Calories)
Sleep, and daytime dozing	500	490
Activities when not at work	1,410	1,420
Activities at work	890	1,750
Total	2,800	3,660

THE INTAKE OF ENERGY

Table 3 gives the average daily intake of energy for each man. Each day the miners took in almost exactly 1,000 Calories more than the clerks. The origin of this energy from protein, fat and carbohydrate is given in Table 4. There is one noteworthy feature. The percentages of the Calories derived from protein, from fat and from carbohydrate are very similar for the miners and the clerks. In other words, the miners, to obtain the 1,000 additional Calories per day, ate more of *all* the proximate principles. Protein and fat are often put in a special

TABLE 3

Average daily expenditure and intake of energy of each subject

Subject	Average daily expenditure and intake of energy (Calories)		Relationship of intake to expenditure (Calories)
	Expenditure	Intake	
	Clerks		
Jimmie R. ..	2,350	2,810	+ 460
David H. ..	2,380	2,500	+ 120
John A. ..	2,330	2,850	+ 520
Joe K.	2,820	3,360	+ 540
George B. ..	2,800	3,360	+ 560
Willie C. ..	3,150	3,130	− 20
Ian C. ..	2,760	2,620	− 140
Bob T. ..	3,100	3,250	+ 150
George C. ..	3,060	2,730	− 330
James L. ..	3,290	3,830	+ 540
Mean	2,800	3,040	+ 240
	Miners		
James D. ..	3,430	3,560	+ 130
Willie B. ..	2,970	3,560	+ 590
Benny L. ..	3,150	3,890	+ 740
Tom A. ..	3,060	4,270	+ 1,210
James C. ..	3,660	3,090	− 570
Pat C.	3,370	3,100	− 270
Dave S. ..	3,870	4,160	+ 290
George M. ..	3,530	4,010	+ 480
James H. ..	3,140	4,110	+ 970
Willie K. ..	3,420	4,050	+ 630
Jock N. ..	3,570	4,420	+ 850
John H. ..	3,780	3,990	+ 210
James W. ..	3,700	4,600	+ 900
Alex W. ..	4,560	4,380	− 180
Jock E. ..	4,090	5,410	+ 1,320
Crawford C. ..	4,320	3,990	− 330
Will J.	3,660	4,180	+ 520
Jock F. ..	3,670	3,320	− 350
Dave W. ..	4,510	4,480	− 30
Mean	3,660	4,030	+ 370

TABLE 4

Contribution of protein, fat, carbohydrate and alcohol to the total energy value of the diets of the miners and of the clerks

Nutrient	Miners		Clerks	
	Average daily intake (Calories per head)	Percentage of total daily Calorie intake	Average daily intake (Calories per head)	Percentage of total daily Calorie intake
Protein	484	12·0	384	12·6
Fat	1,350	33·5	1,074	35·3
Carbohydrate ..	2,001	49·7	1,505	49·6
Alcohol ..	195	4·8	77	2·5
Total	4,030	100·0	3,040	100·0

category as body-building and protective foodstuffs. It has been thought that when the human body has obtained the necessary minimum of these, it draws the energy required for physical exertion from carbohydrate, which is classified as primarily a fuel. Our subjects did not conform to this theory.

In the account of the dietary survey in Part II of this Report, the diets of the men are analysed in very considerable detail and the significance of the findings is discussed.

THE BALANCE

Table 3 sets out also the average daily expenditure of energy of both clerks and miners. Two-thirds of the men showed a clear excess of intake over expenditure. This difference is statistically significant at a low level (see Part III, p. 47). In view of the difficulties of collecting the basic information and the fluctuations inherent in physiological processes, the lack of complete agreement was not unexpected and could be accounted for by one or more of the following factors:

(a) The time spent in the more energetic activities may have been underestimated. This did not arise to an appreciable extent during the working time, the whole of which was under direct observation; but outside working hours we had to rely to some extent on the subject's own estimate of the duration of his activities.

(b) When wearing the respirometer the men may have worked less energetically than normally. There was, however, no sign of any such restriction on muscular activity, and, as already mentioned, the men had no difficulty with the apparatus.

(c) The estimate of the energy available from alcoholic drinks may have been too high.

(d) The dietary survey may have put the wives of our subjects on their mettle. They may have given their menfolk rather more ample meals than usual.

Some of our subjects might have shown a small net gain in weight at the end of the week. We did not find it practicable to weigh the men under exactly comparable conditions at the beginning and end of the survey. It is very doubtful

if a change in weight corresponding to the observed excess in intake of Calories would have been detectable in view of all the other possible minor variations.

Discussion

OTHER STUDIES ON COALMINERS

Although for both geological and mechanical reasons comparisons may be misleading, it is interesting to compare our present findings with the results obtained by Moss (1935) in the West Midlands of England and by Lehmann, Müller and Spitzer (1949) in two German coalfields. The Germans do not give figures to enable the metabolic cost of separate activities to be estimated, but Moss, in Table V of his paper, gives average values, from observations on seven to twelve men, for oxygen consumption and for the Respiratory Quotient when the men carried out specific mining activities. If these figures are converted into Calorie expenditure per minute using Zuntz and Schumburg's factors they are not unlike our own for similar activities (Table 5). The average weight of the subjects was the same in both investigations, so the slightly higher values found by Moss may be due to minor variations in the working conditions below ground. Table 6 gives two sets of values from the eleven sets given by the Germans. These are compared with the findings of Moss and of ourselves. The resemblances and the differences are worthy of note.

TABLE 5

The mean metabolic cost of typical activities in underground mining: a comparison of results from Moss's study in the West Midlands of England with figures from the present study

Area of study	Mean metabolic cost of the following activities: (Calories per minute)		
	Hewing	Loading	Timbering
West Midlands of England	7·3	7·7	7·1
Fife	7·0	7·1	5·7

A study of different types of work in some Italian coalmines should also be mentioned (Granati and Busca, 1941). These results are essentially similar to those reported by Moss in this country, and seem to show a total energy expenditure somewhat higher than that in the more modern surveys. Their figures are of the order of 4,500 Calories per day. These mines were not mechanized.

There is a general impression today that brawn is becoming progressively less important in industry. We have not yet, however, become a nation of machine minders, and, by any standards, our miners were spending a considerable number of Calories at their work. Paradoxically enough, had mechanization below ground been more reliable, the men would have had the opportunity to use their muscles even more. We were impressed by the amount of time the miners were forced to be inactive underground, usually because of a breakdown in the mechanical haulage system. Fig. 6 (p. 11) illustrates a typical working

TABLE 6

A comparison of some results from other studies of coalminers with figures from the present study

	(1)	(2)	(3)	(4)	(5)
Area and date of study	Length of working week hr. min.	Length of average shift hr. min.	Total time working (including travelling) underground per man per shift hr. min.	Average energy expenditure underground per man per shift (Calories)	Average daily expenditure of energy per head over a 7-day week (Calories)
England (West Midlands) 1935	— —	7 30	5 21	2,800	4,070
Germany (Ruhr) 1943–4	50 24	8 24	4 36	2,140	3,820
Germany (Mahrisch Ostrauer) 1943–4 ..	48 0	8 0	5 30	1,650	3,360
Scotland (Fife) 1952 ..	47 14	7 44	4 14	1,990	3,660

Since the results of the different surveys have not been recorded in an identical manner these figures need a little explanation. Moss states that during a period of 5 hours 30 minutes at the coal-face, an average of 27 per cent of the time was spent at rest, and 12 per cent eating a 'snap'. This means that, in the whole shift, 2 hours were spent travelling and 3 hours 21 minutes working, with a combined travelling and working time of 5 hours 21 minutes. The figures for Calorie expenditure underground per shift and average daily expenditure (on a 7-day basis) are taken from the table on p. 150 of his paper.

For the German figures, two sets of observations from Table 1 of their paper have been chosen. The figures in columns 1, 2, 3 and 5 above are straight from columns 3, 4, 5 and 9 in this table. The figures in column 4 have been obtained by multiplying their figures for working Calories per hour by total hours worked and adding an arbitrary 1 Cal./min. for basal metabolism.

The figures for Fife in columns 1, 2 and 3 are taken from the averages in Tables 31 to 35, and those in columns 4 and 5 from averages in Tables 2, 36 and 37.

shift and shows the very spasmodic nature of the work. It also gives in simple diagrammatic form the relative times spent in travelling to and from the place of work, in sitting and at work. Our subjects were inactive for 38 per cent of their total sojourn underground. The corresponding figure for an English mine is 29 per cent, and those for the German mines 45 and 31 per cent.

Table 7 shows the total time spent underground each week by our nineteen miners. It also shows the time spent travelling underground and the actual time at work with pick and shovel. From the colliery records we obtained the figures of output at each face, the number of strippers at the faces, and the weekly output per stripper. These are given in Table 8. The figures for the Dip mine are not comparable with the others since in this part of the pit some men primarily on development work also helped in production. The output per stripper per shift lay within the limits of 6 to 9 tons. This is a very usual output in a mine of this type and such figures indicate that the men we studied are fairly representative of the mining community of this country.

TABLE 7

Details of the hours spent underground by each miner during one week

Week beginning:	Subject	No. of shifts worked	Total time underground		Total time travelling underground		Total time at work with pick and shovel	
			hr.	min.	hr.	min.	hr.	min.
July 28, 1952	Tom A. ..	6	46	9	12	0	13	31
	James W. ..	6	46	0	12	0	14	9
	George M. ..	6	45	52	14	12	16	30
August 4, 1952	James D. ..	6	48	0	9	41	15	17
	James H. ..	6	48	43	9	48	17	39
	Dave W. ..	7	53	27	10	18	23	1
	Will J. ..	7	53	17	10	37	19	26
August 11, 1952	Jock F. ..	6	49	31	12	41	16	48
	Pat C. ..	6	45	32	12	6	18	16
	Alex W. ..	6	47	57	8	23	23	46
	Crawford C.	6	45	35	8	12	19	13
August 18, 1952	Willie K. ..	5	40	5	10	39	12	27
	Jock E. ..	8	60	17	16	30	15	8
	John H. ..	6	44	9	11	55	20	11
	Dave S. ..	6	44	40	11	56	18	36
August 25, 1952	Benny L. ..	6	44	31	12	19	17	40
	Jock N. ..	6	44	28	12	6	16	7
	Willie B. ..	6	44	58	12	0	17	46
	James C. ..	6	45	10	12	2	22	39

TABLE 8

Details of coal production at Wellesley Colliery over several periods of one week

Week beginning:	Section of pit	Amount of coal produced at face (tons)	No. of strippers working at face	Average production of coal per stripper (tons)
July 28, 1952	Basin	1,243	34	37
	Basin West	874	22	40
August 4, 1952	Barn Craig 5 East ..	1,470	31	47
August 11, 1952	Dip	390	6+	—
	Dysart East	830	17	49
August 18, 1952	Bow House	1,233	27	46
	Chemyss 5 East ..	964	20	48
August 25, 1952	Barn Craig 5 East ..	1,682	32	52
	Dip	545	6+	—

Table 9 shows that the English miners spent more energy per minute when underground than did the miners in Scotland and in Germany. The figures for all groups include expenditure of energy while travelling to and from the face and while resting; the expenditure of energy during actual production is much higher.

TABLE 9

Mean rate of expenditure of energy during the underground shift in the English, German and Scottish mines

Area of study	Mean rate of energy expenditure per man during the entire underground shift (Calories per minute)
England (West Midlands) ..	6·2
Germany (Ruhr)	4·2
Germany (Mahrisch Ostrauer)	3·4
Scotland (Fife)	4·3

We cannot, unfortunately, answer the question as to how much work a miner could do if given full opportunity. Our miners were already spending a very considerable amount of energy day in, day out. Economy could have been exercised by enabling them to travel to and from the coal-face with less physical labour. At the face itself there were many interruptions which were frustrating rather than restful, and as a consequence the activity after such pauses may have been, on occasion, uneconomically energetic. With steadier working conditions the men might have been able to carry out more work with the same, or little more, energy expenditure and perhaps with less fatigue.

To make this survey, more than thirty investigators were required for a period of five weeks; only nineteen miners and ten clerks were studied. With experience it might have been possible to reduce the number of investigators somewhat. Nevertheless, it is obvious that a study of this kind is costly in manpower. A simple survey of food intake, which would have given us very similar figures for total energy requirements, would have been much less costly in effort and in personnel. It is clear, however, that only a study of the actual course of the expenditure of energy, as in the present survey, can answer the question "Where do the Calories go?" And only when the answer to this question is known is it possible to obtain a picture of the pattern of the metabolic demands made upon workers in industry.

Summary

In August 1952 a survey was carried out on nineteen underground miners and ten clerks in a colliery in Fife, Scotland. Each man's intake of energy and of essential nutrients over a period of one week was gauged by a dietary survey employing the individual inventory method. Simultaneously each man's expenditure of energy over the same period was determined by indirect calorimetry using the Kofranyi-Michaelis respirometer.

The results were as follows:

1. The total daily expenditure of energy by the underground miners, all of whom were performing hard physical labour during their working hours, was, on the average, 3,660 Calories. This expenditure was 860 Calories greater than the average daily expenditure of the clerks, whose work was of a sedentary nature.

2. While at rest and while off duty and at home the two groups of workers had an almost identical expenditure of energy.

3. The difference in energy expenditure between the two groups over the 24-hour period was due wholly to the additional demands on energy made by the miners during their working hours.

4. The intake of energy in the form of food reflected the difference in expenditure of energy. The miners took in 990 more Calories than the clerks each day.

5. In the case of both clerks and miners the mean daily intake of energy was somewhat greater than the mean daily expenditure. This result may have been due to some circumstance associated with the methods used in the survey.

6. Although the miners obtained many more Calories from their diets than did the clerks, the percentages of the Calories derived from protein, from fat and from carbohydrate were essentially the same in the two groups. There was no suggestion that the miners drew the additional energy required for their hard physical work from foods predominantly composed of carbohydrate.

Brit. J. Pharmacol. (1956), **11**, 35.

THE EFFECT OF 5-HYDROXYTRYPTAMINE ON THE EXCRETION OF WATER IN CONSCIOUS DOGS

BY

V. C. ABRAHAMS AND MARY PICKFORD

From the Department of Physiology, University of Edinburgh

(RECEIVED OCTOBER 12, 1955)

5-Hydroxytryptamine (5-HT) is present in a number of tissues, but its physiological role is still undetermined. Erspamer (1954a, b,) found that 5-HT was powerfully antidiuretic and that, in rats, the subcutaneous route of administration was the most effective, as little as 4 μg./kg. producing definite and fairly long-lasting antidiuresis in the most sensitive individuals. By means of clearance measurements he found that glomerular filtration rate (GFR) was reduced and that there might also be some reduction in renal plasma flow (RPF). He concluded that 5-HT antidiuresis was due to preferential constriction of the afferent glomerular arterioles and, further, that the posterior pituitary played no part in the antidiuresis. He believes that 5-HT is a hormone designed for the physiological regulation of renal function (1953). Since most work on 5-HT has been done on rats, and little on dogs (Erspamer and Ottolenghi, 1951 ; Sala and Castegnaro, 1953), and since it is important to determine whether 5-HT is a renal hormone, as Erspamer believes, or merely an antidiuretic substance, we have carried out a number of different studies on the dog.

METHODS

Observations were made on conscious bitches during water diuresis. The methods of collecting urine and inducing diuresis have previously been described (Abrahams and Pickford, 1954). 5-HT was administered in the form of the creatinine sulphate dissolved in 1.0 ml., or less, of 0.9% NaCl solution. All doses are calculated and given as weight of base/kg. body weight of dog. The drug was injected subcutaneously, intramuscularly, intra-arterially or intravenously. The intra-arterial injections were made either into the sinus-denervated carotid artery lying in a previously prepared van Leersum loop, or into the aorta through a catheter inserted, under local anaesthesia, *via* the femoral artery. The catheter was inserted to a measured length immediately before giving the experimental dose of water. The position of its tip was ascertained later by reintroducing the catheter when the animal was killed. When intra-aortic injections were made the substance to be injected was dissolved in a volume of 0.9% NaCl solution less than that of the dead space of the catheter, namely, 0.8 ml. The test solution was injected into the catheter and then washed into the circulation with 1 or 2 ml. 0.9% NaCl solution.

Some observations were made on the renal clearances of diodone and creatinine before and after the intravenous injection of 5-HT. The methods used have already been described (Pickford and Ritchie, 1945). They avoid interference with the dog except for the collection of blood samples, and provide a period of 20 to 25 min. at the plateau of diuresis when the plasma concentrations of diodone and creatinine remain relatively constant. If antidiuresis occurs the period of steady plasma concentration is prolonged.

When required, blood pressure was measured in the conscious dog through a side-arm of the aortic catheter, and recorded by means of a mercury manometer.

RESULTS

Subcutaneous and Intramuscular Administration of 5-HT.—Only 4 observations were made on the effect on water diuresis of subcutaneously administered 5-HT. One bitch (Angela) received 22 μg./kg. 36 min. after the ingestion of water. The course of the diuresis thereafter was not noticeably different from normal. It was, however, possible that there had not been sufficient time for the drug to be absorbed and produce its effect. Therefore, in another animal (Darkie) 5-HT was given at the time of water administration. On one day the dose was 32 μg./kg. and on 2 others 74 μg./kg. subcutaneously. On no occasion did antidiuresis occur (Fig. 1).

Two animals were given 13.5 and 36 μg./kg. 5-HT intramuscularly and showed no hint of antidiuresis. On the other hand, 32 μg./kg. given intramuscularly to Darkie caused on this occasion an immediate and marked antidiuresis. The short latency of the response (1.0 min.) makes it probable that this positive response of Darkie to an

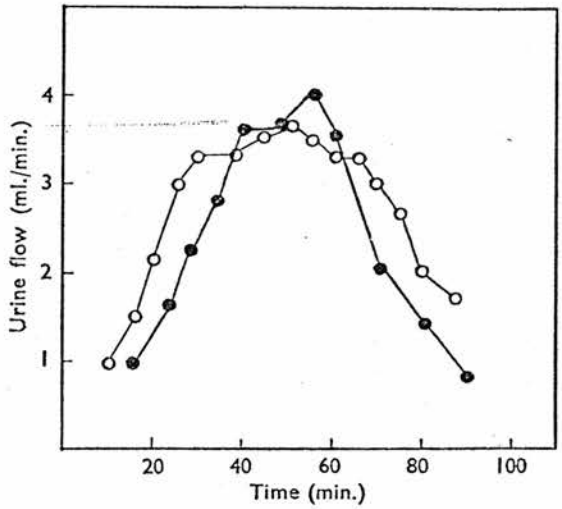

FIG. 1.—Record of water diuresis (Darkie). 300 ml. water by mouth at zero time. ●——● normal water diuresis. O——O, water diuresis when 74 μg./kg. 5-HT was given subcutaneously at zero time. In all graphs, ordinate is rate of urine flow in ml./min., and abscissa is time in min.

intramuscular injection when this animal gave consistently negative responses to subcutaneous injections was coincidental.

Administration of 5-HT into the Malleolar Vein.—A single injection of 5-HT given into the malleolar vein during water diuresis caused antidiuresis to a variable extent. The doses tested ranged from 0.6 μg./kg. to 84 μg./kg. Since the effect in any one dog varied from time to time (Fig. 2) despite apparently constant conditions, which included the time of day and the anoestrous condition of the animal, it was difficult to determine the minimum effective dose. Some dogs showed more variability of response than others. It was, however, clear that, in 11 normal dogs, true antidiuresis, as contrasted with momentary cessation of flow due to ureteric spasm (Abrahams and Pickford, 1956a), was not regularly seen with doses under 10 μg./kg. (Table I). Only one animal (Smokey) showed a sensitivity greater than this, and at post mortem this dog was found to have only the left kidney. On the right there was nothing but a fibrous cord in place of the ureter. The one kidney was large, dimpled on the surface, and showed, on histological examination, many areas of considerable fibrosis. In life there had been nothing to suggest that this dog was congenitally abnormal. This animal was one of the first on which observations were made, and unfortunately, before it was killed, information was given to Professor Erspamer that, in dogs, the sensitivity to intravenously administered 5-HT ranged from 4 to 20 μg./kg. Later experience amends these figures to 10–20 μg./kg.

TABLE I

MINIMUM INDIVIDUAL EFFECTIVE ANTIDIURETIC DOSES OF 5-HT WHEN INJECTION WAS MADE INTO THE MALLEOLAR VEIN

No. of Observations	Dog	Dose 5-HT μg./kg.	No. of Observations	Dog	Dose 5-HT μg./kg.
15	Frisk	10	6	Bouncer	14
2	Topsy	18·5	4	Darkie	16
3	Sheila	20	8	Angela	21·8
5	Jess	12·7	5	Kit	9·4
4	Chris	13·5	10	Judy	10
4	Lady[1]	18·5	5	Thisbe	13
6	Smokey[2]	4·0			

[1] With diabetes insipidus. [2] Congenital absence of right kidney.

Some observations were made on one dog at a time when it had a prolapsed uterus. It could be seen that, when antidiuresis occurred in response to an injection of 5-HT, there was a perceptible paling of the vaginal mucous membrane, lasting about 5 min.; when antidiuresis did not occur there was no pallor. Furthermore, all dogs which subsequently showed antidiuresis sighed, panted, or

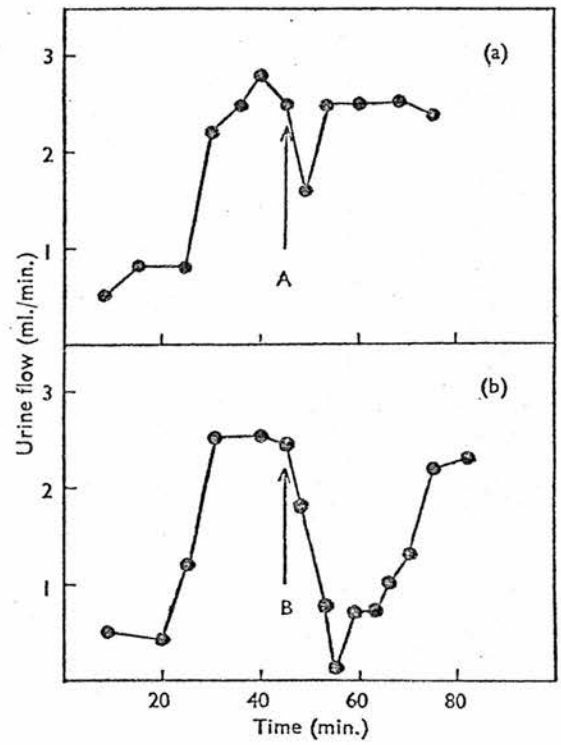

FIG. 2.—Record of water diuresis (Judy). 250 ml. water at zero time. In (a), at A, 34.8 μg./kg. 5-HT. In (b), at B, 17.4 μg./kg. 5-HT. Injections made into malleolar vein on both occasions.

yawned some seconds after the injection of 5-HT. If this respiratory response was minimal or absent antidiuresis did not occur.

Administration of 5-HT into the Carotid Artery.—Intracarotid injections of 5-HT were given 4 times in the following doses: 8.6 μg./kg., 9.2 μg./kg., 9.4 μg./kg., and 18.8 μg./kg. The results were variable, but it was clear that the effect of 5-HT was no greater nor was the minimum effective dose smaller when the drug was given into the common carotid instead of the malleolar vein (Fig. 3). These findings agree with those of others, who state that the action of 5-HT is not a central one (Erspamer, 1954a). With regard to the respiratory effects, these were no greater, and perhaps less, on intracarotid than on intravenous injection. This agrees with the findings of Douglas and Toh (1953) and of Schneider and Yonkman (1954).

Renal Clearances Following the Administration of 5-HT.—Renal clearances of diodine and creatinine were measured 15 times on 5 dogs before and after the injection into the malleolar vein of doses of 5-HT ranging from 9.2 to 18.5 μg./kg. The responses varied considerably, antidiuresis being observed in 9 of the 15 experiments. In the 9 experiments in which antidiuresis occurred, its degree varied from one or more transitory depressions to a prolonged inhibition of urine flow

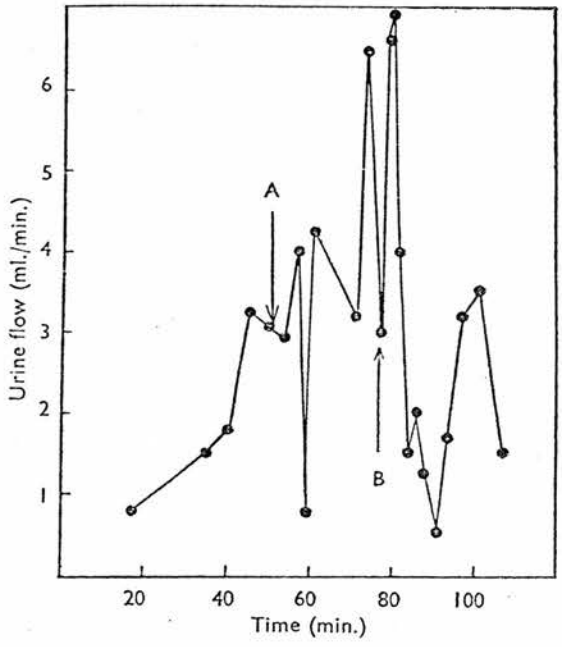

FIG. 3.—Record of water diuresis (Angela). 300 ml. water at zero time. At A, 18.8 μg./kg. 5-HT into the left common carotid artery. At B, 18.8 μg./kg. 5-HT into the malleolar vein.

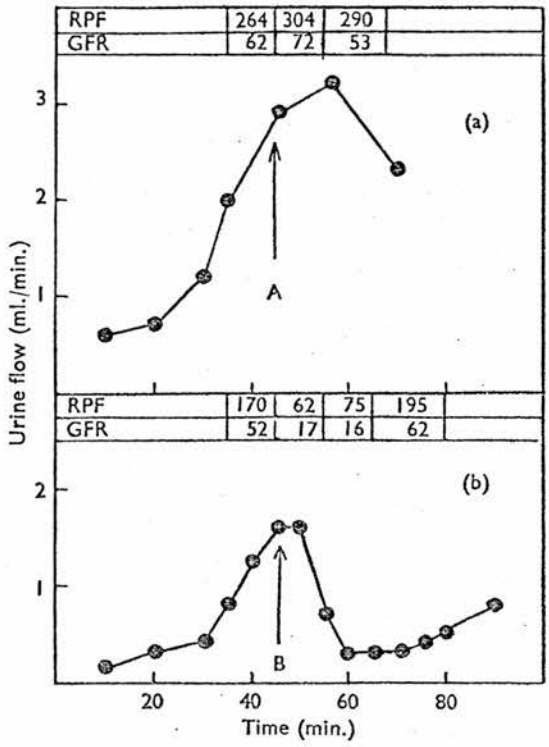

FIG. 4.—Record of water diuresis and renal clearances in ml./min. of diodone (RPF) and creatinine (GFR) in 2 dogs before and after injection into the malleolar vein of 5-HT. (a) Bouncer; at A, 10.2 μg./kg. (b) Judy; at B, 15 μg./kg.

(Fig. 4). When depression was transitory both clearances rose and fell roughly to the same extent and in parallel with the urine flow, suggesting a purely vascular origin for the alterations in renal activity. When there was prolonged inhibition of urine flow, both clearances fell in 7 out of 9 experiments; once the GFR alone and once the RPF alone decreased. After the initial depression which lasted for 20 to 25 min. the RFP rose towards or above the preinjection level, even though the urine flow was still inhibited. On three occasions the GFR also increased (Fig. 4).

When urine flow was not inhibited by the administration of 5-HT both clearance values rose to a greater or lesser extent (Fig. 4).

Thus 5-HT, in contrast to posterior pituitary lobe extract, is uncertain and variable in its action on dog kidney unless given in fairly large doses. The initial phase of 5-HT antidiuresis seems to depend on decreased plasma flow and glomerular filtration rate, the latter especially tending to persist. The fact that antidiuresis persisted after recovery of both clearances suggested that the preliminary reduction of blood supply to the kidney

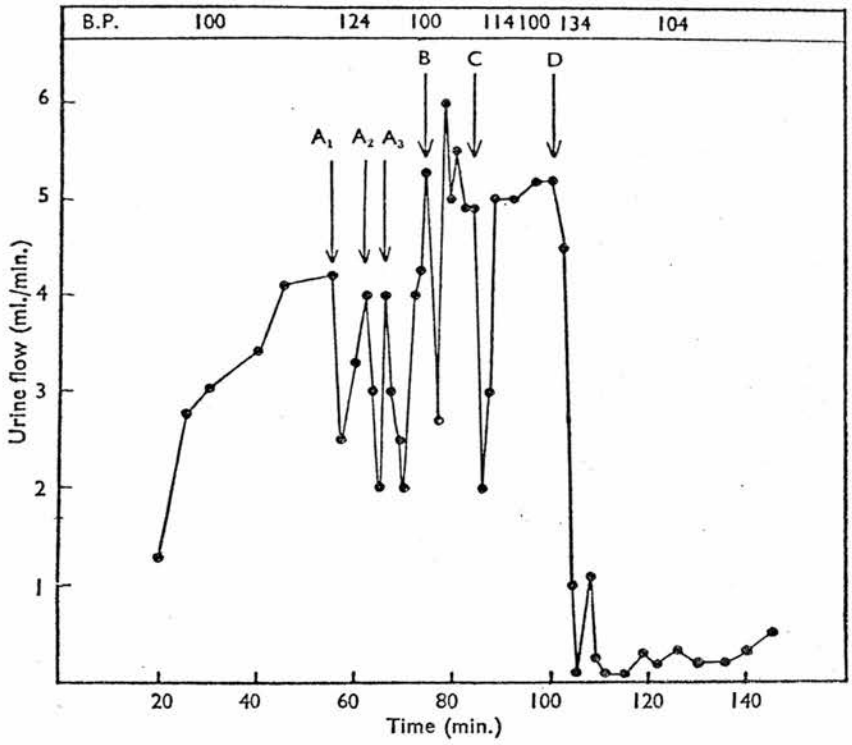

FIG. 5.—Record of water diuresis (Thisbe). 300 ml. water at zero time. A_1, A_2, and A_3, three separate intra-aortic injections of 10 μg. adrenaline. B, 8 μg./kg. 5-HT intra-aortically. C, 40 μg./kg. 5-HT intra-aortically. D, 20 μg./kg. 5-HT into the cubital vein. At top, blood pressure in mm. Hg.

gave rise to some other and more long-lasting reaction (Verney and Vogt, 1938, 1943), possibly reflex. Therefore, the reaction of the kidney to 5-HT injected into either the aorta or the cubital vein was examined. By this means information might be obtained about the origin of the vascular changes occurring in the kidney, i.e., whether the kidney shows a particular sensitivity to the drug, or whether it simply responds to a change in vascular conditions elsewhere in the body.

Administration of 5-HT into the Aorta.—The minor operation of aortic catheterization did not usually delay the onset of water diuresis. In five out of six experiments there was no difference from the normal pattern of diuresis; only once was its onset delayed by 30 min. In all instances the catheter tip was one or more inches above the origin of the right renal artery. Control observations showed that the injection of 2 ml. saline alone into the aorta had no effect on the rate of urine flow.

One animal (Jess) received an intra-aortic injection of 22 μg./kg. 5-HT. Three min. after this dose the urine flow fell from its previous rate of 6 ml./min. to 5.3 ml./min., and 3 min. later was again 6 ml./min. This was the only noticeable response. Respiration was in no way disturbed. Owing to a clot in the catheter it was not possible to repeat the injection. The negative result could have occurred because the 5-HT was swept past the renal arteries and did not reach the kidneys. Another dog (Thisbe) received 8 μg./kg. 5-HT into the aorta, showed no change in blood pressure and inhibition of urine flow for one minute; 40 μg./kg. caused a small rise of blood pressure and another transitory fall in the rate of urine flow (Fig. 5). On both these occasions the reduction in the rate of urine flow seemed to be due to ureteric spasm. In this dog it was unlikely that the 5-HT failed to reach the kidneys, since three consecutive injections of 10 μg. adrenaline given intra-aortically (Fig. 5) immediately before the 5-HT injections caused the type of urinary inhibition always seen after the intravenous injection of adrenaline. In a third dog (Chris) the intra-aortic injection of 36 μg./kg. 5-HT caused a large rise in blood pressure and inhibition of urine flow (Fig. 6).

Administration of 5-HT into the Cubital Vein.—When 5-HT was injected into the malleolar vein the appearance of antidiuresis was correlated to the degree of the respiratory response (see top of p. 37). It was therefore possible that both antidiuresis and panting depended on changes in the vascular bed in the thorax, or on direct stimulation of sensory

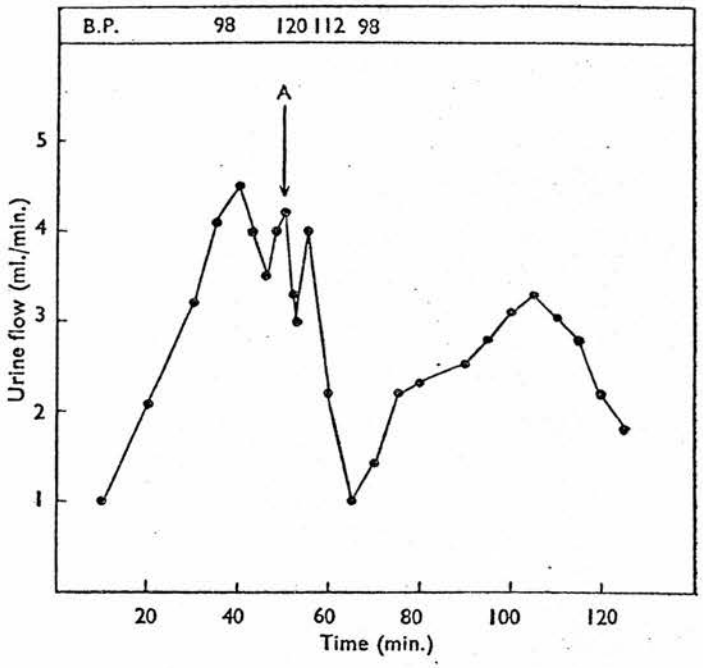

FIG. 6.—Record of water diuresis (Chris). Effect of intra-aortic injection, at A, of 36 μg./kg. 5-HT. At top, blood pressure in mm. Hg.

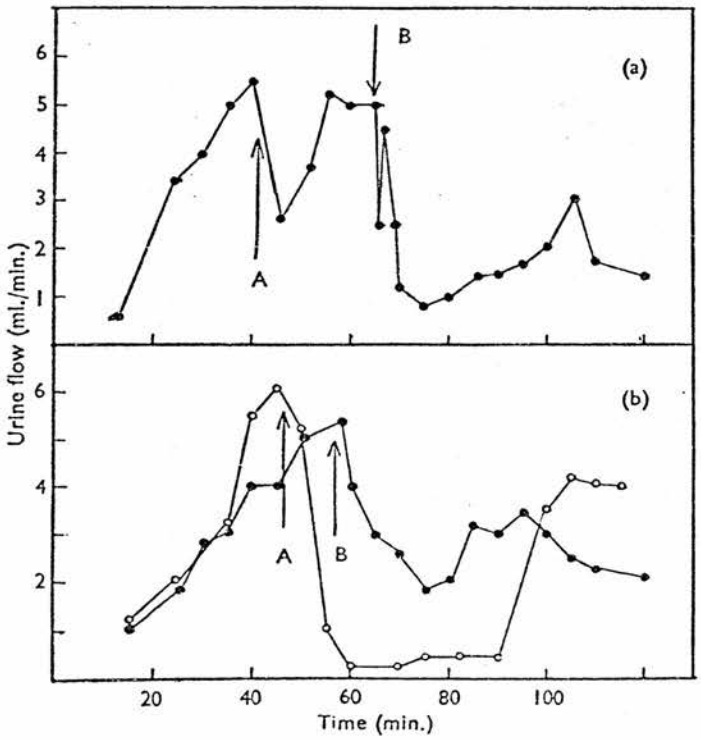

FIG. 7.—Record of water diuresis (Jess). Comparison of the effect of 5-HT injected into the malleolar and cubital veins. (a) At A, 13.4 μg./kg. into malleolar vein; at B, 13.4 μg./kg. into cubital vein. (b) O——O at A, 13.4 μg./kg. into cubital vein; ●——● at B, 13.4 μg./kg. into malleolar vein.

receptors there (Comroe, Van Lingen, Stroud, and Roncorini, 1953; MacCanon and Horvath, 1954; Mott and Paintal, 1953; Schneider and Yonkman, 1953, 1954)—that is, that both phenomena were caused by reflexes arising in the same area. It was also possible that the variability of the response after injection of 5-HT into the malleolar vein depended on the rate at which this substance was picked up by the platelets, and therefore on the final concentration of 5-HT reaching some part of the thoracic viscera. If these hypotheses were valid, then an injection into the cubital vein should be more effective than one into the malleolar, because the path and time to the thorax are shorter; and

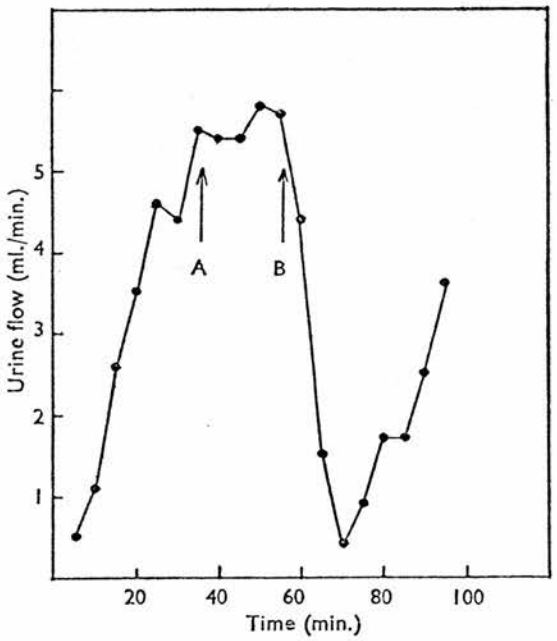

FIG. 8.—Effect on activity of 5-HT of contact with blood before injection into the circulation. Record of water diuresis (Chris). At A, 18 μg./kg. after 16 sec. contact with dog's own blood; at B, 18 μg./kg. without preliminary admixture with blood. Both injections into cubital vein.

5-HT should be inactivated by contact with blood for a short time. It has been confirmed in 5 dogs that a given dose of 5-HT is more effective as an antidiuretic when given into the fore- as compared with the hind-limb vein, and that a smaller dose is effective by the former route (see Fig. 7). Furthermore, a dose injected into the fore-limb vein was more powerfully antidiuretic than twice that amount injected into the aorta earlier the same afternoon (Fig. 5).

Fig. 8 shows the effect of an injection of 5-HT after contact with blood. For the first injection at A, after inserting the hypodermic needle in the cubital vein, 2 ml. blood was withdrawn into the syringe which already contained 18 μg./kg. 5-HT in 1.0 ml. 0.9% NaCl solution. The mixture of blood and 5-HT containing saline was then immediately re-injected. The whole procedure was completed in 16 sec. For the second injection at B, 18 μg./kg. 5-HT in 1.0 ml. 0.9% NaCl solution was given into the same vein without preliminary admixture with blood. In the first instance the change in rate of urine flow was negligible, but in the second there was marked antidiuresis. This shows that contact with blood for 16 sec. is sufficient to reduce the amount of free 5-HT below that necessary to produce antidiuresis. This interval is roughly equal to that between the injection into the hind-limb vein of 5-HT or acetylcholine and the respiratory response—that is, the circulation time from leg to thorax or head in dogs of 12 to 16 kg. body weight is about 14–16 sec.

The Effect of 5-HT on Blood Pressure.—It was evident from the intra-aortic records of blood pressure that in the conscious dog 5-HT produced the same response as in the anaesthetized animal—that is, a sharp rise in pressure lasting 2 to 3 min. and then a gradual fall. In the conscious dog the preinjection level was re-attained in 11 to 13 min. It was also clear that there was no inhibition of urine flow unless there was a change in general blood pressure (Figs. 5 and 6).

The Effect of 5-HT in the Dog with Diabetes Insipidus.—Fig. 9 shows that, in a diabetic dog (Lady), drinking between 5 and 6 l. of water daily, an intravenous injection of 15.5 μg./kg. 5-HT caused a brief inhibition of urine flow, probably due to ureteric spasm, followed by recovery and then a moderate but longer-lasting inhibition. 31 μg./kg. produced a similar response. Unfortunately there is no information about the prediabetic response of this dog. Post-mortem histological examination of the hypothalamus and pituitary showed that few supraoptic or paraventricular cells remained. Those cells present, when stained with toluidine blue, were pale and agranular, and when stained with chrome-alum-haematoxylin and phloxin were loaded with dark material. The posterior lobe and median eminence were atrophic.

The Effect of 5-HT after Renal Denervation.—In one dog (Jess) the kidneys were denervated by stripping the renal pedicles and freeing the kidneys from all peritoneal connexions. 11 days later an intravenous injection of 13.5 μg./kg. 5-HT induced antidiuresis.

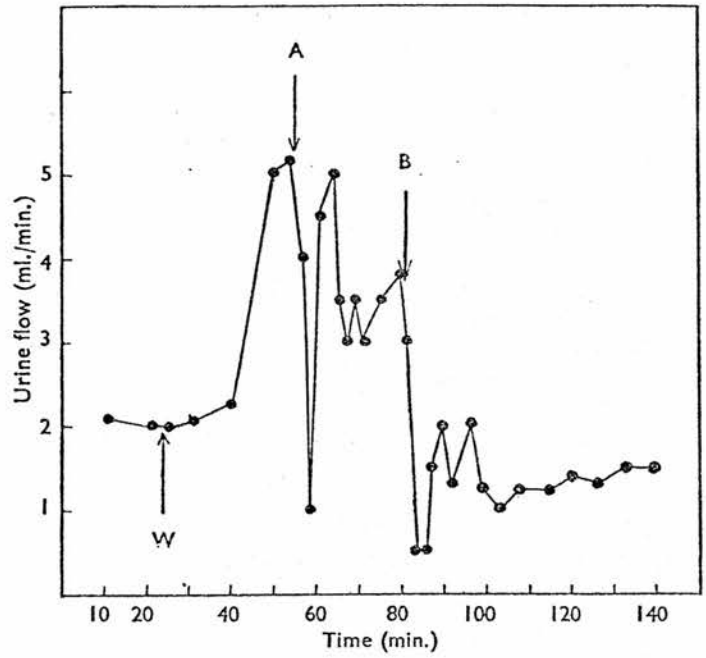

FIG. 9.—Effect of intravenous 5-HT on water diuresis in dog (Lady) with diabetes insipidus. At W, 350 ml. water by mouth. At A, 15.5 μg./kg. 5-HT. At B, 31 μg./kg. 5-HT. Both injections into malleolar vein.

Appearance of the Kidney after the Administration of 5-HT.—Once, immediately before killing an anaesthetized dog (Bouncer), the abdomen was opened and the kidney watched during the injection into the malleolar vein of 26 μg./kg. 5-HT. Some seconds after the injection the surface of the organ paled markedly. The colour began to return 2 to 3 min. later. After waiting 15 min. to allow time for recovery, a further dose of 26 μg./kg. was injected intravenously, followed 1 min. later by a concentrated solution of bromphenol blue in 0.9% NaCl solution. As quickly as possible after this the left renal pedicle was clamped, the kidney removed and bisected, and examined under low-power binocular magnification. Dye was seen plentifully in the medulla, but in only a few radial streaks in the cortex. Juxtamedullary glomeruli were empty of dye. These findings were confirmed later by histological examination of both kidneys.

DISCUSSION

The experiments described show that, in the conscious dog, single intravenous injections of 5-hydroxytryptamine (5-HT) given during water diuresis cause an inhibition of urine flow. In normal dogs, the minimum individual effective dose given into the malleolar vein varied from 10 to 20 μg./kg. In one dog with congenital absence of the right and fibrosis of the left kidney, the minimal effective dose was only 4 μg./kg. In any one dog the degree of antidiuresis induced by a given dose varied from day to day, even under constant conditions, and on some days no antidiuresis would be seen. Subcutaneous doses of up to 74 μg./kg. caused no antidiuresis. These results in the dog are similar to those of Erspamer (1954a and b) working on rats. They differ, however, in the degree of sensitivity of the two species, in the most effective route of administration and in the regularity of response. Erspamer found that in some rats as little as 4 μg./kg. 5-HT given subcutaneously caused antidiuresis. Clearly dogs do not exhibit anything like this degree of sensitivity to subcutaneously administered 5-HT. On the other hand, rats appear to be fairly insensitive to its intravenous administration (Erspamer, 1954b), though we have been unable to trace any published work on the intravenous use of a salt of the pure base in a study on water diuresis in rats.

Measurements of diodone and creatinine clearances in dogs showed that during antidiuresis the values of both RPF and GFR fell rapidly. 20 to 25 min. later RPF rose towards or above pre-injection levels, even though urinary inhibition persisted. The GFR, too, might recover during the phase of urinary inhibition, but usually it remained depressed for a longer period than the RPF. When antidiuresis failed to appear in response to an injection of 5-HT the clearances of

diodone and creatinine rose. Here, then, dogs and rats showed similar reactions, in that, when antidiuresis occurred, it seemed to depend on a reduction in GFR rather than in RPF. That blood pressure rose and vaginal mucous membrane paled when antidiuresis followed injection of 5-HT, and, on the other hand, that clearance values rose when antidiuresis failed to occur, all suggest that vasoconstriction, both generally and in the kidney, is a prerequisite for antidiuresis. In other words, the kidney is no more sensitive to 5-HT than are other areas, and 5-HT, unlike the vasopressor fraction of posterior pituitary extracts, does not specifically induce water reabsorption. This agrees with Erspamer's conclusion that the antidiuresis is vascular in origin, but is contrary to the idea that 5-HT is a specific renal vascular hormone. Sala and Castegnaro (1953) believe it is a water reabsorbing hormone. They used large doses given subcutaneously to dogs, and report that antidiuresis occurred, on occasion, in the absence of any change in GFR. There was, however, always a reduction in RPF. They interpret this as meaning that 5-HT induces specific reabsorption of water. Unfortunately no observations were made on the blood pressure which would be greatly increased by such doses, which would cause complex and variable changes in the kidney and possibly other remote effects such as release of antidiuretic hormone.

Why, in the present experiments, the reaction of the kidney to 5-HT varied from day to day is unknown. There seemed to be no relationship to the size of dose used. The variability may have depended on the proportion of injected 5-HT affecting the circulatory system and the proportion finally reaching the kidney.

No observations were made on the effect of infusions of 5-HT as was done by Corcoran, Masson, Del Greco, and Page (1954). They infused dogs intravenously at rates of 2 to 13 μg./kg./min. 5-HT, and found no hint of antidiuresis with the smaller doses and hypotension with the larger. Judging from the present results the low doses were probably too small to cause antidiuresis, even when given steadily over a period of time, since 5-HT is so rapidly removed from the circulation (Gaddum, Hebb, Silver, and Swan, 1953) that its concentration is unlikely to rise. The higher doses were within the antidiuretic range for some dogs, but hypotension would confuse the issue. The last complication was absent from the experiments described in this paper.

The present results on intracarotid injections in a dog with diabetes insipidus accord with those of Erspamer (1954a, b) and Barac (1953), who concluded that an intact posterior lobe was unnecessary for an antidiuretic reaction to 5-HT administration, and that no direct central stimulation was involved.

Certain observations make it unlikely that 5-HT can be considered a renal hormone. In the dog the uncertainty and variability of its effect on the kidney raise the suspicion that its most important action is at some other site. Compared with the kidney, the response of the systemic blood pressure, the ureter (Abrahams and Pickford, 1956a), and uterus (Abrahams and Pickford, 1956b) show greater consistency and sensitivity. When 5-HT is in contact with blood its activity is rapidly reduced, as was shown by Gaddum, Hebb, Silver, and Swan (1953) and in the present experiments. When 5-HT is given by intravenous injection the time during which it is in contact with blood before it reaches the ureter, uterus or kidney must be nearly the same ; yet the smallest injected dose to which the kidney responds varies from 10 to 20 μg./kg., whereas the other two organs react to doses of 0.5 μg./kg. and 5 μg./kg. respectively—and are thus more sensitive than the most sensitive kidney. Yet it might be expected that a hormone would act on its target organ in lower concentration than that necessary to affect other organs. The systemic blood pressure is altered by doses of 5-HT which are wholly without effect on the rate of urine flow (Abrahams and Pickford, 1956a). Further, when 5-HT is injected into the aorta, and there was reason to suppose that the drug was reaching the kidney, the renal reaction to 40 μg./kg. was insignificant, but there was a marked response to the intravenous injection of 20 μg./kg. (Fig. 5). Furthermore, antidiuresis only occurred when the general blood pressure was raised, and was never seen unless there was evident respiratory reaction to the injection. All these facts made it necessary to look beyond the kidney for the origin of the effect of 5-HT on urine flow. Mott and Paintal (1953) found that 5-HT stimulates vagal fibres whose origins lie between the great veins and left atrium ; and 5-HT is more strongly antidiuretic when injected into the cubital vein instead of the malleolar vein or aorta (Fig. 7). It would thus appear that 5-HT produces antidiuresis mainly by setting up reflexes from the thorax, probably from the pulmonary bed, which, by altering the general blood pressure, affect the kidney.

This does not exclude other modes of action as well. Page (1952) found that the perfused kidney shows vasoconstriction in response to 5-HT, though the reaction is less marked than that to

adrenaline. There is, too, the present observation that the denervated kidney responded by antidiuresis to an injection of 5-HT. This could be explained as due, in part, to the direct vasoconstrictor action of 5-HT ; but also, almost certainly, to the powers of autoregulation displayed by the denervated kidney (Sellwood and Verney, 1955). No substance can strictly be called a renal hormone because it is active on the denervated kidney, unless, at the same time, the general blood pressure is unchanged.

Finally, there is the problem of why the antidiuretic effect of 5-HT sometimes greatly outlasts an effective blood concentration. Verney and Vogt (1938, 1943) found that in some dogs a long-lasting suppression of urine flow followed short periods of renal arterial occlusion. It may be that injected 5-HT, by depriving the renal cortex of blood for as long as 2 or 3 min., which it was observed to do, has much the same effect as renal arterial occlusion. Possibly, too, the sharp changes in blood pressure may cause the release of antidiuretic hormone from the posterior lobe of the pituitary.

Summary

1. Observations were made on conscious dogs of the effect during water diuresis of injecting 5-hydroxytryptamine (5-HT) subcutaneously, intramuscularly, into the malleolar or cubital vein, or the aorta or carotid artery.

2. 5-HT was most effectively antidiuretic when injected into the cubital vein, less so when injected into the malleolar vein or carotid artery, and still less so when given into the aorta at a point above the origin of both renal arteries. It was least effective when administered subcutaneously or intramuscularly.

3. When injected into the malleolar vein the minimum effective antidiuretic dose varied from 10 to 20 μg./kg.

4. Antidiuresis was not regularly seen after a given dose of 5-HT. When it did occur it was accompanied by obvious respiratory reactions. When aortic pressure was measured this was seen to rise at the onset of antidiuresis. The vaginal mucous membrane also paled. If the respiratory and vascular reactions were inconspicuous or absent antidiuresis did not occur.

5. During antidiuresis the renal clearances of diodone and creatinine fell. One or both values might increase towards or above preinjection level before urine flow recovered. When 5-HT failed to cause antidiuresis both clearance values rose.

6. The meaning of the results is discussed ; it is concluded that there are several reasons for the antidiuretic action of 5-HT, but that it is not a specific renal hormone.

We wish to record our gratitude to the Upjohn Company for a generous supply of 5-hydroxytryptamine creatinine sulphate.

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THE EFFECT OF 5-HYDROXYTRYPTAMINE ON THE URETER AND ON THE BLOOD PRESSURE OF DOGS; AND OF ADRENALINE, NORADRENALINE, AND POSTERIOR PITUITARY EXTRACTS ON THE URETER

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When observing the action of 5-hydroxytryptamine (5-HT) on the rate of urine flow in conscious dogs it was often noticed that almost immediately after an intravenous injection of this substance there was a brief marked slowing of urine flow, followed by an equally brief period of rapid flow before the appearance of the long-lasting antidiuresis described by Erspamer (1954) (Fig. 1). These fluctuations in rate of urine flow lasted no more than 2–3 min. and could be most easily explained as due to temporary spasm of the ureters. It was decided, therefore, to examine the response of the ureter *in situ* to 5-HT. Moreover, it was of interest in connexion with the antidiuresis seen after 5-HT to know whether renal function was affected by smaller or greater doses than those which had an action on smooth muscle such as that of ureter, uterus and of the vascular system. At the same time, the opportunity was taken of observing the effect of some other drugs on the ureter, and of the effect of 5-HT on the blood pressure of the anaesthetized dog.

METHODS

Nine dogs were anaesthetized either with chloralose. 0.1 g./kg., or with pentobarbitone sodium, 0.033 g./kg., intravenously. The abdomen was opened through a midline incision and a length of ureter, of 2 inches or more, was prepared for perfusion through its lumen by inserting one cannula into its upper end just below the renal pelvis, and another into its lower end either immediately above the bladder, or, from within the bladder, through the ureteric orifice. Care was taken when tying in the cannulae to leave the main ureteric blood vessels outside the ligatures so that the ureter kept its normal blood supply and, to some extent, its normal innervation. This arrangement made it possible for solutions injected into distant veins to reach the ureter by the normal route. The lumen of the ureter was perfused with 0.9% NaCl solution from a Mariotte bottle connected with one or other cannula, so that the pressure and direction of perfusion could be controlled. Inflow and outflow from the ureter were connected to the exterior by fine rubber tubes

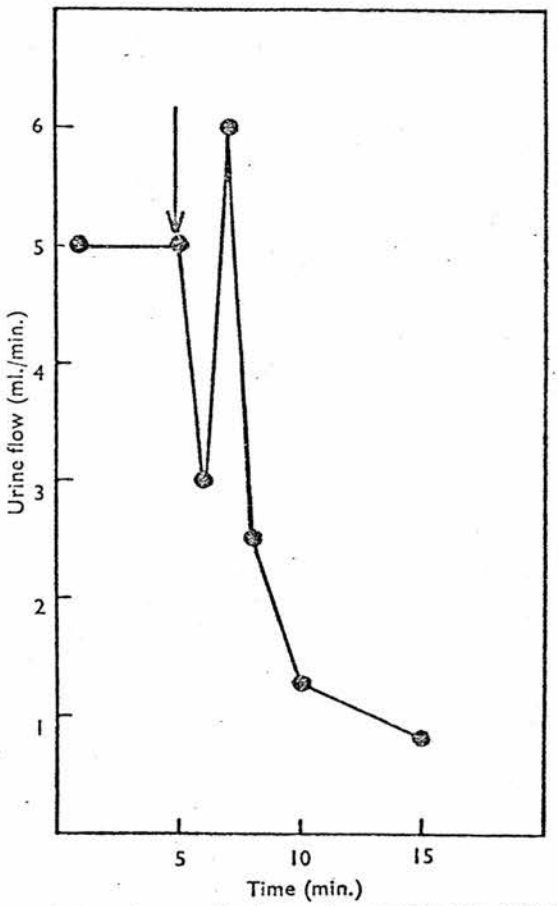

FIG. 1.—Part of a water diuresis curve in a conscious dog. 350 ml. water given by mouth 65 min. before the intravenous injection, at arrow, of 12.7 μg./kg. body weight 5-HT. Ordinate, urine flow in ml./min. Abscissa, time in min.

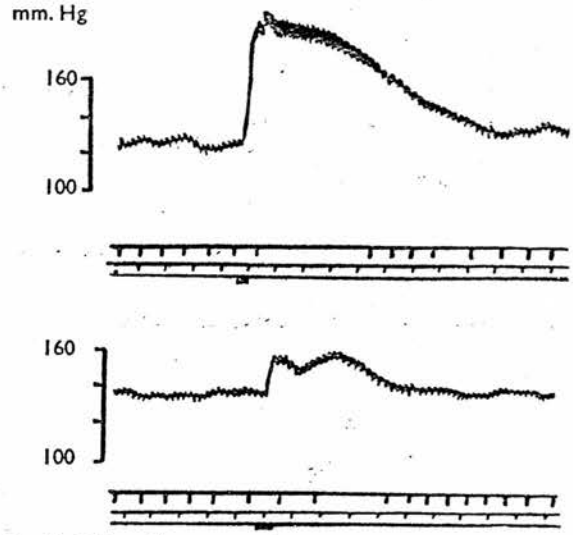

FIG. 4.—Effect of intravenous adrenaline and noradrenaline on flow through the ureter. Upper record, 5 μg. (−)-noradrenaline Lower record, 5 μg. (−)-adrenaline. In each record: top line, arterial blood pressure; 2nd line, ureteric flow; 3rd line, time in 10 sec.; bottom line, signal marker.

Intermittency was not due to resistance in the perfusion system, since at any pressure above zero a steady, more or less rapid, stream of fluid passed. If the blood supply of the ureter was occluded, or if anaesthesia was deep, there was no intermittency of flow, and instead a continuous series of drops emerged from the lower cannula. The results were the same whether chloralose or pentobarbitone sodium was the anaesthetizing agent. On two occasions the ureter was perfused from its lower end. Despite this reversal of direction the pattern of flow was unchanged.

With this information available, observations on the actions of drugs on the ureter were made at perfusion pressures between 25–30 cm. water unless otherwise stated, and only on ureters that showed intermittency of flow.

Effect of 5-HT.—Fig. 3 shows the effect on flow through the same ureter of three different concentrations of 5-HT given into the saphenous vein. With increase in amount of the drug, flow was held up for a longer period. Since, in the conditions of the experiments, the pressure above a ureteric block did not build up as it would have done in natural circumstances (see Fig. 1), no secondary rush of fluid could be expected, and none was seen. The minimum intravenous dose observed to have an effect on the ureter was 3.6 μg./kg. (Fig. 3). It can also be seen from Fig. 3 that the cessation of ureteric flow began just after the onset of the blood-pressure rise, and that flow returned before the blood pressure fell to its initial level. If, during the inhibition of flow caused by 5-HT, the perfusion pressure was quickly raised to 55 or 60 cm. water, then ureteric flow started again immediately.

Effect of Adrenaline and Noradrenaline.—Fig. 4 shows that both adrenaline and noradrenaline caused a short-lasting cessation of transureteral flow which occurred during the period of increased arterial pressure. On one occasion, 2 min. after the intravenous administration of 2 mg. of phentolamine, (2-*N*-*m*-hydroxyphenyl-*p*-toluidinomethyliminazoline, "Rogitine") neither ureter nor blood pressure reacted to 10 μg. adrenaline.

Effect of Extracts of Posterior Lobe of Pituitary.—Doses of vasopressor extract up to 10 mU. were without effect on the pattern of flow through the ureter. On the other hand, 50 sec. after 50 mU. of the oxytocic fraction had been given there was a temporary decrease in the rate of flow, whilst following 100 mU. the number of drops in a series

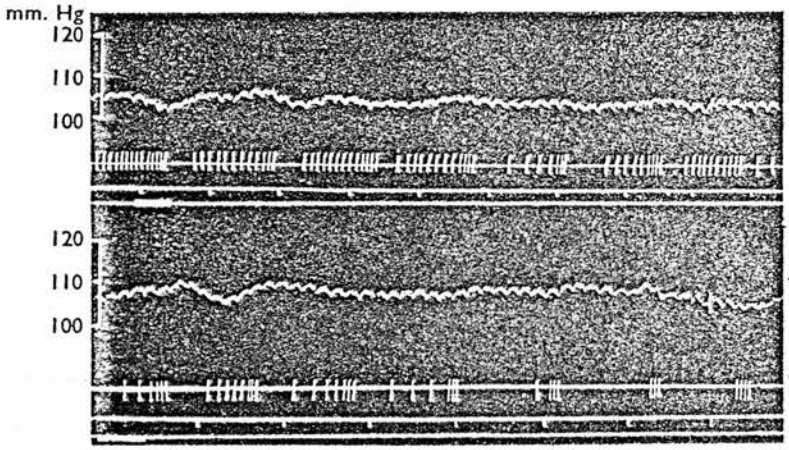

FIG. 5.—Effect of intravenous oxytocin on flow through the ureter. Upper record, 50 mU. at signal; lower record 100 mU. at signal. In each part: top line, arterial pressure; 2nd line, drop record of ureteric flow; 3rd line, time in 10 sec.; bottom line, signal marker.

passing either through the midline incision or through stab wounds in the flank. The rate of flow of fluid through the ureter was measured by means of a drop recorder. Carotid or femoral arterial pressure was recorded from a mercury manometer. The abdomen was closed before observations were begun.

5-HT was administered in the form of the creatinine sulphate, but all doses are calculated and given as the weight of base/kg. body weight. Posterior lobe extracts used were "Pitressin" and "Pitocin" of Parke, Davis & Co. Synthetic (−)-adrenaline and (−)-noradrenaline bitartrate were made up shortly before use in 0.9% NaCl solution in such concentration that the volume injected was never more than 1.0 ml. All injections were made intravenously.

Results

It was found that no fluid passed through the ureter when the perfusion pressure was below a critical level which varied somewhat in different dogs. Usually the limiting pressure was about 20 cm. water, but in some dogs it was as low as 15 cm. Just above the minimum effective pressure, drops appeared at regular intervals in ones, twos or threes. At somewhat higher pressures a number of drops passed through quickly, regularly followed by a short pause (Fig. 2). Further increase of perfusion pressure increased the number of drops between pauses until a point was reached when the drops came steadily and continuously. This pressure varied greatly between dogs, ranging from 40–60 cm. water. In any one experiment the pattern of flow through the ureter remained constant for a given perfusion pressure.

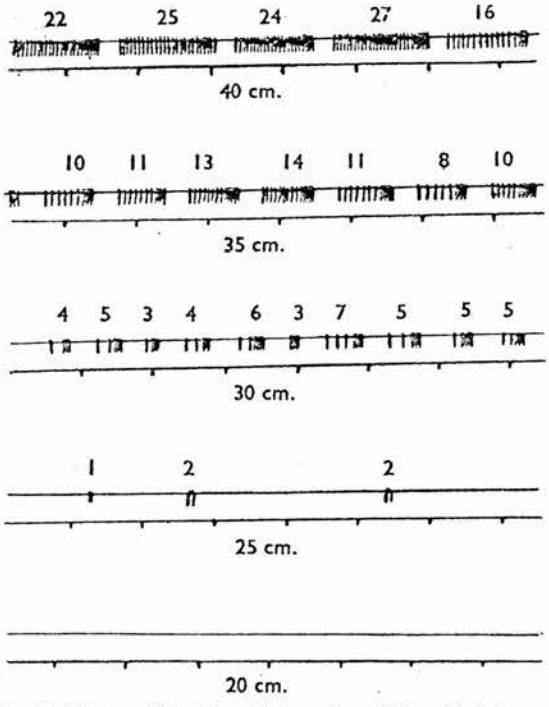

FIG. 2.—Pattern of flow through the ureter at different intralumenal pressures. Upper line is drop record of ureteric flow, the numerals above which indicate the number of drops appearing from the ureter at each outflow-spurt of the perfusion fluid. Lower line, time in 10 sec. Intralumenal pressures in cm. water.

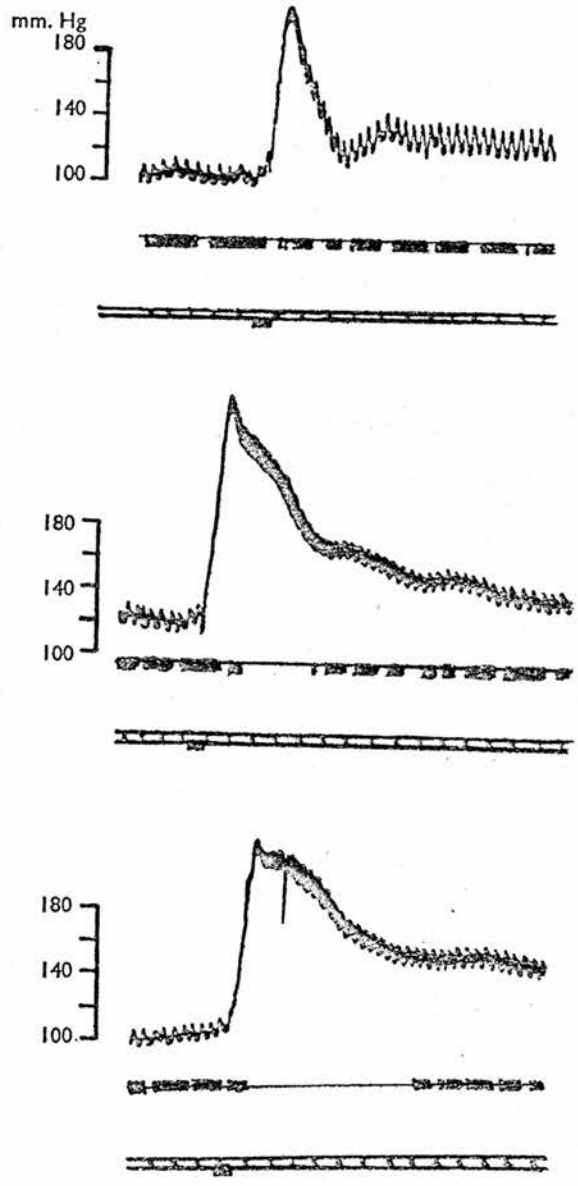

FIG. 3.—Effect of intravenous 5-HT on flow through the ureter, and on arterial pressure. Top record, 3.6 μg./kg. 5-HT; middle record, 10.8 μg./kg. 5-HT; bottom record, 18 μg./kg. 5-HT. In each record: top line, arterial pressure; 2nd line, drop record of ureteric flow; 3rd line, time in 10 sec.; bottom line, signal marker.

decreased and the length of the pauses between series increased for more than 5 min. (Fig. 5).

Effect of 5-HT on Arterial Blood Pressure.—In 9 dogs given 41 injections of 5-HT ranging from 3.6 μg. to 84 μg./kg., only twice (Fig. 6) was there any fall of blood pressure. Both occurred in the same dog following the injection of 4 μg./kg. 5-HT. Later, this animal gave a pure

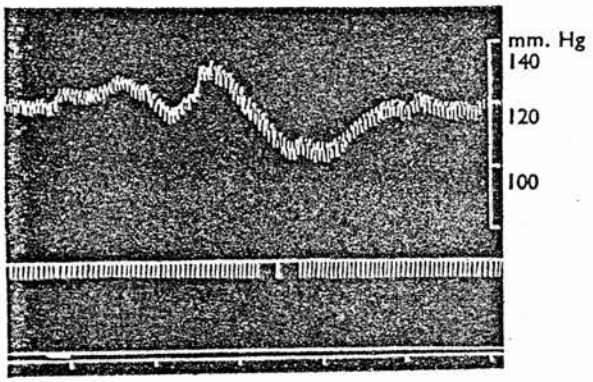

FIG. 6.—Blood-pressure record. Dog, pentobarbitone sodium. At signal, 3.6 μg./kg. 5-HT intravenously. Illustrates the only occasion on which any fall of pressure occurred after the initial rise.

pressor response to the same or larger doses. Two other dogs given 4 μg. and 2 μg./kg. of 5-HT showed only a rise of blood pressure. On all other occasions 5-HT caused an immediate rapid rise in blood pressure which lasted about 60 to 70 sec. The pressure then fell to within 8–10 mm. Hg of the preinjection level, thereafter falling slowly over some minutes. This occurred after moderate as well as after large doses of 5-HT (Fig. 7). Table I gives four examples, picked at random from the records, showing the time before the blood pressure recovered completely. Thus, in anaesthetized dogs, the intravenous administration of 5-HT had a pure pressor action which lasted some minutes. The response to the intracarotid injection of 5-HT was always a marked, but short-lived, pressor effect (Fig. 7).

TABLE I

TO SHOW TIME FOR FALL OF BLOOD PRESSURE TO NORMAL AFTER INTRAVENOUS 5-HT

Four examples picked at random

Dose (μg./kg.)	Time (min.)
7·3	5·0
7·3	3·0
21·0	4·8
49 0	6·0

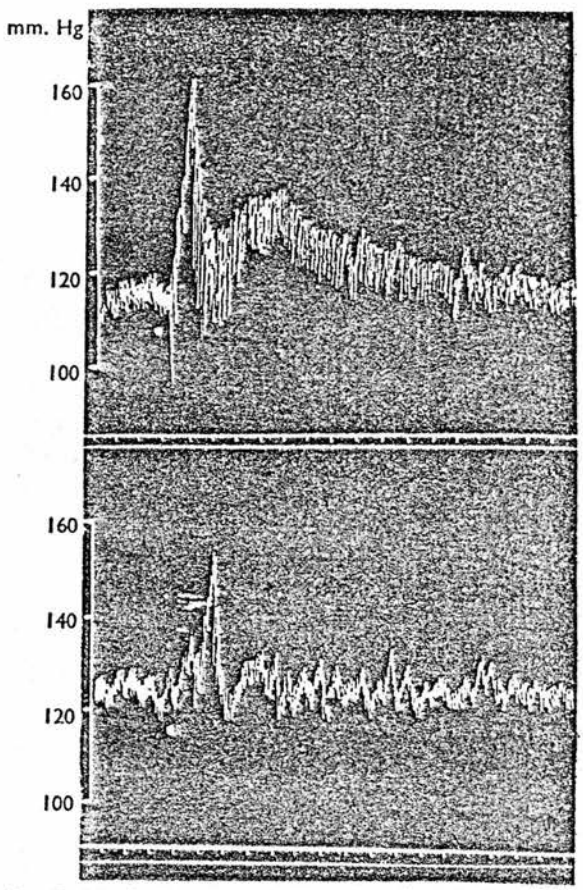

FIG. 7.—Blood pressure record. Dog, pentobarbitone sodium. Comparison of the effect in the same dog of 12.5 μg./kg. 5-HT given into the femoral vein (upper record), and into the carotid artery (lower record).

DISCUSSION

The preliminary observations on ureteric activity showed that between certain perfusion pressures fluid emerged from the lower end of the ureter in small rushes with regular pauses between. Since the abdomen was closed, peristalsis was not actually seen; but the observations are in accord with clinical and experimental work showing that the ureters have peristaltic activity, and that urine enters the bladder in spurts. Much of the experimental work on the ureter has been done on the excised organ observed in a bath, but Trattner (1932) noted the response of the human ureter *in situ* and found that contractions appeared in it with an intralumenal pressure of 0–12 cm. water and were maximal between 3 and 18 cm. water. He also found that peristalsis disappeared at 38–70 cm. pressure. Lucas (1908) found that a pressure of 15 cm. water applied at the lower end of the isolated

ureter of dogs was not transmitted to a higher level —that is, the muscle was able to remain contracted against this force. Morales, Crowder, Fishman, and Maxwell (1952) observed in dogs with exteriorized trigones that at high rates of urine flow, which presumably means at high renal pelvic pressures, the urine came in a continuous jet and not in spurts. All these pressure values and reactions are similar to those recorded here, namely, that intermittent flow began at about 15 cm. water and became continuous at various pressures from 40 cm. upward. It is surprising that the ureter seems to work as well in reverse as in the normal direction, but Gruber (1930) noticed the same phenomenon in the isolated ureter of pigs. The preparation used may thus be considered a reasonably normal one, and consequently suitable for study of the action of 5-HT and other drugs.

The ureter of the dog was sensitive to as little as 3.6 μg./kg. 5-HT given intravenously, and reacted by a spasm lasting 1 to 2 min. which began almost immediately after injection. It appeared that the ureteric muscle relaxed sooner than that of the vascular system, but it may be that the perfusion pressures used were sufficient to force open the ureter during the phase of recovery from spasm. It seems clear that the rapid swings in rate of urine flow seen immediately after the intravenous injection of 5-HT can be fully explained by its action on the ureter; the contraction holds back the urine, which accumulates in the renal pelvis, so that, when relaxation occurs, a considerable volume is delivered in a short time.

Several workers (Gruber, 1930; Lucas, 1908; Roth, 1917) have found that the isolated ureter responds to adrenaline by an increase in tone or strength of contraction. This accords with the present findings on the intact ureter, that both adrenaline and noradrenaline temporarily inhibit transureteral flow during the phase of greatest increase in arterial pressure.

Ross and Stehle (1930) showed that an intravenous injection of 1 mg. of a laboratory-made posterior pituitary lobe extract had a constrictor action on the ureter that was inadequate to account for antidiuresis. The observations described above show that an antidiuretic dose (10 mU.) of the vasopressor fraction has no perceptible effect on the ureter, whereas the oxytocic fraction, in amounts that may well occur in a limited number of normal circumstances (Abrahams and Pickford, 1954), does cause contraction of the ureteric, as of the uterine, muscle.

With regard to the effect of 5-HT on blood pressure, the results were only in part the same as those obtained by other workers. Only in one dog was there any secondary hypotensive effect. On all other occasions the response was purely pressor, and no late sustained fall of pressure was seen even after large doses (Page and McCubbin, 1953). Why these results were different is unknown. Intracarotid injections of 5-HT caused a sharp rise of blood pressure, with return to normal in about 1 min. The response was immediate, and no secondary fall of pressure was ever seen. Thus 5-HT may have a transient central action (cf. Heymans and Heuvel-Heymans, 1953). That the responses to intracarotid and intravenous injection of 5-HT were somewhat different may depend on the rate at which 5-HT is removed from the circulation (Gaddum, Hebb, Silver, and Swan, 1953).

The object of the observations on blood pressure was not to re-examine the mechanism of action of 5-HT but to determine the dose which, given intravenously, had a measurable effect on the blood pressure, and to compare it with the dose to which other systems or tissues reacted. In Fig. 3 the response of the ureter is clearly a threshold one, yet the pressor response is well developed; thus it may be concluded that the ureter is less sensitive than the arterial pressure to 3.6 μg./kg. The uterus of the conscious and anaesthetized dog contracts in response to an even lower concentration than that which affects the blood pressure (Abrahams and Pickford, 1956a). As described in another paper (Abrahams and Pickford, 1956b), antidiuresis is rarely seen after single intravenous doses of 5-HT of less than 10 μg./kg. Thus, whatever may be the reason or reasons for the hypertensive action of 5-HT, a rise in general blood pressure is provoked by a smaller dose of 5-HT than affects either ureter or kidney, but needs a larger one than causes contraction of the uterus.

Summary

1. In the anaesthetized dog observations were made on the activity of the ureter left *in situ*, with blood supply intact, and the lumen perfused with 0.9% NaCl solution.

2. At perfusion pressures of about 15 cm. water, or more, small volumes of fluid at regular intervals passed through the ureter.

3. At pressures of 40 cm. water and upward, the actual pressure varying with the individual, a continuous series of drops passed through the ureter.

4. Intravenous 5-hydroxytryptamine, by causing contraction of the ureteric muscle, completely prevented the passage of fluid through its lumen for

1 to 2 min. and slowed the flow for a further period of 1 to 2 min.

5. Both adrenaline and noradrenaline caused a transitory contraction of the ureteric muscle.

6. Given in adequate antidiuretic dose, the vasopressor fraction of posterior lobe extract was without effect on the ureter. The oxytocic fraction caused contraction of the muscle.

7. In dogs, anaesthetized with either chloralose or pentobarbitone sodium, 5-hydroxytryptamine rarely caused other than a pure pressor response, whether given into the carotid artery or intravenously.

A few days before our manuscript was submitted a paper appeared in *J. Physiol.* (1955, **129**, 436) on "The Behaviour of the Intact Ureter in Dogs, Rabbits and Rats" by D. W. Gould, A. C. L. Hsieh and L. F. Tinckler. There is no disagreement between the conclusions reached by these authors and by us.

We wish to express our gratitude to the Upjohn Company for a generous supply of 5-hydroxytryptamine creatinine sulphate, and to Messrs. Ciba for a supply of "Rogitine."

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THE EFFECT OF 5-HYDROXYTRYPTAMINE ON THE UTERUS OF CONSCIOUS AND OF ANAESTHETIZED DOGS

BY

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(RECEIVED OCTOBER 12, 1955)

The following observations were made on the response of the uterus of the dog to 5-hydroxytryptamine (5-HT) in order that it might be possible to compare the minimum dose to which this organ reacts with the minimum effective dose for the ureter, the kidney, and the systemic blood pressure, in the same species.

METHODS

Observations were made on conscious and anaesthetized bitches. The former were ovariectomized and provided with a fistula of one uterine horn ; the spontaneous and induced movements of the uterus were recorded by means of a balloon inserted into the lumen. The method has previously been described (Abrahams and Pickford, 1954). Four dogs were observed under anaesthesia which was induced by the intravenous administration of 0.1 g./kg. chloralose in 0.9% NaCl solution. For 7 to 10 days before observation the dogs received 0.25 to 0.5 mg. stilboestrol dipropionate in oil (British Drug Houses preparation) given subcutaneously on alternate days. Two of the 4 animals were subjected to a preliminary operation 2 days before the experiment. One uterine horn was exteriorized so that its movements could be recorded by means of a balloon, as in the conscious dogs. In the other 2 dogs the preliminary operation was omitted, and, instead, uterine contractions were recorded by tying a thread to the upper end of one uterine horn and passing it over pulleys to a lever writing on a kymograph. The lower end of the uterus was fixed by gripping the cervix in volsellum forceps passed up the vagina, the forceps then being clamped to a stand. The abdominal incision was closed round a wide glass tube filled with warm paraffin which guarded the uterine horn and its attached thread. Unless otherwise stated all injections were made into the malleolar vein. 5-HT was administered in the form of the creatinine sulphate dissolved in 0.9% NaCl solution. All doses are calculated and given as μg. base/kg. body weight.

RESULTS

Conscious Dogs.—Observations were made on 3 dogs, whose uteri all showed regular spontaneous contractions. In all 3 the uterus gave the same type of response to an intravenous injection of 5-HT, namely, a contraction which was usually larger than the spontaneous ones, followed by a period of quiescence during which spontaneous contractions were suppressed and tone sometimes diminished (Fig. 1). The minimum effective dose was less than 1 μg./kg.; 17 μg./kg. was a supramaximal dose, since the contraction induced was no larger, nor the inhibition longer in duration, than after the injection of 10 μg./kg. The longest observed period of inhibition was 7 min.

Anaesthetized Dogs.—In all 4 animals, regardless of the recording system used, the response to

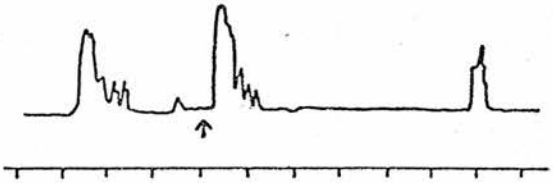

FIG. 1.—Tracing from record of uterine contractions in conscious dog. Effect of 1.4 μg./kg. 5-HT intravenously at arrow. Upward movement indicates contraction. Upper line, uterine contractions. Lower line, time in 50 sec.

the intravenous injection of 5-HT was the same as that of the conscious dog, with the addition that a loss of tone was apparent (Fig. 2). The minimum effective dose in 3 dogs was less than 1 μg./kg., and in one was 0.45 μg./kg. The effect of intravenous and intracarotid injections was compared. An intravenous dose of 3 μg./kg. caused a contraction, followed by an inhibition lasting 5.5 min. The same dose given into the carotid artery caused a contraction followed by an inhibition lasting 2 min.

DISCUSSION

These experiments show that the uterus of the dog, when *in situ*, is highly sensitive to 5-HT and responds to the intravenous injection of 1 μg./kg., or even less, by a contraction, followed by some minutes' inhibition. No tachyphylaxis was shown

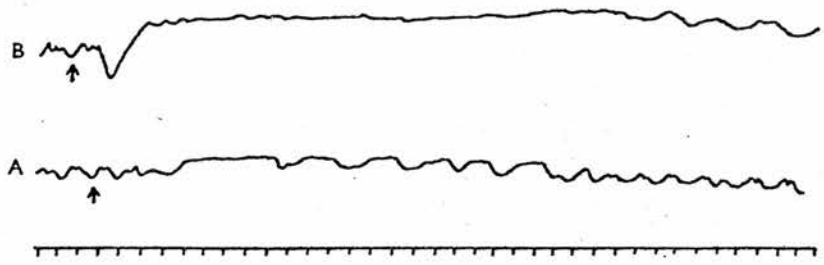

FIG. 2.—Tracing from record of uterine contractions in anaesthetized dog. Effect of intravenous administration of 5-HT. Downward movement indicates contraction. Upper two lines, uterine contractions. Lowest line, time in 10 sec. In A, 0.45 μg./kg. 5-HT at arrow; in B, 2.5 μg./kg. 5-HT at arrow.

to injections given repeatedly at 10 to 15 min. intervals. The action of 5-HT appears to be wholly peripheral, since intravenous injections were more effective than intracarotid ones, and the results were the same whether or not the dog was anaesthetized. The response of the uterus differs from that of the ureter, since, in the latter, there was no sign of any inhibitory phase following the contraction of the muscle (Abrahams and Pickford, 1956a). It is clear that the uterus of the dog is more sensitive to intravenous injections of 5-HT than is the ureter, kidney or general blood pressure (Abrahams and Pickford, 1956a and b).

SUMMARY

1. The effect of 5-HT on the uterus of dogs was observed, both in conscious animals and in those anaesthetized with chloralose.

2. In response to an intravenous injection of 5-HT the uterus gave a contraction which was often greater than those occurring spontaneously. This was followed by a period of inhibition which might last for as long as 7 min.

3. In all dogs tested the minimum effective intravenous dose was less than 1 μg./kg. In one dog it was 0.45 μg./kg.

We wish to record our gratitude to the Upjohn Company for a gift of 5-HT.

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OBSERVATIONS ON A CENTRAL ANTAGONISM BETWEEN ADRENALINE AND ACETYLCHOLINE

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In a previous paper (Duke & Pickford, 1951) experiments were described in which unanaesthetized dogs received intravenous injections of adrenaline and acetylcholine, either singly, together, or in rapid sequence during the course of water diuresis. About half the number of times that adrenaline was given with or just before acetylcholine the reduction in rate of urine flow, usual after injection of acetylcholine alone, was prevented. At other times adrenaline seemed to reinforce the acetylcholine action, or had no effect on the normal response to it. Reasons were given for thinking that adrenaline interacted with acetylcholine in the central nervous system, possibly at the supraoptic nuclei, and that the inconstancy with which acetylcholine was inhibited by adrenaline was owing to the intravenous route of administration, which made impossible any control of the amount of the drugs reaching the central nervous system.

In the work to be described here adrenaline and acetylcholine either singly, together, or in sequence were injected into the carotid artery of conscious dogs. The object was to test whether the response to the drugs given by this route was more regular than when they were given intravenously: i.e. to determine whether their combined action was a central one as previously suggested.

METHODS

Seventy observations were made on four bitches (weights, 10, 10, 11·5 and 13 kg), mainly on two of them. In all animals the dorsal perineum had been slit to facilitate catheterization of the urethra. They were also provided with denervated carotid loops. On any day when observations were made the dogs were hydrated in the morning and 2½ to 3 hr later diuresis was induced with 250–300 ml. water by mouth. About 10 min after the water 2 mg atropine sulphate was injected subcutaneously, and 45–60 min after the water they received the intracarotid injections. The amount of acetylcholine given was always 200 μg because this was found to give a maximal and reproducible inhibition of water diuresis in dogs of this size. The quantity of adrenaline HCl varied from 0·5 to 40 μg. The drugs were made up in 0·9% NaCl solution, and the volume injected was always 0·2 ml.

Since the drugs were given in rapid succession, both injections were made through the same hypodermic needle so that the carotid artery was punctured only once in each experiment. The injection apparatus was assembled as follows. A short length of soft rubber tubing was tied at one end to the hypodermic needle and at the other to a short length of glass tube. The glass in turn was joined to the syringe by 4 in. of polythene tube. Polythene was chosen since its inelasticity allowed injections to be made rapidly against the considerable resistance of the fine needle and the carotid blood pressure. The glass junction made it easy to see blood withdrawn when making certain that the artery had been entered. The procedure was then as follows. The syringe, tubing and needle were filled with the solution that was to be injected first. As soon as the needle was in the artery the first injection was made, then as quickly as possible mosquito forceps were put on the rubber tubing close to the needle, partly to prevent reflux of blood from the artery, and partly to ensure that the second injection went forward into it. At the chosen time the second injection was made with a second syringe and needle through the rubber directly into the socket of the intra-arterial needle. In this way dead space was reduced to less than 0·02 ml.

The adrenaline used was the 1 mg/ml. mixed DL solution supplied by Messrs Parke Davis and Co. Acetylcholine solutions were made up freshly each day immediately before use.

RESULTS

Fig. 1*a* (curve A) shows the effect, on the rate of urine flow during water diuresis, of 200 μg acetylcholine injected intra-arterially. The effect of this amount of acetylcholine is almost the same as that of 1 m-u. of vasopressor activity of the posterior pituitary gland given intravenously (Fig. 1*a*, curve *P*). Fig. 1*b* shows the effect of 2 μg adrenaline in solution injected into the carotid artery of the same dog. These responses are typical of those seen in all the dogs used. Fig. 2 shows the effect of injecting 2 μg adrenaline followed in 11 sec by 200 μg acetylcholine. The injections caused a fleeting irregularity in the rate of urine flow, but no inhibition comparable to that seen after acetylcholine alone. Provided certain conditions mentioned below were observed adrenaline in this dose never failed to prevent the inhibition of urine flow which occurred when acetylcholine alone was injected.

Timing of the injections relative to each other. The time between the injections was varied from 8 to 60 sec, 8 sec being the least time in which the necessary manipulations could be made. The drugs were also given simultaneously, having been mixed in the syringe immediately before use. When the drugs were given simultaneously the urine flow was inhibited as though acetylcholine alone had been given. With an 8 sec interval between the injections of adrenaline and acetylcholine sometimes urine flow was inhibited and sometimes it was not. When at least 10 sec was allowed between injections the usual inhibitory effect of acetylcholine was never seen.

When the time between the two injections was increased up to 38 sec adrenaline still invariably antagonized the effect of acetylcholine. Fig. 3*a* shows the type of response seen when the interval was 30 sec. If the time was longer than 38 sec, and up to about 45 sec, one of two things might happen: either there was moderate reduction in the rate of urine flow (Fig. 3*b*), or rapid

temporary fluctuations appeared (Fig. 4*a*). If the interval between injections was increased beyond 45 sec and up to 60 sec then the urine flow was always inhibited to the same extent as if acetylcholine had been given alone (Fig. 4*b*).

Thus, in order that the inhibition of urine flow normally caused by acetylcholine might with certainty be antagonized, either wholly or in part, it was necessary to inject adrenaline from about 8 to 45 sec before the acetylcholine.

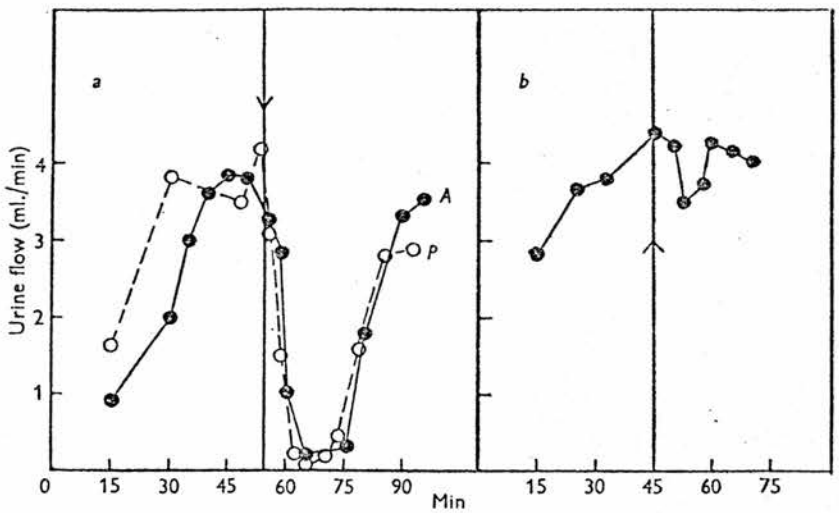

Fig. 1. Three water diuresis curves in the same dog. (*a*) At the arrow in curve *A*, 200 μg acetylcholine injected into the carotid artery. At the arrow in curve *P*, 1 m-u. vasopressor extract injected intravenously. (*b*) At the arrow, 2 μg adrenaline injected into the carotid artery.

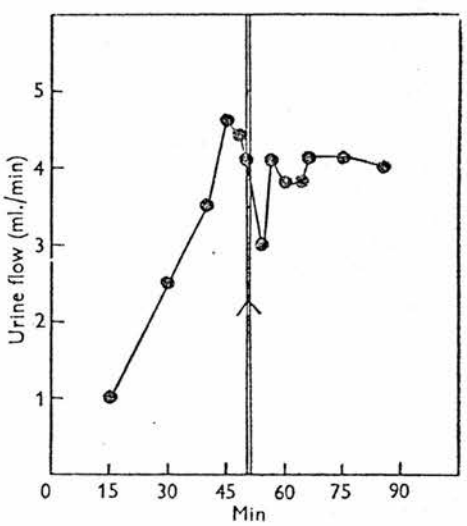

Fig. 2. Water diuresis curve. At the arrow, 2 μg adrenaline followed 11 sec later by 200 μg acetylcholine injected into the carotid artery.

The dose of adrenaline necessary to antagonize acetylcholine. The quantity of acetylcholine injected was kept constant at 200 μg, and the amount of adrenaline was varied. The time between injections of the two drugs was in the range 10–30 sec. In all four dogs 2 μg adrenaline always prevented acetylcholine inhibition. In one of them 1 μg (Fig. 5*a*), and on occasion, even 0·5 μg adrenaline was sufficient. In the other three 1 μg only partially antagonized acetylcholine, or more often entirely failed to do so.

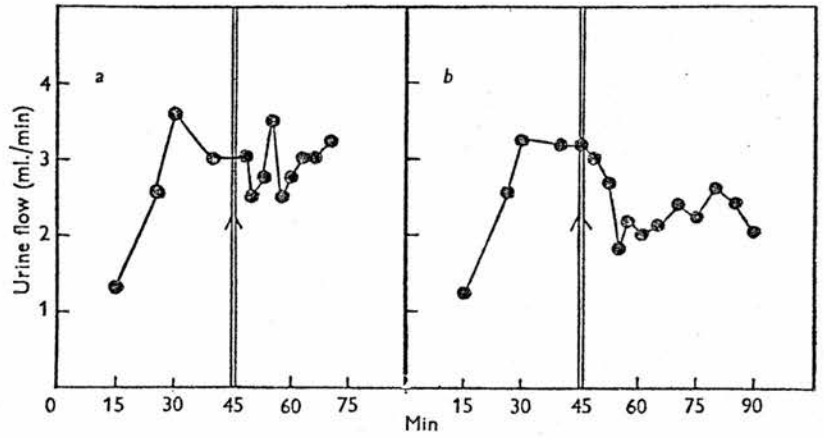

Fig. 3. Two water diuresis curves from the same dog. (*a*) At the arrow, 2 μg adrenaline followed 30 sec later by 200 μg acetylcholine injected into the carotid artery. (*b*) At the arrow, 2 μg adrenaline followed 45 sec later by 200 μg acetylcholine injected into the carotid artery.

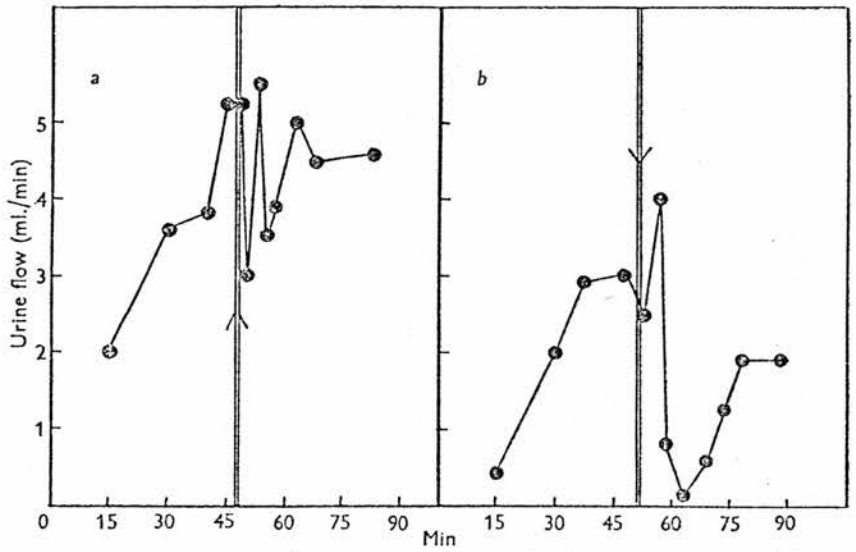

Fig. 4. Two water diuresis curves from the same dog. (*a*) At the arrow, 2 μg adrenaline followed 40 sec later by 200 μg acetylcholine injected into the carotid artery. (*b*) At the arrow, 2 μg adrenaline followed 60 sec later by 200 μg acetylcholine injected into the carotid artery.

On two occasions adrenaline in a dose of $3\mu g$ was as effective as $2\mu g$ in inhibiting the action of acetylcholine. Three times, however, this amount only partially prevented the effect of acetylcholine. Once a slight antagonism was seen using $5\mu g$ adrenaline. More commonly, with the 4 and $5\mu g$ doses of adrenaline, and certainly with a $10\mu g$ dose, followed by $200\mu g$ acetylcholine, the rate of urine flow was inhibited to the same extent as by acetylcholine alone (Fig. 5*b*).

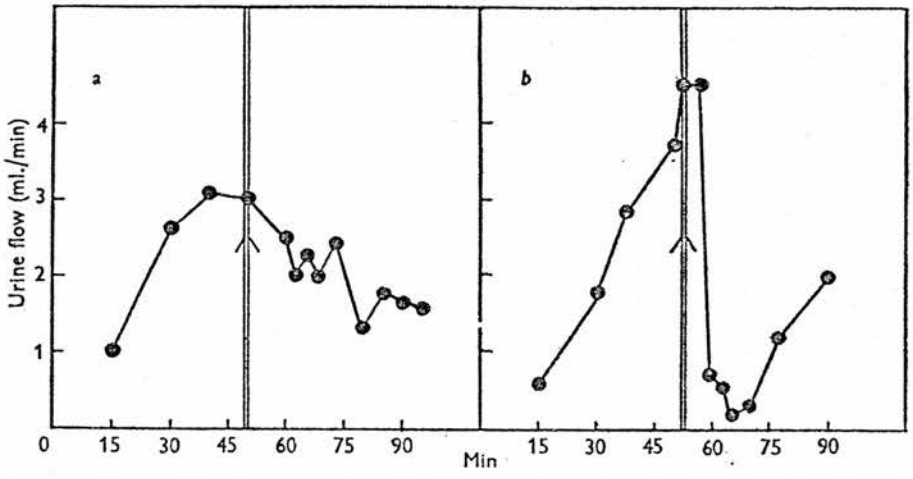

Fig. 5. (*a*) Water diuresis curve in dog 102. At arrow, $1\mu g$ adrenaline followed 18 sec later by $200\mu g$ acetylcholine injected into the carotid artery. (*b*) Water diuresis curve in dog 104. At arrow, $4\mu g$ adrenaline followed 30 sec later by $200\mu g$ acetylcholine injected into the carotid artery.

Given alone intracarotid injections of 10, 20 and $40\mu g$ adrenaline in solution caused either no inhibition of urine flow, or a brief spiked inhibition similar to that seen when the same dose was injected intravenously (Fig. 6).

Effect of adrenaline and acetylcholine on respiration. When a dose of acetylcholine, sufficient to inhibit urine flow, was injected into the malleolar vein of an atropinized dog there always followed, 12–16 sec later, a sighing respiration. When $200\mu g$ acetylcholine was given into the carotid artery the sigh followed almost immediately. Since the carotid sinus and body were always denervated the respiratory response could not be a reflex arising from the latter. By its timing the sigh appeared to be of central origin and not due to any change in general blood pressure. It was noticed that the sigh still occurred even when the intracarotid injection of acetylcholine was preceded by a dose of adrenaline adequate to antagonize the renal action of acetylcholine. Adrenaline by itself induced no sigh.

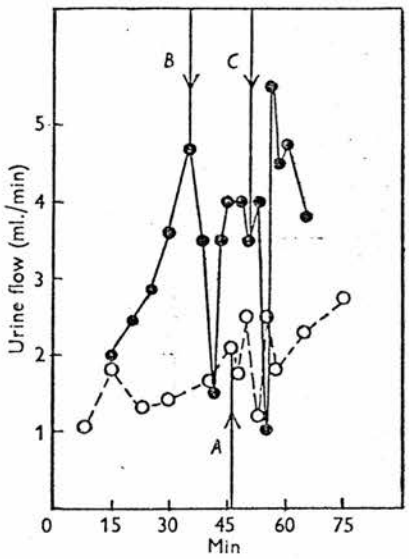

Fig. 6. Water diuresis curves from 2 dogs. O---O, At *A*, 10 μg adrenaline injected into the carotid artery; ●—●, at *B*, 40 μg adrenaline injected into the carotid artery; at *C*, 40 μg adrenaline injected intravenously.

DISCUSSION

Normally an intracarotid injection of 200 μg acetylcholine caused a decrease in the rate of urine flow during water diuresis by releasing antidiuretic hormone from the posterior pituitary (Pickford & Watt, 1951). The results recorded above show that when the intracarotid route was used an injection of adrenaline consistently antagonized the effect of acetylcholine given later, provided that the size and timing of the doses of the two drugs were within certain limits. For clear-cut antagonism the shortest time interval between injections of adrenaline and acetylcholine was about 8 sec, and the longest 38–45 sec. The effect of a 200 μg dose of acetylcholine was always annulled by 2 μg adrenaline, sometimes by 1 μg or (in one dog) by 0·5 μg. Using greater amounts of adrenaline, 3 μg sometimes completely antagonized the action of acetylcholine. More often 3, 4 and 5 μg adrenaline only partly prevented the inhibition of urine flow seen when acetylcholine alone was given. With a 10 μg dose of adrenaline followed by acetylcholine the urine flow was inhibited to the same extent as by the latter alone.

These results strengthen the earlier conclusions (Duke & Pickford, 1951) that adrenaline and acetylcholine interact centrally, and that the former can prevent the release of posterior lobe hormone usually caused by the latter. Time, about 8 sec, is necessary for this action of adrenaline to develop, and it then endures for not more than 35–40 sec. How these two drugs interfere with each other

is unknown. The adrenaline could, by a constrictor effect on the capillaries round the supraoptic cells, simply prevent access to acetylcholine. Such a constrictor action would be highly specific, since acetylcholine is not prevented from reaching the centre responsible for the disturbance of respiration. Since, further, it is difficult to see why 2 μg adrenaline should constrict the supraoptic capillaries, and 4, 5 and 10 μg fail to do so, this seems an unlikely cause for the antagonism between these two drugs. Possibly the adrenaline directly antagonizes the action of acetylcholine on the supraoptic cells. If so, it is difficult to explain the disappearance of the local antagonism when the dose of the antagonist is increased. A further possibility is that the neurones whose axons terminate on the supraoptic cells and there exert an inhibitory action, are stimulated by small doses of adrenaline, but paralysed by high concentrations. This would explain how small doses of adrenaline, by remote action, block the effect on the supraoptic cells of the later arriving acetylcholine, whilst large doses leave these neurones free to respond to acetylcholine.

Since the respiratory effect of acetylcholine was not abolished by any dose of adrenaline used in these observations it is evident that these two substances are not antagonistic at all sites in the central nervous system.

SUMMARY

1. During water diuresis in dogs provided with carotid loops adrenaline and acetylcholine were injected into the carotid artery either singly, together, or in sequence.

2. An injection of 100 μg acetylcholine caused an antidiuresis equivalent to that due to the intravenous injection of 1 m-u. vasopressor extract of the posterior pituitary. An injection of 2–40 μg adrenaline caused a brief antidiuresis.

3. If 2 μg adrenaline and 200 μg acetylcholine were simultaneously injected the resulting antidiuresis was similar to that seen when acetylcholine alone was injected.

4. The antidiuretic action of an injection of 200 μg acetylcholine was prevented by a previous intracarotid injection of about 1–3 μg adrenaline, provided that the adrenaline preceded the acetylcholine by not less than about 8 sec and not more than about 45 sec.

5. The interference between the actions of adrenaline and acetylcholine occurs in the central nervous system, but its site and mode of action is unknown.

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THE EFFECT OF ANTICHOLINESTERASES INJECTED INTO THE SUPRAOPTIC NUCLEI OF CHLORALOSED DOGS ON THE RELEASE OF THE OXYTOCIC FACTOR OF THE POSTERIOR PITUITARY

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In a paper by Duke, Pickford & Watt (1950) experiments are described in which diisopropylfluorophosphate (DFP) injected amongst the supraoptic nerve cells of dogs gave rise, after an immediate and profound inhibition of the rate of urine flow, to a temporary diabetes insipidus lasting from 4 to 19 days, and then an inability lasting 89–139 days to respond by antidiuresis to an intravenous injection of acetylcholine. Thereafter there was an apparently complete return to normality. From these observations, and some earlier ones (Pickford, 1947), it was concluded that acetylcholine was the transmitter at the supraoptic neurones. Since antidiuretic hormone (ADH) always seems to be released in company with the oxytocic factor of the posterior lobe of the pituitary it seemed probable that the oxytocic factor, too, depends for its liberation on a similar cholinergically transmitted stimulus.

In the experiments described in this paper eserine and DFP were injected amongst the supraoptic neurones of anaesthetized dogs and the effect on uterine movements noted. The results showed that spontaneous uterine activity increased following the injection of these two drugs into the supraoptic nuclei, but that it was unaffected by the injection at the same site of small volumes of 0·9% NaCl solution.

METHODS

The first stage of the experiments was to give each animal four doses of 0·5 mg stilboestrol diproprionate in oil (British Drug Houses) subcutaneously on alternate days in order to sensitize the uterus to the action of posterior pituitary oxytocic factor and to induce spontaneous activity. The day after the last stilboestrol injection a preliminary aseptic operation was performed under sodium pento-barbitone anaesthesia to expose the pituitary gland and optic chiasma. Early the following morning when the animal was well and lively it was given half a pint of milk to which water and glucose were added. Two or three hours later anaesthesia was induced either by giving 0·14 g chloralose per kg body weight by mouth in 250–300 ml. water, or by the intravenous injection of 0·1 g chloralose per kg body weight in 0·9% NaCl solution. Two methods were used

for recording uterine movements. In the first, a balloon inserted into the uterus contained water in continuity with that in a small reservoir held 25 cm above the level of the uterus. Changes in water-level in the reservoir caused by uterine contractions were transmitted by air to a 5 ml. volume recorder provided with a stylus writing on a kymograph. The abdomen was closed after insertion of the uterine balloon. In the second method, the upper end of one uterine horn was freed and tied to a thread passing over pulleys to a recording lever. The uterus was prevented from cooling and drying by passing it through a glass tube filled with warm paraffin. The abdomen was closed round the tube. In both methods the lower end of the uterus was fixed by gripping the cervix in volsellum forceps passed up the vagina, the forceps being clamped to a stand. The DFP was made up immediately before use as a part solution–part emulsion in 0·9% NaCl solution and well shaken. The eserine salicylate was also made up immediately before use. The volume of the injections was 0·002 ml. The method of making the injections has already been described (Pickford, 1947).

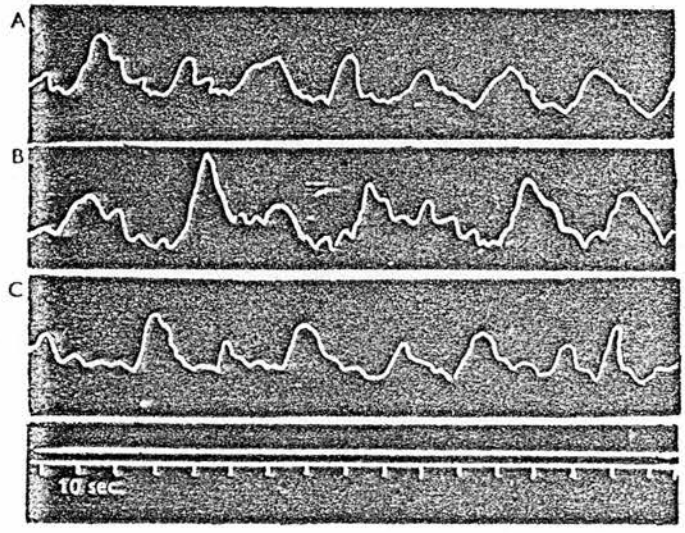

Fig. 1. Record of uterine contractions. A, normal spontaneous movements; B, 30 min after injection of 40μg eserine salicylate into the left supraoptic nucleus; C, 60 min after the injection. Time, 10 sec.

RESULTS

Observations were made on a total of seven dogs. The results were the same whichever system of recording or anaesthesia was used. Following the pretreatment with stilboestrol all the uteri showed steady spontaneous activity (Figs. 1A and 2A).

Effect of 0·9% NaCl solution. The injection of 0·002 ml. 0·9% NaCl solution into the area of the supraoptic nuclei was wholly without effect on the pattern of uterine activity.

Effect of eserine salicylate. On two occasions 40μg eserine salicylate in 0·9% NaCl solution was injected into one or other supraoptic nucleus. In both instances the result was the same. Four to five min after the injection the spontaneous uterine contractions increased in size and remained large for

45–50 min, they then began diminishing until in about an hour after the injection they were once more the normal size (Fig. 1).

Effect of DFP. DFP was used on three occasions in doses of 40, 80 and 100μg, and each time the result was the same. Four to five min after the injection into the supraoptic nuclei uterine contractions began to increase in size, attained their maximum in about 15 min and remained large for the rest of the experiment. The longest time of observation was 4½ hr after the injection was made.

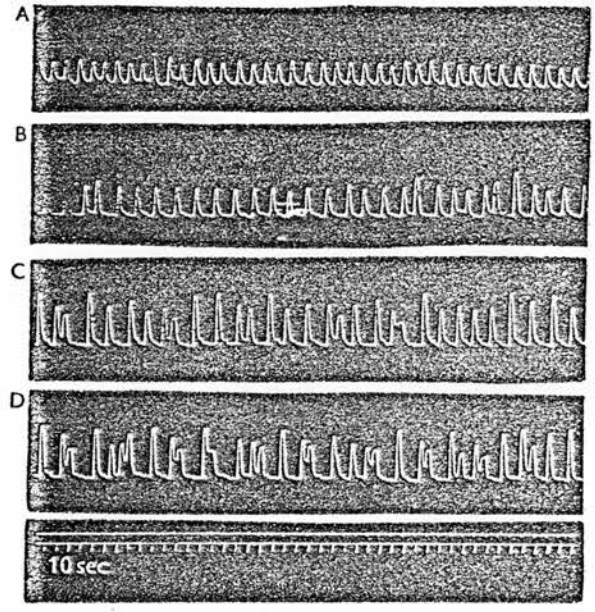

Fig. 2. Record of uterine contractions. A, normal spontaneous movement; B, 4 min after injection of 80μg DFP into left supraoptic nucleus; C, 1½ hr after the injection; D, 4 hr after the injection. Time, 10 sec.

In the two remaining experiments the injection of eserine and DFP was without effect. Histological examination of the hypothalamus showed that the injections had been badly placed, in one instance the tip of the needle had been lateral to one fornix, and in the other it had penetrated to the posterior hypothalamus.

Owing to the impossibility of maintaining the uterus in a steady state of spontaneous activity for several weeks no attempt was made to follow the after-effect of DFP injection on the uterus to see if it paralleled the effect on the excretion of water by the kidney.

DISCUSSION

Injection of eserine or DFP into the supraoptic nuclei of chloralosed dogs led to a rapid increase in size of spontaneous uterine contractions. After the former drug the increased activity lasted for about an hour, after the latter it lasted for the duration of the experiment, i.e. for at least 3–4½ hr. No uterine reaction followed injection of anticholinesterases into two other sites in the hypothalamus. Since the anticholinesterase action of eserine is short-lived and that of DFP prolonged, if the oxytocic factor, like ADH, is liberated by a cholinergic mechanism, then the above results are to be expected. The findings, then, support the view that a cholinergic transmitter to the supraoptic neurones is responsible for the release of the oxytocic, as of the antidiuretic, factor of the posterior lobe. The results are also in agreement with others showing that any stimulus causing the release of one posterior pituitary active substance also causes the simultaneous release of the other (Abrahams & Pickford, 1954; Harris, 1955), but they offer no solution to the problem of how differing proportions of the two factors may be liberated at different times.

SUMMARY

1. Observations were made of the effect on spontaneous uterine contractions of injecting eserine salicylate and DFP into the supraoptic nuclei of chloralosed dogs.

2. Both eserine and DFP caused an increase in size of uterine contractions. The effect of eserine lasted for about an hour, and that of DFP for at least 3–4½ hr.

3. These findings support the view that the oxytocic, like the antidiuretic factor, of the posterior pituitary can be released by cholinergic transmission to the supraoptic neurones.

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HISTOCHEMICAL DEMONSTRATION OF CHOLINESTERASES IN THE HYPOTHALAMUS OF THE DOG

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(*Received* 1 *July* 1957)

In the experiments to be described a histochemical study has been made of the distribution of true and pseudo-cholinesterase in the hypothalamus of the dog. There is considerable evidence to indicate that cholinergic neurones are involved in the release of neurohypophyseal hormones. Pickford (1939) showed that the intravenous injection of acetylcholine (ACh) produced antidiuresis in atropinized dogs, and that this antidiuresis was reduced or abolished by surgical removal of the posterior lobe of the pituitary. She therefore concluded that the ACh caused release of the antidiuretic hormone. Later experiments (Pickford, 1947; Duke, Pickford & Watt, 1950) showed that the ACh most probably acted on the neurones of the hypothalamus, since injections of ACh, as well as anticholinesterases, into the supraoptic nuclei, produced antidiuresis in chloralosed dogs, but only as long as the posterior lobe of the pituitary was intact. The injection of the irreversible anticholinesterase, diisopropylfluorophosphate (DFP), into the supraoptic nucleus produced immediate antidiuresis followed by prolonged polyuria. This prolonged polyuria was attributed to permanent paralysis of neurones resulting from local excess of ACh.

There is some evidence that the neurones concerned with the release of oxytocin are also cholinergic, since the injection of DFP into the supraoptic nucleus increases spontaneous uterine activity (Abrahams & Pickford, 1956). So far no histochemical studies have been performed concerning the distribution of cholinergic neurones in the hypothalamus. From histochemical studies it is known that the peripheral and central nervous system cholinergic neurones contain high concentrations of true cholinesterase, whereas non-cholinergic

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neurones are characterized by low concentrations of this enzyme. It seemed therefore possible that the histochemical localization of true cholinesterase in the hypothalamus would aid in the identification of the cholinergic neurones concerned in these secretory systems.

METHODS

Adult dogs of both sexes weighing from 5 to 20 kg were anaesthetized with sodium pentobarbitone and then bled to death from one carotid artery. A block of brain containing the hypothalamus and still attached to the base of the skull was quickly removed and placed in a freezing cabinet for 30–60 min. By this procedure the brain became firm enough (without being frozen) to allow dissection without distortion of the hypothalamus. Sections of the hypothalamus were cut on the freezing microtome at 10 μ, and before thawing were transferred to slides for the demonstration of cholinesterases, using the method of Koelle & Friedenwald (1949) as modified by Koelle (1951, 1955). In this method the sections are incubated in a medium containing a substrate of either acetylthiocholine or butyrylthiocholine, together with copper glycinate. The thiocholine liberated as a result of enzyme action is precipitated as a white mercaptide which is converted to copper sulphide by subsequent treatment with ammonium sulphide. Hence the site of enzyme activity is shown by a dark brown precipitate. The use of the two different substrates allows a distinction to be made between true and pseudo-cholinesterase activity. With both substrates, controls were carried out using the following inhibitors: DFP, and the dimethyl carbamate of (2-hydroxy-5-phenylbenzyl)-trimethyl-ammonium bromide (Nu 683) which were used as selective inhibitors for pseudo-cholinesterase, and 1-5-bis (4-allyldimethylammonium-phenyl)-pentan-3-1-dibromide (BW 284c51), which was used as selective inhibitor for true cholinesterase. Eserine was used in high concentrations to inhibit both cholinesterases, thereby excluding errors due to other esterases or to artifact.

The only difference between the procedures used in this investigation and those developed by Koelle (1955) for the cat brain was to use a weaker concentration of DFP for the selective inhibition of pseudo-cholinesterase. In preliminary experiments using homogenates of dog brain in the Warburg apparatus, it was found that the concentration of DFP used for the cat would cause inhibition of the true cholinesterase as well. The concentration of DFP used for the inhibition of pseudo-cholinesterase was therefore reduced to 5×10^{-8}. In all incubation solutions high concentrations of sodium sulphate were included to reduce enzymic diffusion. This procedure causes partial inhibition of cholinesterase activity, and to compensate for this the incubation time was extended to 2 hr.

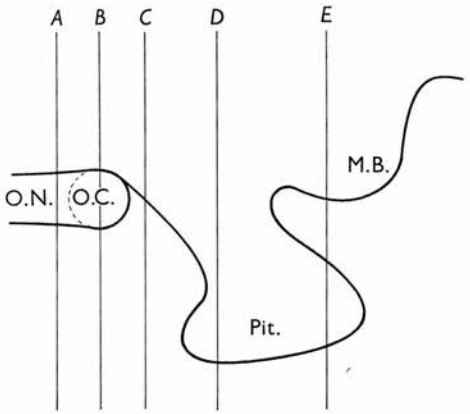
Groups of serial sections were cut from five regions of the hypothalamus as indicated in Text-fig. 1. These regions were:

(*A*) immediately anterior to the optic chiasma;
(*B*) at the level of the optic chiasma;
(*C*) just posterior to the optic chiasma;
(*D*) in the infundibular region; and
(*E*) through the mamillary bodies.

Of the nine brains examined, sections from the first two were incubated in the full range of ten incubation solutions. Sections from the other seven brains were incubated in only three solutions, since it was found that this allowed adequate discrimination to be made. These solutions were as follows: (1) for the demonstration of both cholinesterases, acetyl thiocholine as substrate, no inhibitors; (2) for the demonstration of true cholinesterases, acetylthiocholine as substrate with DFP as inhibitor of pseudo-cholinesterase; and (3) for the demonstration of pseudo-cholinesterase, butyrylthiocholine as substrate, with no inhibitor present.

One section in every group of serial sections was stained by the chrome-alum haematoxylin method of Gomori (1941) to aid in the identification of the exact area sectioned. The identification

was based on the anatomical accounts of the hypothalamus by Rioch (1929, 1931), Kappers, Huber & Crosby (1936) and Clark (1938). In a few experiments, sections, after being incubated for the demonstration of cholinesterase, were refixed in Bouin's fixative and stained by the method of Gomori to demonstrate neurosecretory material.



Text-fig. 1. Diagrammatic sagittal section through the hypothalamus to indicate the five planes of section referred to in the text. O.N., optic nerve; O.C., optic chiasma; Pit., pituitary; M.B., mamillary bodies.

RESULTS

The two types of cholinesterase were found to be discretely localized in the hypothalamus of the dog. True cholinesterase was confined to the cell bodies and axons of three nuclear groups only: the supraoptic nuclei, the suprachiasmatic nuclei, and the paraventricular nuclei. Pl. 1, fig. 1, shows the distribution of true cholinesterase in the region of the right paraventricular nucleus on the side of the third ventricle. A lateral detachment of cells of the paraventricular nucleus also shows the presence of true cholinesterase. Along the side of the third ventricle, cells showing the presence of true cholinesterase extend down to the suprachiasmatic nucleus. This can be seen in Pl. 1, fig. 2, which also shows the presence of true cholinesterase in the cells of the supraoptic nucleus, and in cells extending from the suprachiasmatic nucleus, to the supraoptic nucleus. The slides from which these photomicrographs were taken were incubated in acetylthiocholine and thus show both cholinesterases. This results in a light background stain due to pseudo-cholinesterase. This light background stain has been deliberately left in to aid orientation of the area. Similar sections, incubated in the presence of appropriate concentrations of DFP or Nu-683 to inhibit pseudo-cholinesterase, fail to show the background precipitation.

Pl. 1, fig. 3, shows cells of the paraventricular nucleus photographed under a higher power. The precipitation due to the true cholinesterase is confined to the cytoplasm of the cells, the nuclei being devoid of any precipitation. True

cholinesterase is also seen in the axons leaving both ordinary and bipolar cells, as well as in structures which appear to be the beginnings of dendritic processes. It is not possible to follow true cholinesterase down an axon for any distance greater than 400 μ; none is visible in the supraoptico-hypophyseal tract.

Sections incubated in butyrylthiocholine, to show the presence of pseudo-cholinesterase, reveal a distribution of the enzyme different from that found for true cholinesterase. There is a general background of precipitation which cannot be associated with any particular structure. Against this background two areas of dense precipitation are seen. The first is in the region of the supra-optic nuclei. As can be seen in Pl. 2, fig. 1, high-power examination of this area shows rings of dense precipitation. When counterstained these rings are shown to be around the bodies of the cells of the supraoptic nucleus. The second area of dense precipitation is around the third ventricle, but only in the region of the paraventricular nucleus. Precipitation can also be seen on some of the ependymal cell walls.

The tuberal and mamillary areas of the hypothalamus do not show precipitation in any of the incubation media used. Other stained areas included in the sections are the caudate nucleus and amygdala. Both of the nuclei appear rich in true cholinesterase. A similar finding has been previously reported for the rat (Koelle, 1954). The optic nerves, chiasma and tracts appear devoid of any precipitation with the exception of some fibres in the dorso-medial part of the chiasma which appear to be rich in true cholinesterase. It is of interest in this respect that Feldberg & Vogt (1948) found this part of the optic chiasma richer in choline acetylase than the ventromedial portion.

In sections counterstained by the Gomori method after the demonstration of cholinesterases, it was possible to see that cells already showing the presence of true cholinesterase also showed the cytoplasmic basophilia, said to represent the neurosecretory substance. This staining could also be seen in cells surrounded by pseudo-cholinesterase.

DISCUSSION

The restricted localization of true cholinesterase within the cells of the supra-optic, paraventricular and suprachiasmatic nuclei of the hypothalamus explains why only moderate concentrations of this enzyme have been found in manometric determinations of homogenates of whole hypothalamus (Burgen & Chipman, 1951). It would appear also that choline acetylase has a similar distribution in the hypothalamus to true cholinesterase, the supraoptic nucleus having a high content when compared with the infundibulum (Feldberg & Vogt, 1948) or with the hypothalamus as a whole (Zetler & Schlosser, 1955). The high concentrations of true cholinesterase in the cells of these nuclei suggests the presence of large numbers of cholinergic neurones. It does

not, however, indicate whether it is a region of termination or of origin of cholinergic neurones, or a region of both origin and termination. From the finding that the true cholinesterase is concentrated in the cell bodies, and can be seen for the first few hundred microns of the axon as it emerges from the cell, one might argue that cholinergic neurones originate in the cells. These cells, at least as far as the supraoptic and paraventricular nuclei are concerned, are the origin of the nerve fibres to the neurohypophysis, and secretory products are supposedly formed within the cells, migrating down the axons to their termination in the neurohypophysis from which they are eventually released (for review, see Scharrer & Scharrer, 1954). If these same neurones were cholinergic, this would produce the unique situation of a neurone's own transmitter substance providing the stimulus for the release of its own endocrine product. An alternative possibility, suggested by Heller (1955), would be that the supraoptic and paraventricular nuclei give rise to separate conducting and secretory fibres, both of which supply the neurohypophysis. The conducting fibres would be cholinergic and supply the pericellular nerve nets in the neurohypophysis described by Brookes & Gersh (1941).

There is another possibility. We know, from the peripheral nervous system, that at a synaptic junction where ACh is the transmitter, cholinesterase can be found at either the presynaptic endings or in the post-synaptic region. For instance, at the sympathetic ganglion, true cholinesterase is found on the presynaptic terminals (Koelle, 1955), but at the neuromuscular junction true cholinesterase is predominantly found post-synaptically, that is, at the muscle end-plate. If the same situation that prevails at the neuromuscular junction were to apply to the situation in the supraoptic and paraventricular nuclei, the histochemical demonstration of large quantities of true cholinesterase in the cells of these nuclei and even at the beginning of their axons might well be a sign of cholinergic axons terminating on these cell bodies. The inability to detect high concentrations of true cholinesterase histochemically in the axons of the supraoptico-hypophyseal tract would be in agreement with this view.

Little is known concerning the function of neurones of the suprachiasmatic nucleus. According to Pate (1937), pathways from here terminate in the retina. However, in other studies with histochemical methods a similarity was found between the supraoptic, paraventricular and suprachiasmatic nucleus, all being rich in phosphatases (Cohn & Richter, 1956).

Burgen & Chipman (1951) found that the hypothalamus, which contains only small amounts of true cholinesterase, contains large quantities of pseudocholinesterase. From its distribution as shown in the present experiments it appears that this enzyme subserves a special function in the hypothalamus. The high concentration of the enzyme in the area of the ventricle known to have a peculiar convoluted ependyma (Kappers, 1929), raises the possibility that it acts as a diffusion barrier preventing the diffusion of ACh from the

paraventricular nucleus into the cerebrospinal fluid. The pseudo-cholinesterase present in high concentration around the supraoptic nucleus would exert a similar function. For instance, if the cholinergic neurones converging on these nuclei were continuously to release large amounts of ACh and this ACh were to escape into the ventricular spaces, widespread effects would result, since it is known that the presence of small amounts of ACh in the ventricles of cats causes prolonged stupor (Dikshit, 1935; Silver & Morton, 1936; Bornstein, 1946; Feldberg & Sherwood, 1953, 1954). There is even the possibility that the pseudo-cholinesterase plays a part in the destruction of the ACh at the site of its release during neuronal activity. This possibility is suggested by the findings of Desmedt & La Grutta (1957) that selective inhibitors of pseudo-cholinesterase in minute doses will produce the 'alerting reaction' in the isolated cat cerebral cortex.

SUMMARY

1. The distributions of true and pseudo-cholinesterases have been examined in the hypothalamus of the dog by histochemical methods.

2. True cholinesterase was found confined to three nuclei of the anterior hypothalamus: the supraoptic, the paraventricular and the suprachiasmatic.

3. Pseudo-cholinesterase was found around the supraoptic nucleus, and around the third ventricle in the region of the paraventricular nucleus.

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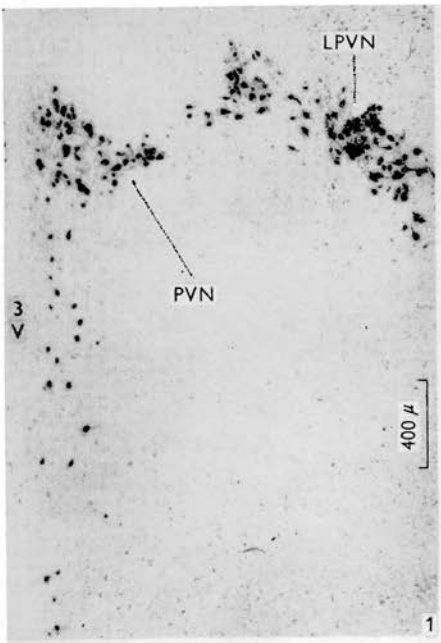

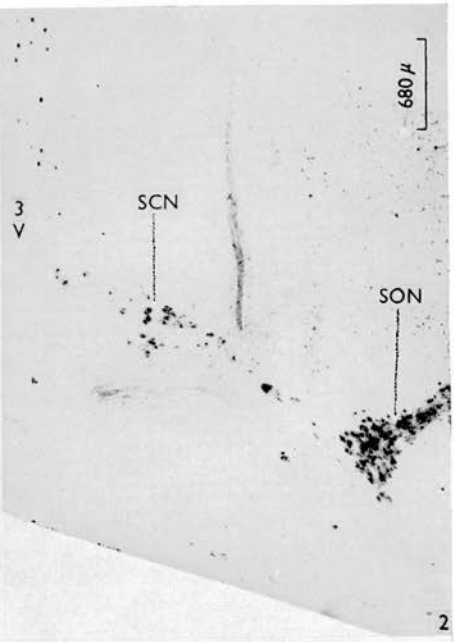

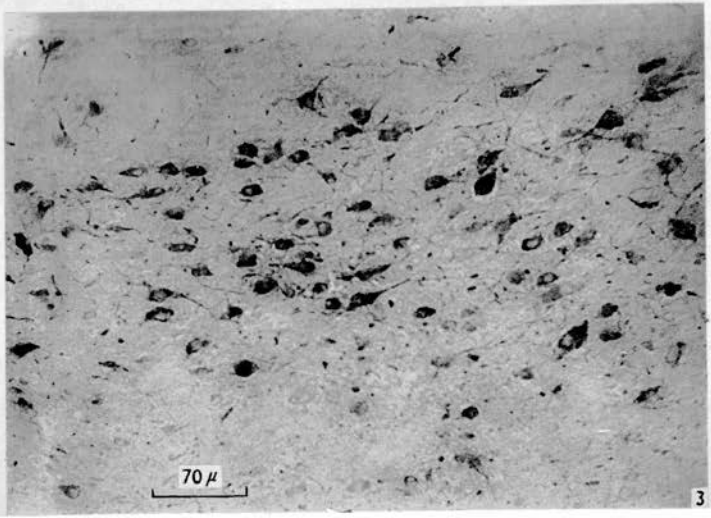

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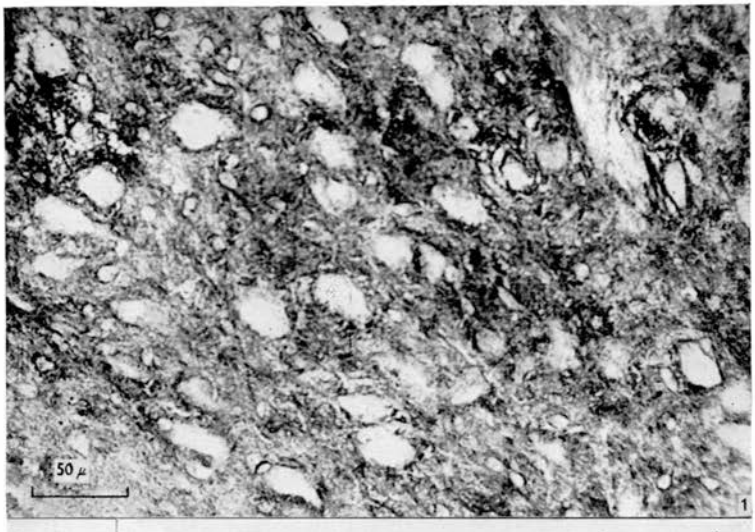
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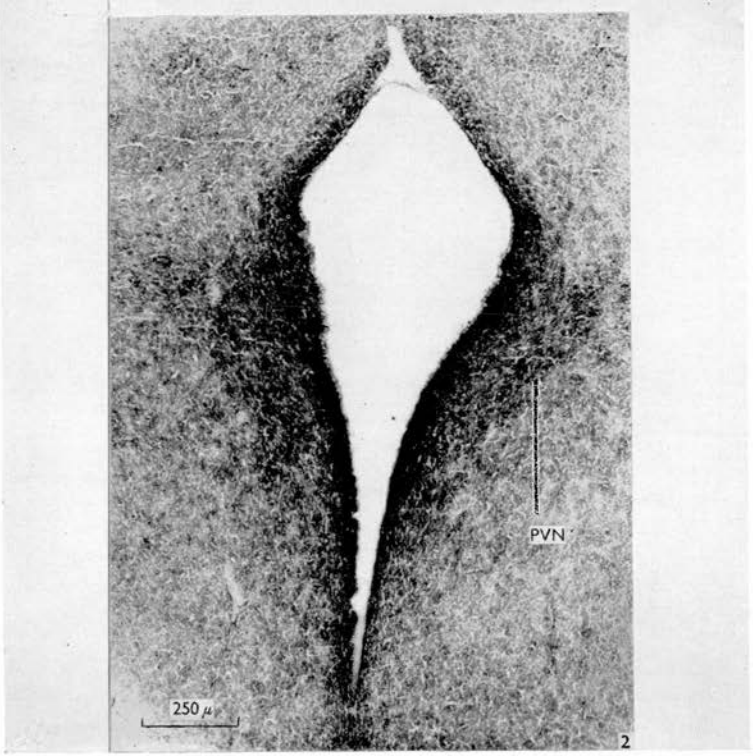
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EXPLANATION OF PLATES

PLATE 1

Sections of dog hypothalamus incubated in acetylthiocholine with no inhibitor present, to show distribution of true cholinesterase.

Fig. 1. Coronal section of the dorsal hypothalamus at the level of the paraventricular nucleus. Precipitation in the cells of the paraventricular nucleus (PVN), in cells of a lateral detachment of the paraventricular nucleus (LPVN) and in cells alongside the third ventricle (3V). This precipitation indicates true cholinesterase as it occurs also in the presence of DFP in the incubation solution. The background precipitation is due to pseudo-cholinesterase, as it is absent in the presence of DFP.

Fig. 2. Coronal section of the ventral hypothalamus at the level of the supraoptic nucleus. Precipitation in cells of the supraoptic nucleus (SON), suprachiasmatic nucleus (SCN), and cells between the two nuclei and along the third ventricle (3V). The precipitate in the cells results from true cholinesterase, the precipitate in the background from pseudo-cholinesterase, being absent in the presence of DFP.

Fig. 3. Cells of the paraventricular nucleus. (For details see text.)

PLATE 2

Sections of dog hypothalamus incubated in butyrylthiocholine with no inhibitor present to show the distribution of pseudo-cholinesterase.

Fig. 1. Supraoptic nucleus. Precipitation is confined to spaces between the cells. Rings of intense precipitation occur around many cells.

Fig. 2. Coronal section of the hypothalamus at the level of the paraventricular nuclei. Intense precipitation around the third ventricle which spreads out to embrace the area of the paraventricular nuclei (PVN).

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Active muscle vasodilatation and its relation to the 'Flight and Fight reactions' in the conscious animal.

By V. C. Abrahams* and S. M. Hilton. *The National Institute for Medical Research, Mill Hill, London, N.W. 7*

Lindgren, Rosen, Strandberg & Uvnäs (1956) have shown that electrical stimulation of certain regions in the hypothalamus, mesencephalon and medulla produce vasodilatation in skeletal muscle, thus providing evidence for a vasodilator pathway in the brain stem. This vasodilatation is due to activation of cholinergic vasodilator fibres (Eliasson, Folkow, Lindgren & Uvnäs, 1951). In the present experiments on anaesthetized cats the hypothalamic vasodilator pathway has been localized more precisely, using unipolar stimulating electrodes of 8–25 μ tip diameter.

Muscle vasodilatation was readily elicited on stimulation of the anatomically undifferentiated grey matter extending from the pre-optic area anteriorly to the mesencephalic central grey matter posteriorly. The excitable zone consisted, on either side of the mid line, of a horizontally flattened strip between the anterior commissure and the supraoptic nucleus in the pre-optic area. It continued posteriorly into the hypothalamus just ventral and medial to the fornix, in some experiments lateral to it also. The zone then continued further as a vertically flattened strip just dorso lateral to the mammillary body and ascended into the central grey matter.

Eliasson *et al.* (1951) noted that the muscle vasodilatation was often but not always accompanied by vasoconstriction in the skin and intestine, tachycardia, rise in blood pressure, pupillary dilatation and retraction of the nictitating membranes; they suggested that the muscle vasodilatation represented a reaction in preparation for muscular exercise. We observed these accompanying effects regularly, and, in addition, saw pilo-erection and widening of the palpebral fissure. In general the more pronounced the muscle vasodilatation, the more prominent were the associated effects.

It is known that stimulation of a localized area in the hypothalamus elicits the 'defence reaction' in the conscious cat (Hess & Brügger, 1943). This localized area appeared to be identical with the hypothalamic zone from which we had obtained our responses in the anaesthetized animal. Accordingly, we stimulated in the conscious cat, with an electrode chronically implanted in the hypothalamic zone from which muscle vasodilatation was elicited, and the typical picture of the 'defence reaction' was obtained. Immediately, the pupils dilated and the animal was alerted; after a few seconds it hissed and snarled, and started to run round the cage. There was massive pilo-erection. If disturbed by a sudden noise, the cat would start to attack in the direction

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of the sound. Stimulation via the chronically implanted electrode under chloralose anaesthesia produced the cardiovascular responses, including the characteristic muscle vasodilatation, and the other sympathetic reactions. It therefore appears that all these responses obtained under anaesthesia, and particularly the pattern of vasodilatation and constriction, are part of the general 'defence reaction', being preparatory to the muscular exertion of flight or fight.

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THE EFFECT OF DISTENSION OF THE UTERUS ON THE COMPOSITION OF URINE OF CONSCIOUS DOGS

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Certain potentially harmful stimuli, such as distension of hollow viscera or pinching of viscera or skin in spinal man, may alter the rhythm of the heart (Guttmann & Whitteridge, 1947) and, in spinal and anaesthetized animal or man may cause a general or localized skin vasoconstriction (Downman & MacSwiney, 1946; Cunningham, Guttmann, Whitteridge & Wyndam, 1953). In normal individuals Robertson & Wolff (1950) found it difficult to demonstrate any appreciable rise in blood pressure with distension of the rectum, but they did not look for the skin vasoconstriction which almost certainly occurred (Carmichael, Doupe, Harper & MacSwiney, 1939), nor therefore for compensatory vasodilatation in other areas. Owing to the intrinsic difficulties of acquiring information, little is known regarding the existence of viscero-visceral reflexes comparable with the viscero-cutaneous reflexes. Mukherjee (1957*a*, *b*) showed in chloralosed cats whose buffer nerves were cut that renal vasoconstriction accompanied the rise in blood pressure which followed distension of the bladder, but that when the buffer nerves were intact the rise in blood pressure was small and the renal vasoconstriction more often absent than present. The renal vascular changes, then, are a part of the general cardiovascular response, and in the animal with intact nervous system the stabilizing mechanisms profoundly modify this vascular response. Mukherjee (1957*a*, *b*) did not determine whether the renal vasoconstriction he observed was general or restricted to a part of the kidney. Using the method of serial angiography, Trueta, Barclay, Daniel, Franklin & Prichard (1947) showed that in rabbits trauma and haemorrhage was followed by renal cortical vasoconstriction with a persisting blood flow in the renal medulla. They made no observations on the effect of distension of viscera. Daniel, Peabody & Prichard (1951, 1952) found that a renal cortical vasoconstriction could be induced in the cat, dog and sheep as well as in the rabbit. Franklin (1952) and Sophian (1955) have suggested that in pregnancy uterine tension may initiate utero-

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renal reflexes which bring about a renal cortical ischaemia, which then leads to the condition of pre-eclampsia. A serious criticism of the work of the last two authors is that they made no tests of renal function and that many of their conclusions are based on the appearance of the kidneys of animals under anaesthesia. As Daniel *et al.* (1952) point out, surface pallor of the kidney may be due to a general renal vasoconstriction as much as to vasoconstriction localized to the renal cortex. Further, neither oliguria nor albuminuria are pathognomic of renal cortical vasoconstriction. Since the problem is an important one, the renal function was tested in conscious dogs during periods when the uterus was put under tension by the sudden inflation of an intra-uterine balloon. A preliminary account of some of the results has already appeared (Pickford, 1956).

METHODS

Dogs were prepared for observation by operations at which the external urethral orifice was exposed by a dorsal slit of the perineum, and at which the upper end of one uterine horn was divided from the ovary and fimbria of that side, brought through to the surface of the flank and stitched in place. No observations were made until healing was complete. The posterior lobe of the pituitary was removed transorally. The balloon for insertion into the uterus was joined by polythene tubing to a hand pump and pressure bottle and a recording Hg manometer, as described by Abrahams & Pickford (1954). The balloon and tubes were filled with 0·9% NaCl solution. With this system it was possible to raise the pressure in the balloon to the desired height in 3–4 sec or less. On the days on which they were to be used, the dogs were given a hydrating dose of water at noon and observations made after a second oral dose of water given at 2.15–2.30 p.m. of the same day. When the renal clearances of creatinine and diodone were to be measured, 3·5–4 g creatinine was given by mouth with the afternoon dose of water, and 6–9 ml. 30% diodone solution injected subcutaneously half an hour before the water was given. In general the methods used were similar to those described by Ali, Cross & Pickford (1958). The diet was maintained fairly, but not exactly, constant. At present no useful comments can be made on the higher post-operative electrolyte loss in the dog which was ovariectomized; in any case, this increase does not directly affect the question under investigation. Estimations of Na and K were made on a flame photometer, chloride by the method of Prout-Winter as described by Cole (1919), creatinine in the blood and urine by the methods of Rehberg (1926) and Folin (1914), respectively, and diodone by the method of Alpert (1941). After death the kidneys were examined histologically and all were found to be normal. Serial sections of the hypothalamus and pituitary confirmed that in the one dog the posterior lobe of the pituitary had been removed at operation.

RESULTS

Control observations showed that in the three dogs used the excretion rates of water, Na, K and Cl were normal; that is, the peak of diuresis was attained in 45–60 min, 80–90% of the water given was excreted in approximately 2 hr, and the excretion rates of Na, K and Cl fell steadily as diuresis progressed (Ali *et al.* 1958). Having established these facts, the effects of uterine distension were then investigated in the anoestrous dog, the oestrogen-treated dog and a dog whose posterior pituitary lobe had been removed; examination was also made of the effect of distension of the lower segment of the uterus.

Effect of uterine distension in the anoestrous dog. It was first determined that distension of the uterus during, and for a time after, the administration of water, in no way altered the normal course of diuresis. Ten experiments were then performed, six experiments being on one dog (Anona, 9·5 kg) and four on another (Maggs, 20 kg). The effect of uterine distension was examined under two sets of conditions. In the first the balloon was inserted and inflated at the peak of diuresis; and in the second, the balloon was inserted immediately following the administration of water, but again inflated only at the peak of diuresis. The pressure to which the balloon was initially inflated was from 30 to 100 mm Hg. Left to itself the intra-uterine pressure declined

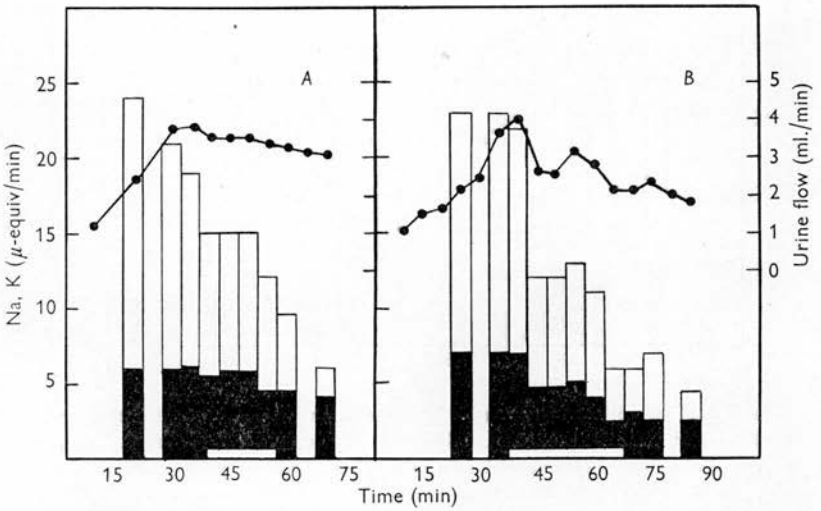

Fig. 1. Records of two observations made on one dog. In both *A* and *B* the continuous line shows the rate of urine flow in ml./min. The histograms show the excretion rate of Na (open columns) and K (black columns) in μ-equiv/min. The open blocks along the abscissa show the time during which the uterus was distended. In *A* the pressure applied was 40 mm Hg, and in *B*, 30 mm Hg. On both occasions 250 ml. water was given by mouth at zero time.

steadily, but the effects on renal function were no different when this fall was prevented by the repeated addition of small volumes of saline to the uterine bag. The results were also unaffected by the time of insertion of the uterine balloon. In seven of the ten experiments inflation of the bag produced no alteration in the course of the water diuresis, nor any deviation from normal in the excretion of Na or K (Fig. 1*A*, Anona). In one experiment uterine distension caused a brief increase in the rate of urine excretion with perhaps a slight delay in the usual fall of Na excretion. In two other experiments on the same dog the urinary excretion rate fell somewhat and the diuresis continued at the lower level (Fig. 1*B*). On one of these last two occasions renal clearances were measured and it was found that although the initial fall in

urinary excretion rate was accompanied by a fall in both glomerular filtration rate (24%) and renal peak flow (42%), within 15 min urine flow and clearance values had returned to normal levels.

Effect of uterine distension after treatment with stilboestrol. It was considered possible that the anoestrous uterus had a low sensitivity to a rise in tension. Experiments were therefore performed on Anona and Maggs after they had been injected with stilboestrol diproprionate (0·35 and 0·75 mg, respectively) in oil. Inspection at laparotomy had previously shown that within 24 hr of the subcutaneous injection of stilboestrol the dog uterus becomes enlarged and

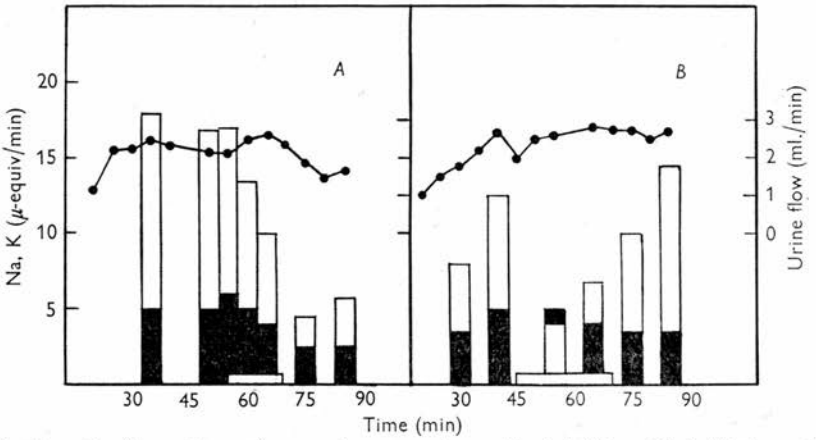

Fig. 2. Records of two observations made on one dog on the 1st (*A*) and 2nd (*B*) days after a subcutaneous injection of 0·35 mg stilboestrol diproprionate. On both occasions the intrauterine pressure was maintained at 50 mm Hg. Conventions as for Fig. 1.

highly vascular. Observations were accordingly made on the two days following injection. In Anona uterine distension 24 hr after stilboestrol administration caused a small rise in the rate of urine flow, but had no significant effect on the normal course of Na and K excretion (Fig. 2*A*). Two days after the injection a transient decrease in the rate of urine flow followed inflation of the uterine bag, and there was some fall in the rate of Na loss (Fig. 2*B*). In Maggs uterine distension 24 hr after the injection of stilboestrol caused a sharp reduction in water, Na and K loss, but excretion returned to the original level in 7 min. The following day uterine distension was without effect on renal excretion.

Effect of distension of the lower segment of the uterus. It is known that the different parts of the uterus vary in sensitivity (see Fitzpatrick, 1957). In the third dog, Little Claus, which had been ovariectomized 12 days previously and whose uterus was showing spontaneous contractions, care was taken to push the balloon as far down towards the cervix as possible. Post-mortem measurements showed that the tip of the bag must have been close to the cervical

orifice. When diuresis was established, a pressure of 140–150 mm Hg was thrown into the balloon and maintained for 1 hr. This high intra-uterine pressure did not alter the normal pattern of water excretion nor the already low rate of Na and K loss, but after some delay the rate of Cl excretion rose for a time (Fig. 3).

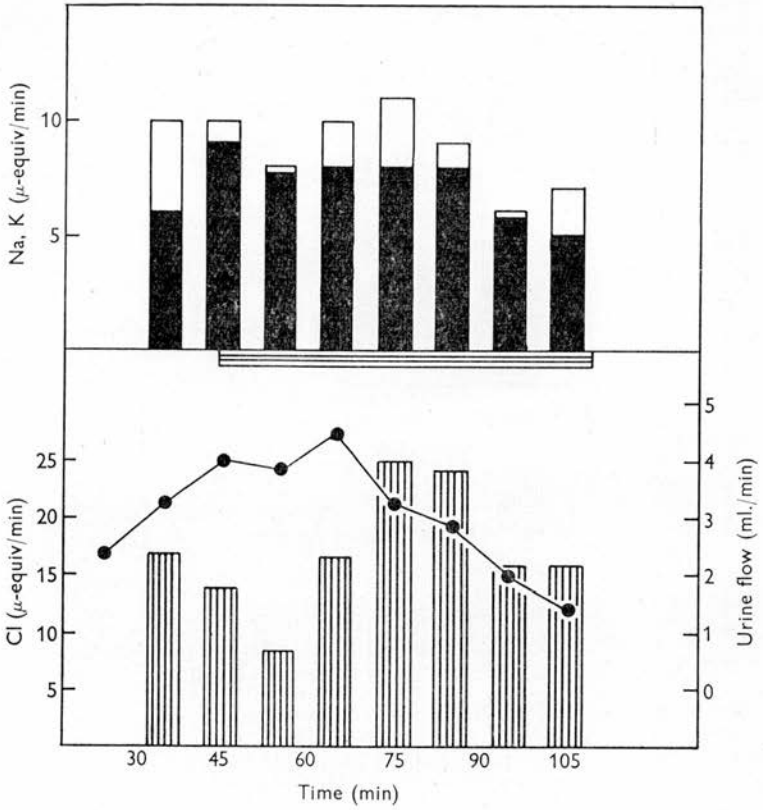

Fig. 3. Effect of distending the lowest part of one uterine horn with a pressure of 150 mm Hg during the period shown by the horizontally lined block. Lower part of graph shows rate of urine flow (continuous line) and Cl loss (columns with perpendicular lines). Upper part of graph shows rate of Na (open columns) and K loss (black columns). 300 ml. water given by mouth at zero time.

Effect of uterine distension after removal of the posterior lobe of the pituitary. Since Dale's observations in 1909 it has been known that the posterior lobe of the pituitary contains a substance which profoundly affects uterine activity. There is no suggestion that this substance directly affects uterine sensitivity. The posterior lobe also contains the vasopressor-antidiuretic factor. Since the opportunity offered, it seemed worth while re-examining Little Claus whose posterior lobe had been removed 11 days previously. The water intake had returned to normal levels. The uterus still showed spontaneous activity; the

renal nerves were intact. In Fig. 4 it can be seen that after raising the pressure in the uterine bag to 150 mm Hg the rate of urine flow increased for a while, and the excretion rates of Na and K rose also, but Cl loss fell. None of these changes persisted despite the maintenance of uterine distension and the intact nerve supply to the kidneys.

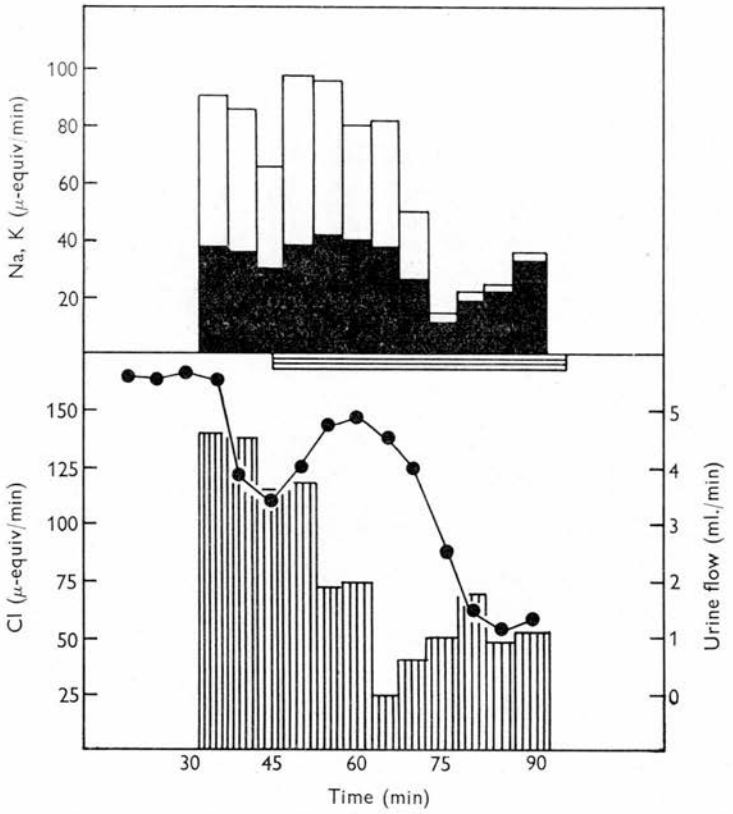

Fig. 4. Observation on same dog as in Fig. 3, but 11 days after removal of posterior lobe of pituitary. Intra-uterine pressure, 150 mm Hg. Conventions as for Fig. 3

DISCUSSION

The results described above suggest that in the dog, whether normal and anoestrous, treated with stilboestrol or ovariectomized, a utero-renal reflex is not highly active, even when a pressure as great as 150 mm Hg is applied in the uterus. The results provide no evidence that a rise in intra-uterine tension causes vasoconstriction localized to the renal cortex, and the inconstant alterations seen in renal function could well be due to variable vascular changes occurring in the whole kidney, these being a part of the general vascular response to distension of a hollow viscus as described by Mukherjee (1957*a*, *b*).

Two sets of workers have previously made observations on changes in renal function when the uterus is under tension. McGaughey, Weller & Anslow (1956), using dogs under chloralose anaesthesia, noted that uterine insufflation caused a 23% reduction in *p*-aminohippuric acid clearance; but a similar reduction followed vaginal manipulation and non-specific sensory stimulation of the skin. Thus, their evidence and that presented here reinforce the impression that the renal effects of uterine stretch, in the dog at any rate, are part of a general response and not specifically related to a utero-renal reflex. It should be mentioned that Daniel *et al.* (1952) found that renal cortical vasoconstriction was more difficult to produce in dogs than in the other animals they tested.

Saurio (1957) made a large number of observations on conscious rabbits and studied the effect of uterine distension on the excretion of water, Na and K by the animal when normal and after section of the splanchnic nerves. She found no evidence of a utero-renal reflex, and concluded that the results seen to follow uterine distension could be matched by the intravenous injection of pituitrin, and were best explained as due to a transient liberation of pituitary antidiuretic hormone. The present results gave no evidence of the release of antidiuretic hormone in normal dogs, but in the single observation on a dog whose posterior lobe had been removed the sharp temporary rise in urine flow which followed uterine distension perhaps gives negative support to Saurio's thesis.

All observations so far made suggest that it is difficult in normal unanaesthetized individuals to cause a renal vasoconstriction limited to the cortex. As previously pointed out (Pickford, 1956) uterine muscle readily accommodates even to a rapid rise in intra-uterine tension, so that any reflex effect occurring should be of short duration, as indeed was noted in the present experiments. However, since renal cortical necrosis does occur, it must be concluded that some unidentified abnormal condition is a prerequisite for the appearance of renal cortical ischaemia. In the presence of this abnormality uterine stretch may be one of a number of adequate stimuli.

SUMMARY

1. Observations were made on conscious dogs in which one uterine horn was fistulated. Uterine movements and the urinary loss of water, Na and K were noted before and after the sudden inflation of an intra-uterine balloon.

2. The dogs were tested when normal, after the administration of stilboestrol, after ovariectomy, and after ovariectomy accompanied by removal of the posterior lobe of the pituitary.

3. No consistent effect followed inflation of the intra-uterine balloon, and ten out of sixteen times renal activity was unchanged.

4. The results provided no evidence that, in dogs, a utero-renal reflex causes renal cortical vasoconstriction.

Addendum. Since this paper was written four observations have been made on the response to uterine distension (pressure, 40 mm Hg) of a dog with diabetes insipidus (induced by transoral section of the supraopticohypophysial tracts). Water and K excretion showed no discernible pattern of response; on every occasion Na excretion fell when the uterine bag was inflated; on all occasions Cl excretion remained steady.

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Venous temperature registration, and its use as an index of muscle blood flow. By V. C. ABRAHAMS.* *National Institute of Medical Research, Mill Hill, London, N.W. 7*

In experiments on cats it was shown that the temperature of the blood leaving a muscle is dependent on the rate of blood flow through the muscle. When muscle blood flow increased, the effluent blood temperature increased and vice versa. It was found that the temperature changes associated with quite small fluctuations in flow, such as those associated with the respiratory cycle, were sufficiently large to be registered by a thermistor introduced into the blood stream.

The procedure used was as follows. In cats anaesthetized with chloralose the femoral vein of one limb was cut and a small chamber was inserted into the cut ends so that the blood could flow through it; the chamber was machined from Araldite block. An unmounted bead thermistor (Standard Telephones Type U. 2361), cemented to the wall of the chamber, was used to detect temperature changes of the blood passing through it. The changes were registered using a conventional bridge circuit, the output of which was recorded by a Speedomax Type 'G' pen recorder. The paw was tied off and the limb was skinned.

In order to study the muscle blood flow in conscious cats the femoral vein temperature was used as an index of muscle blood flow. In a preliminary operation the animals were anaesthetized with ether and the femoral vein was cut for the insertion of the small Araldite chamber. In these experiments the paw was not tied off and the limb was not skinned. The use of the temperature of the whole outflow from the femoral vein as an index of muscle blood flow is feasible when responses to nociceptive stimuli are studied, because an increase in muscle blood flow is accompanied by a fall in skin blood flow.

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Active muscle vasodilatation elicited by mesencephalic stimulation. Its relation to the defence reaction. By V. C. Abrahams*, S. M. Hilton and A. Zbrozyna†. *The National Institute for Medical Research, Mill Hill, London, N.W. 7*

Stimulation in the diencephalon and mesencephalon of the cat has been shown to produce active vasodilatation in muscle (Eliasson, Folkow, Lindgren & Uvnäs, 1951; Lindgren, 1955; Abrahams & Hilton, 1958). In the present experiments the mesencephalon at the level of the superior colliculi has been extensively explored. At this level, vasodilator responses had hitherto only been obtained from stimulation of a restricted region deep in the tectum. We could also regularly obtain large vasodilator responses from the central grey matter and from a well-defined area overlying each cerebral peduncle. The latter is small in cross-section. It was traced anteriorly to the level of the mammillary bodies where it connects with the hypothalamic area from which vasodilatation is obtained. Smaller vasodilator responses were obtained from other tegmental regions which appeared to connect it with the region in the tectum and central grey matter. Since this peduncular region is continuous with those in the hypothalamus, tectum and central grey matter, it is suggested that it constitutes the main vasodilator pathway in the mesencephalon and that this pathway is not in the tectum, as proposed originally by Lindgren (1955). This conclusion is supported by the finding that vasodilator responses produced by hypothalamic stimulation are abolished by small electrolytic lesions in the peduncular region, whereas extensive lesions in the superior colliculi or tegmentum did not abolish the response except in one experiment.

The diencephalic area from which active muscle vasodilatation is obtained is identical with that from which defence reactions are elicited (Abrahams & Hilton, 1958). Such reactions are also elicited from the central grey matter and the superior colliculi (Hunsperger, 1956; Spiegel, Kletzkin & Szekely, 1954) from areas corresponding to those from which we obtained muscle vasodilatation. However, stimulation of the peduncular vasodilator pathway in the conscious cat did not lead to the complex co-ordinated defence reaction, but to partial responses such as rolling over on to one side with claws extended, ipsilateral ear-flattening or closure of the eye. This finding further supports the conclusion that the peduncular region is the main vasodilator pathway.

Chronic decorticate cats show a stereotyped reflex response to nociceptive stimuli indistinguishable from the defence reaction (Dusser de Barenne, 1920). Similar but incomplete reactions are obtained from decerebrate cats (Wood-

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worth & Sherrington, 1904; Bazett & Penfield, 1922). These findings, together with our results, suggest that the regions defined in the hypothalamus, central grey matter and colliculi are reflex centres concerned in the response to nociceptive and other stimuli, the peduncular region constituting the efferent pathway for the concomitant muscle vasodilatation.

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Sensory input to hypothalamic and mesencephalic regions subserving the defence reaction.

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The defence reaction, which is a complex behavioural and visceral response, can be elicited by electrical stimulation of regions of the hypothalamus, central grey, superior colliculi and tegmentum. It commences with an alerting reaction and with sufficiently strong stimulation culminates in fight or flight. The evidence suggests that these regions are concerned with the reflex elicitation of the reaction (Abrahams, Hilton & Zbrozyna, 1959). We have now shown that these regions have connexions with afferent pathways from the skin, the ears and the eyes. These connexions appear not to be localized, but to be part of a diffuse system relaying to the hypothalamus as a whole and to extensive areas of the mesencephalon.

In cats anaesthetized with chloralose evoked electrical responses were recorded with tungsten micro-electrodes.

Direct electrical stimulation of the skin, or stimulation of the superficial radial nerve evoked large responses in all regions of the hypothalamus, extending from the pre-optic area anteriorly to the mammillary bodies posteriorly. Large responses were also evoked in the central grey matter, smaller responses in the superior colliculi and tegmentum. The latencies of the responses varied between 10 and 40 msec. The response to unilateral nerve stimulation appeared bilaterally and was practically identical on both sides. The responses resulted from stimulation of A fibres as was shown by monitoring the action potential of the stimulated nerve. This is in agreement with Feldman, Van der Heide & Porter (1959), who showed that responses recorded in the hypothalamus of cats immobilized with Flaxedil after sciatic nerve stimulation were also due to A fibre stimulation.

The responses to crystal generated clicks appeared less consistently except in the tuberal region where the responses were always large. In all other regions explored, responses were obtained irregularly only and the size of the responses varied greatly. The latencies of the responses were always greater than 10 msec.

Responses to a light flash were recorded from all explored regions of the hypothalamus and the mesencephalon. They were large and uniform in the hypothalamus and had a latency of about 50 msec. In the mesencephalon they varied greatly, the largest responses being obtained from the superior colliculi and the underlying tegmentum. The latency here was about 40 msec. These latencies are much longer than those found by Ingvar & Hunter (1955)

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in corresponding experiments on unanaesthetized cats immobilized with curare. The demonstration of these afferent connexions adds further support to our hypothesis that these regions are concerned with the reflex mediation of the defence reaction in the conscious cat.

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Reflex activation of vasodilator nerve fibres to skeletal muscle in decerebrate and intact cats.

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Certain regions of the hypothalamus and mesencephalon appear to act as reflex centres for the defence reaction (Abrahams, Hilton & Zbrozyna, 1959; Abrahams, Hilton & Malcolm, 1959). Part of this reaction is a cardiovascular response which includes active muscle vasodilatation.

Active muscle vasodilatation has now been obtained as a reflex response in high decerebrate cats, in which the hypothalamus and mesencephalic structures have been spared. When the ether has worn off, electrical stimulation of the skin produces most of the signs of the defence reaction including the features observed in similar experiments by Woodworth & Sherrington (1904) and termed the pseudaffective reflex.

In our decerebrate cats venous outflow was recorded from an innervated gastrocnemius muscle perfused by a heparinized donor cat. Movements of the perfused muscle were prevented by section of the spinal cord between L5 and L6. It was found that the reflex response to skin stimulation included a large and prolonged increase in muscle blood flow due to activation of cholinergic vasodilator nerve fibres.

When decerebration removed a substantial part of the hypothalamus the skin stimulation no longer produced muscle vasodilatation but still elicited many features of the behavioural response.

If the defence reaction is produced in the intact non-anaesthetized cat by electrical stimulation of the hypothalamus, the response is graded according to the strength of stimulation. Threshold stimulation does not produce fight or flight. It produces alerting, but already with pronounced muscle vasodilatation, as shown when muscle blood flow is registered using the venous temperature technique (Abrahams, 1959). Confirmation that this vasodilatation, like that in the decerebrate cat, is due to activation of cholinergic vasodilator fibres was obtained by showing that it is reduced or abolished by atropine.

These same behavioural and cardiovascular responses are obtained reflexly in the intact cat on nociceptive stimulation such as electric shocks to the foot pads. Any stimulus which produces the alerting reaction also elicits the active muscle vasodilatation. For instance, a loud note sustained for 10 sec usually produces these responses at least at the first trial. On repetition of the note, however, the responses usually diminish and are absent by the 5th trial. In a few cats this habituation to repeated stimulation was not seen.

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When an auditory stimulus does not itself produce responses it is possible to show that a conditioned vasodilator response can be developed to such a stimulus, by combining it with nociceptive stimulation 4 or 5 times.

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ACTIVE MUSCLE VASODILATATION PRODUCED BY STIMULATION OF THE BRAIN STEM: ITS SIGNIFICANCE IN THE DEFENCE REACTION

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The existence of a sympathetic vasodilator nerve supply to the skeletal muscles of the cat and dog has been known for some time (Bülbring & Burn, 1935; Folkow & Uvnäs, 1950). More recently it was discovered that these vasodilator nerve fibres can be activated by electrical stimulation in the hypothalamus, in regions of the mid-brain tegmentum and medulla, and, in the dog, by stimulation of a small region of the motor cortex (Eliasson, Folkow, Lindgren & Uvnäs, 1951; Eliasson, Lindgren & Uvnäs, 1952; Lindgren & Uvnäs, 1953; Lindgren, 1955). These findings led Lindgren, Rosén, Strandberg & Uvnäs (1956) to postulate the existence of a vasodilator pathway, originating in the anterior sigmoid gyrus and passing to the spinal cord via the hypothalamus, mid-brain tegmentum and medulla. They suggested that this pathway was involved in the cardiovascular adjustments of muscular exercise.

Previously, Eliasson *et al.* (1951) had noted that the muscle vasodilatation produced by hypothalamic stimulation was accompanied by vasoconstriction in the skin and intestine, and by other signs of sympathetic activity. In view of the known role of the hypothalamus in the expression of 'fear' and 'rage', they suggested that the vasodilator fibres were normally activated as part of the patterned response characteristic of these emergency reactions. According to the view of Eliasson, Lindgren & Uvnäs (1954), the hypothalamic areas were to be regarded as relay stations on a vasodilator pathway which could be activated not only in cases of emergency, but indeed whenever a sudden increase in muscle blood flow would be required for muscular effort.

In the conscious cat, systematic stimulation of points in the hypothalamus enabled Hess to define a region from which co-ordinated behaviour characteristic of the emergency reactions could be evoked (Hess & Brügger, 1943). This behaviour pattern, which commenced with alerting and culminated in flight or attack, was termed the defence reaction. In

* Work carried out during tenure of Beit Memorial Fellowship. † Rockefeller Fellow.

the present experiments we have located those parts of the hypothalamus and mid-brain from which the muscle vasodilatation is evoked and have shown that they are in fact the regions responsible for the whole integrated defence reaction in the conscious cat. Thus, strictly speaking, these are not vasodilator areas, but co-ordinating centres which produce muscle vasodilatation as a part of the defence reaction. In addition, we have located a separate vasodilator pathway which is activated by this extensive defence centre and which runs in close relation to the cerebral peduncles.

Woodworth & Sherrington (1904) showed that stimulation of peripheral nerves in the high-decerebrate cat elicited a response comprising many features of the defence reaction, which they termed the pseudaffective reflex. Cannon & Britton (1925) showed that all the autonomic features of the defence reaction which were known at the time could be produced reflexly in such preparations. In the present experiments, the active muscle vasodilatation has been found to be a prominent feature of these reflex responses, the reflex centre presumably being those regions of the hypothalamus and mid-brain from which the defence reactions are obtained.

METHODS

All experiments were performed on cats. The acute experiments were carried out under chloralose anaesthesia, a dose of 60–80 mg/kg being injected intravenously after anaesthesia had been induced with ethyl chloride and ether. To prepare a hind limb for registration of changes of muscle blood flow, a longitudinal incision was made in the skin along the inner side of the leg from the inguinal region to the ankle, the skin was separated from the underlying tissues, the circulation through the foot was occluded by a stout ligature round the limb just above the ankle joint and the skin was then sewn back over the limb. The femoral venous outflow was passed via polythene cannulae through a photo-electric drop-recorder and returned to the femoral vein more centrally. Changes of skin flow in the forepaw were registered similarly by recording the outflow from the cephalic vein. To record intestinal flow the circulation through several arcades supplying a section of the small intestine was isolated and the outflow from the appropriate branch of the superior mesenteric vein was passed through the drop-chamber and back into a branch of the portal vein. Heparin 1000 u./kg) was injected 1 hr after all dissections had been completed, and before the veins were cannulated for recording venous outflow.

Blood pressure was recorded by a mercury manometer connected to a carotid artery. Respiratory movements were registered by a piston recorder connected to corrugated rubber tubing held round the animal's chest.

Deep structures of the brain stem were stimulated by the use of stereotactically placed monopolar steel micro-electrodes. They were prepared from steel sewing needles by electrolytic erosion (Bishop & Collin, 1951) to a tip size of 5–20 μ, and were varnished except for the tip, either with a single coat of Dulux varnish, or with four coats of 'Araldite' 985E. The indifferent electrode was a similar steel needle pushed under the skin of the neck. A square-wave stimulator was used, the output being isolated from earth by means of an RF unit.

Electrolytic lesions were produced with similar electrodes, the insulation having been scraped off the tip to leave the last 0·5 mm bare. In some experiments, these electrodes were inserted in pairs, 2 mm apart, at the same antero-posterior level, and lesions were made by

passing current from one electrode to the other. In some, a single electrode was used, current being passed between this electrode and an indifferent electrode under the skin.

In a number of experiments electrodes were implanted into the brain stem in sterile operations under pentobarbitone anaesthesia. These electrodes were no. 6 or no. 4 intestinal needles made from austenitic steel and coated with 'Araldite' 985E. The needles used had a tip diameter of about 50 μ. The electrodes were implanted either singly or in assemblies. In such an assembly the needles, spaced at 2 mm intervals, were previously fixed in a small fluon (Polytetrafluoroethylene, Imperial Chemical Industries Ltd.) plate. The electrodes were implanted by means of the stereotactic apparatus. To aid fixation of the electrodes, 2 or 3 small (10 BA) stainless steel screws were screwed into the outer table of the skull, one being used for holding the indifferent electrode, a small stainless steel plate. Acrylic resin (Dental Fillings, Ltd.) was used to cover the electrodes and screws, and to cement them together. The leads were brought under the skin of the neck and out between the scapulae, to be fixed to a connector carried by the animal on a leather or polythene jacket.

Exact location of the needles was determined histologically at the end of each experiment. The brain was fixed by perfusion with alcoholic formol saline. Sections 25 μ thick were cut on the freezing microtome and stained with luxol fast blue and cresyl violet (Klüver & Barrera, 1953).

In some experiments muscle blood flow was registered in decerebrate cats. Under ether anaesthesia the calvarium was removed and the thalamus, basal ganglia and cerebral hemispheres were extirpated with a spatula. The anaesthetic was then discontinued. Two methods were used to record muscle blood flow. In one it was recorded directly by a cross-circulation technique. The circulation of one gastrocnemius muscle of the decerebrate cat was isolated and the muscle was supplied with blood from a heparinized donor cat. The venous outflow from the muscle was registered by the drop-recorder on its return to the donor. The spinal cord of the decerebrate cat was tied below the emergence of the L6 roots, so as to eliminate movements of the gastrocnemius while preserving its sympathetic innervation. The other method enabled the preparation to be more rapid and avoided the use of heparin. The changes of muscle blood flow were registered by a modification of the venous temperature method described by Abrahams (1959). In the original method a small chamber bearing a thermistor bead is inserted between the cut ends of the femoral vein. In the present modification cutting the vein was avoided by mounting the thermistor bead on the surface of a small strip of 'Araldite' which was held close to the external surface of the vein by an 'Araldite' cuff placed round the vessel. In this way adequate records were obtained of the changes in venous blood temperature which result from changes in muscle blood flow.

RESULTS

The finding of Eliasson *et al.* (1951) and Lindgren (1955), that active vasodilatation in skeletal muscle is elicited by electrical stimulation of regions of the hypothalamus and mid-brain, was readily confirmed. This vasodilatation usually appeared after about 5–10 sec of stimulation and reached a maximum within a few seconds. When stimulating with voltages just above the threshold (2–5 V under the present experimental conditions) the muscle blood flow often approached the value seen after maximal tetanic contraction. In some experiments, there was almost a tenfold increase in flow. As was shown by Lindgren (1955), square-wave stimulation at a frequency of 70–80 pulses/sec and with a pulse width of 2 msec is optimal for this response. With these parameters it was possible to

elicit reproducible responses from the same point in the hypothalamus at intervals of 3–4 min. We have elicited up to 13 successive responses without any obvious diminution.

At most points in the hypothalamus from which vasodilatation was elicited, the stimulation also produced moderate rises of arterial blood pressure, up to 40 mm Hg. On stimulation in some parts of the mid-brain these blood-pressure rises were much higher. It was clear, however, that these rises in blood pressure made little if any contribution to the increase in blood flow. Almost invariably the rise of blood pressure occurred before the onset of vasodilatation, and the pressure was back to normal when the vasodilatation was at its peak. Moreover, small doses of atropine (0·1 mg/kg) abolished or greatly reduced the vasodilator response (Eliasson *et al.* 1951), while the blood pressure rise then produced was if anything greater.

As was noted by Eliasson *et al.* (1951), the vasodilatation was usually accompanied by vasoconstriction in the skin and intestine, by tachycardia, pupillary dilatation and retraction of the nictitating membrane. In addition, we observed widening of the palpebral fissure, pilo-erection along the tail and mid line of the back, and changes in respiration. Respiratory movements were usually rapid, though much reduced in depth, the chest being held predominantly in the inspiratory position. The respiratory change made no significant contribution to the cardiovascular responses; for they persisted when the animals were artificially respired. With light anaesthesia stimulation often produced swishing movements of the tail, arching of the back and small movements of the jaws and limbs. In general, these associated effects were most prominent on stimulation of the brain-stem regions which produced the largest vasodilator responses.

Localization in the hypothalamus

The excitable area was sharply localized. As an example, the three panels in Fig. 1 show the effects of stimulating three points 1 mm apart, along a single electrode track in the tuberal region or the hypothalamus. The muscle vasodilatation obtained on stimulation at the middle point (marked 0) was much greater than that produced by stimulation 1 mm above or below it (marked X' and X). Figure 2 shows a hemisection of the brain from this experiment, in which there are four electrode tracks, the diagrammatic hemisection on the right illustrating the position of the electrode tip when the results shown in Fig. 1 were obtained. With the electrodes used stimulation probably only occurred close to the tip. From results such as these it was possible to construct maps of the restricted areas of the hypothalamus from which large vasodilator responses in skeletal muscle were obtained. The maps shown in Fig. 4 incorporate the results of twenty-two experiments in which the hypothalamus was

systematically explored, and of fifteen experiments in which electrodes were chronically implanted. At each level the results are included from planes 1 mm anterior and posterior to the level of section. These maps give a somewhat less precise localization than that actually obtained in individual experiments, because the borders of the area vary a little from

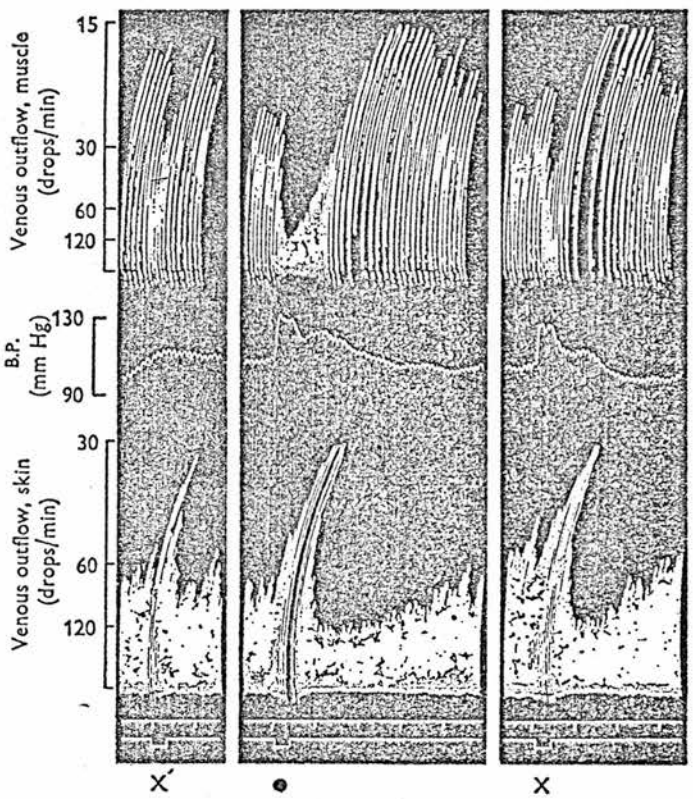

Fig. 1. Cat, chloralose. Records of venous outflow from skinned lower leg (top) and skin of the fore-paw (bottom), and of arterial blood pressure (B.P.). At X′, ● and X, stimulation at 3 successive points, 1 mm apart, in one electrode track in hypothalamus. Position of electrode tip at each point is shown in Fig. 2. Time marker, 30 sec.

one animal to another. The levels of the four illustrative sections through the hypothalamus are shown on the paramedian sagittal section in Fig. 3.

The hypothalamus anterior to the optic chiasma was explored in six experiments. Large vasodilator responses were obtained in three of them. The excitable region extended horizontally in the undifferentiated grey matter just above the base of the brain, from 1 to 3 mm lateral to the mid line, as illustrated in Fig. 4*A*. Smaller responses were obtained from

stimulation more dorsally, close to the anterior commissure. At the level of the optic chiasma the excitable area was sharply localized about 1–2 mm lateral to the third ventricle, and just dorsal to the chiasma itself (Fig. 4*B*). In the remaining three experiments this region was the most anterior from which large vasodilator effects could be obtained.

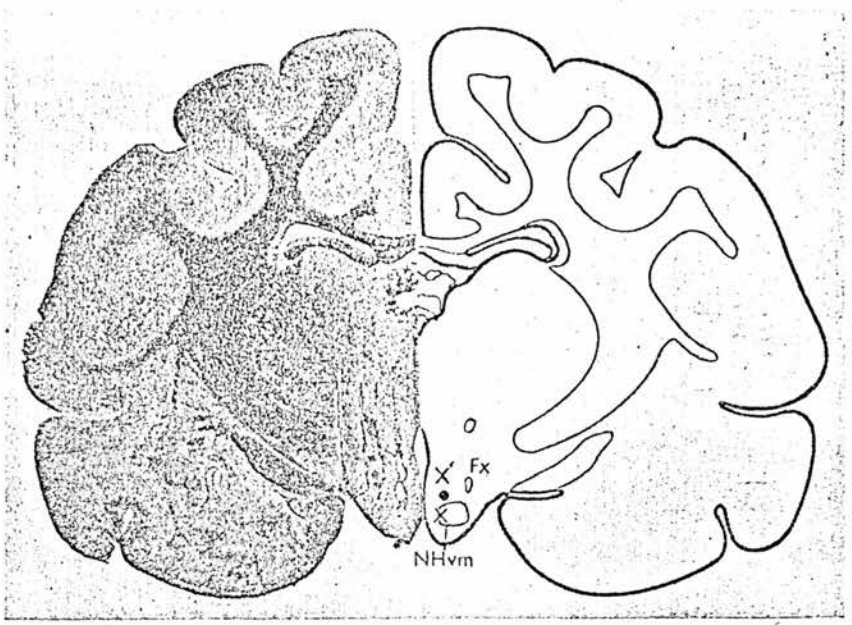

Fig. 2. Left: photomicrograph (coronal section) of cat's brain from same experiment as Fig. 1, showing four tracks left by stimulating electrode. Right: diagrammatic reproduction of section; X', ● and X indicate position of electrode tip when records of Fig. 1 obtained. Fx, fornix; NHvm, ventromedial nucleus.

On stimulation more posteriorly, at the tuberal level, the vasodilator responses were even larger. In all five of the animals in which this part of the hypothalamus was studied, the excitable area was in the perifornical region and extended laterally as a narrow band towards the zona incerta (Fig. 4*C*). In one animal the largest responses were obtained from a region dorsal to the fornix. Stimulation at the level of the mammillary bodies, carried out in five experiments, produced large responses from a strip dorsal and lateral to the mammillary bodies themselves. In four experiments, large responses were obtained from a region just dorsal to the cerebral peduncles (Fig. 4*D*).

Throughout the hypothalamus the most excitable region for these responses never appeared to be associated with any of the hypothalamic

nuclei. When stimulating in the tuberal region, for example, the vasodilator response was greatly reduced when the electrode tip passed into the ventromedial nucleus (as seen from Figs. 1 and 2). Likewise, stimulation in the mammillary bodies, supraoptic nucleus, paraventricular, suprachiasmatic and dorsomedial nuclei never produced a large vasodilatation. In different experiments the descending columns of the fornix were stimulated from the septal region to their termination in the tuberal region: the vasodilator effect was never pronounced.

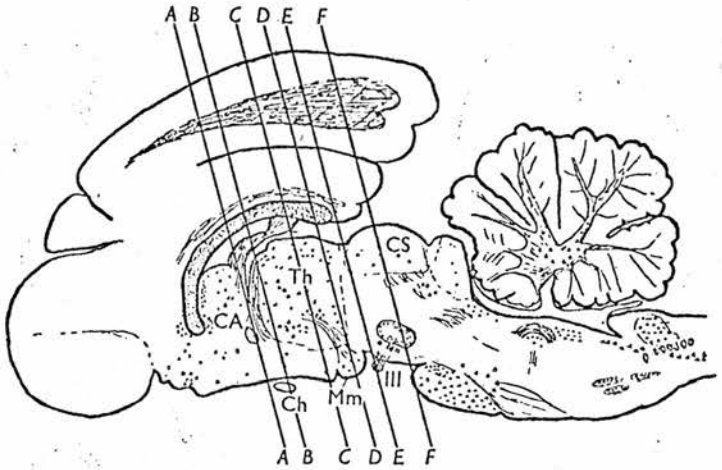

Fig. 3. Diagrammatic paramedian sagittal section of cat's brain indicating levels of coronal sections in Figs. 4 and 5. CA, anterior commissure; Ch, optic chiasma; CS, superior colliculus; Mm, mammillary body; Th, thalamus; III, oculomotor nerve. Section reproduced by permission of Editor of *Brain*.

Thus, the excitable zone in the hypothalamus consists of a narrow strip in the anatomically undifferentiated grey matter on both sides of the mid line, and extending through the whole length of the hypothalamus. In the tuberal region, where the largest responses are obtained, a lateral extension of the zone is apparent. A little more posteriorly, in the region of the mammillary bodies, this leads to a small, sharply localized area just dorsal to the cerebral peduncle, from which large responses are obtained.

Localization in the mesencephalon

An exploration was made posterior to the mammillary bodies, as far back as the intercollicular level, in 22 acute experiments and in 7 experiments with chronically implanted electrodes. As illustrated in the diagrammatic sections *E* and *F* of Fig. 5, the most readily excitable regions were in the tegmentum ventral to the superior colliculus (explored in 9 experiments), in the dorsolateral part of the central grey matter (explored in 10 experiments) and in a restricted area dorsal to each cerebral peduncle (explored

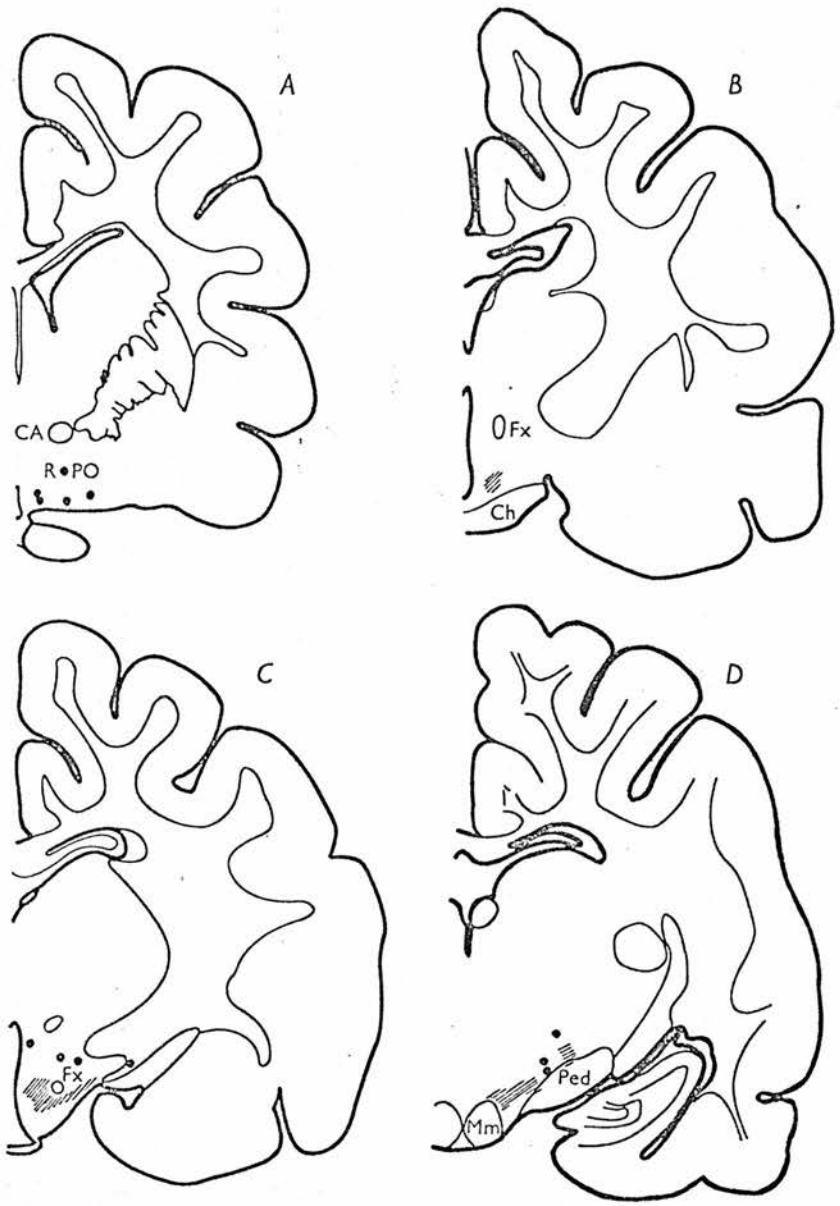

Fig. 4. Diagrammatic coronal sections showing regions in cat's hypothalamus from which active muscle vasodilatation is obtained on electrical stimulation. Hatching indicates regions from which vasodilatation regularly obtained. Dots indicate sites of large responses obtained in individual experiments. Sections *A*, *B*, *C* and *D* are at levels indicated in Fig. 3. Results are included from explorations in planes 1 mm anterior and posterior to each level of section. CA, anterior commissure; Ch, optic chiasma; Fx, fornix; Mm, mammillary body; Ped, cerebral peduncle; RPO, pre-optic area.

in 14 experiments). From these three regions, which appear to be well defined, large vasodilator responses were regularly obtained. Stimulation of the tegmentum between these regions sometimes also produced large vasodilator responses, but the excitable points were scattered and more difficult to find, and they varied from one animal to another. In Fig. 5*E* and *F* the dots indicate the points from which vasodilator responses were

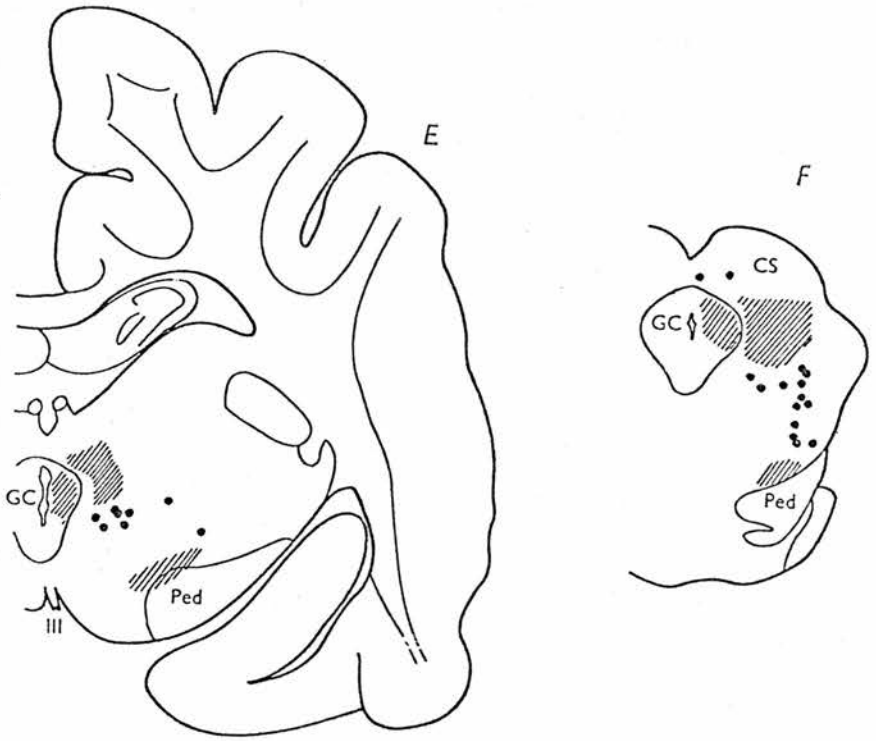

Fig. 5. Diagrammatic coronal sections showing regions in mesencephalon from which active muscle vasodilatation is obtained on electrical stimulation. Hatching indicates regions from which vasodilatation regularly obtained. Dots indicate sites of large responses obtained in individual experiments. Sections *E* and *F* are at levels indicated in Fig. 3. Results are included from explorations in planes 1 mm anterior and posterior to each level of section. CS, superior colliculus; GC, central grey matter; Ped, cerebral peduncle.

obtained in one or another experiment. These points may well be the sites of tracts which connect the central grey matter and tegmentum with the peduncular region.

At the level of the habenular nucleus, vasodilator responses could be elicited on stimulation of points in the pre-tectal region, presumably an anterior extension of the excitable region in the tegmentum ventral to the superior colliculi.

The separation of the three main regions is based not only on the ease with which the largest vasodilator responses were obtained, but also on differences of the associated autonomic effects. The effects produced from the central grey matter resembled those seen on hypothalamic stimulation: there was pronounced pilo-erection, pupillary dilatation, retraction of the nictitating membranes, and a moderate rise in arterial blood pressure and heart rate. The response to stimulation in the tegmental region was characterized by a particularly large rise of arterial blood pressure, often exceeding 200 mm Hg and associated with bradycardia. On stimulation of the peduncular region the responses differed from those of the other two regions, in that the associated effects were minimal: pilo-erection and retraction of the nictitating membranes were hardly ever seen and arterial blood pressure either fell or rose only very slightly.

Effect of mesencephalic lesions on active muscle vasodilatation produced by hypothalamic stimulation

The fact that stimulation of the peduncular region resulted in a large vasodilatation with few of the other accompanying autonomic effects suggested that this region, already seen at the level of the mammillary bodies, and extending through the mesencephalon in the same position relative to the cerebral peduncles, is the main efferent pathway to the medulla for the muscle vasodilatation. Direct evidence for this view was obtained by examining the effect of discrete mid-brain lesions on the vasodilator response to hypothalamic stimulation.

In these experiments a stimulating electrode was introduced into the excitable region at the tuberal level of the hypothalamus and fixed at a point from which large vasodilator responses were obtained. Two lesion electrodes were then introduced into the mesencephalon. As this pair of electrodes was racked down, electrolytic lesions were made at all points on each track where vasodilator responses could be obtained. The adequacy of each lesion was tested by passing the stimulating current again. If a vasodilator response was still obtained, the lesion was extended. Histological examination showed most of the lesions to be less than 1 mm in diameter.

After each lesion had been made the effectiveness of stimulating the hypothalamic point was tested. In only one experiment did the response to hypothalamic stimulation remain greatly reduced after a tegmental lesion had been made, and in this case the lesion electrode had produced a large haemorrhage occupying the whole of one colliculus and the underlying tegmentum. In 6 other experiments, even after multiple lesions in the superior colliculi, the tegmentum underlying them and the central

grey matter, the response to hypothalamic stimulation was much the same as it had been at the beginning of the experiment. There was often a transient reduction of the response, but this lasted for a few minutes only. Results from an experiment are shown in Fig. 6. The mid-brain section at the top shows some of the lesions made in four electrode tracks. Each track extended through the whole tegmentum, the lesions being made at all points where vasodilator responses were obtained. At the bottom are shown the vasodilator response to hypothalamic stimulation before (*a*) and 1 min after (*d*) a tegmental lesion had been made. The lesion was made at the upper end of the section of the track seen just to the right of the central grey matter. Stimulation at this point produced strong vasodilator and pressor responses before the lesion (*b*) and practically no response after it (*e*). Althogether 9 lesions were made during the course of this experiment and after all of them the hypothalamic response remained substantially the same. In striking contrast with the results of experiments such as this, a single small lesion restricted to the peduncular region from which vasodilator responses were obtained always abolished the hypothalamic response in 3 consecutive experiments. A typical result is shown in Fig. 7, in which the hypothalamic response was abolished by the lesion seen in the section.

Stimulation of the brain stem in the conscious cat

Defence reactions are obtained in conscious cats on stimulation of a well-defined region in the hypothalamus (Hess & Brügger, 1943). Similar reactions have been obtained from a localized region of the central grey matter (Hunsperger, 1956) and from points deep in the colliculi (Spiegel, Kletzkin & Szekely, 1954). From our experiments in anaesthetized cats the regions concerned appeared to be identical with those from which active muscle vasodilatation is most readily obtained. This identity was demonstrated in experiments in which electrodes were implanted in the hypothalamic and mid-brain areas which had been located on the basis of the vasodilator responses. Except in those animals with electrode implanted in the peduncular region, subsequent stimulation in the conscious cat gave a fairly constant pattern of behaviour. Threshold stimulation produced an alerting, or orientation, reaction. The resting animal would raise its head and look around, with moderate pupillary dilatation. As the voltage was increased, the animal would stand up with increasing pupillary dilatation and pilo-erection of the tail and back. It then began to hiss and snarl, with ears flattened and claws unsheathed. Finally, the cat would move rapidly around the observation chamber or would suddenly attack, particularly in response to a visual or auditory stimulus. The optimal stimulation parameters were the same as those for muscle vaso-

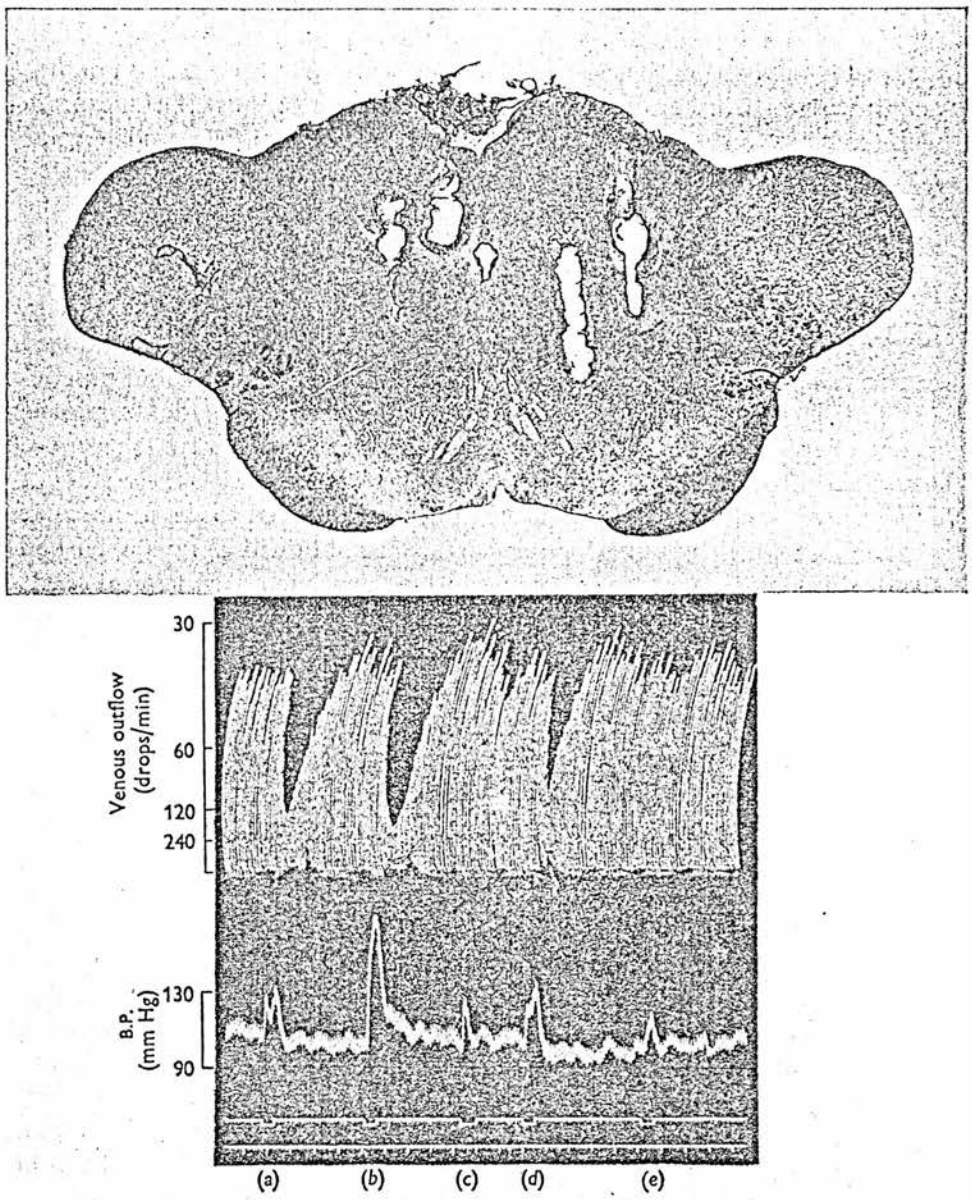

Fig. 6. Top: photomicrograph (coronal section) of cat's brain at level of superior colliculus, showing electrolytic lesions in four electrode tracks. Bottom: records of venous outflow from skinned hind limb and of arterial blood pressure. At (*a*) and (*d*), electrical stimulation of same hypothalamic point. At (*b*) and (*e*) stimulation at same point in mesencephalic tegmentum, at top of electrode track just on right of central grey matter. At (*c*) electrolytic lesion made at this mesencephalic point. Time marker, 30 sec.

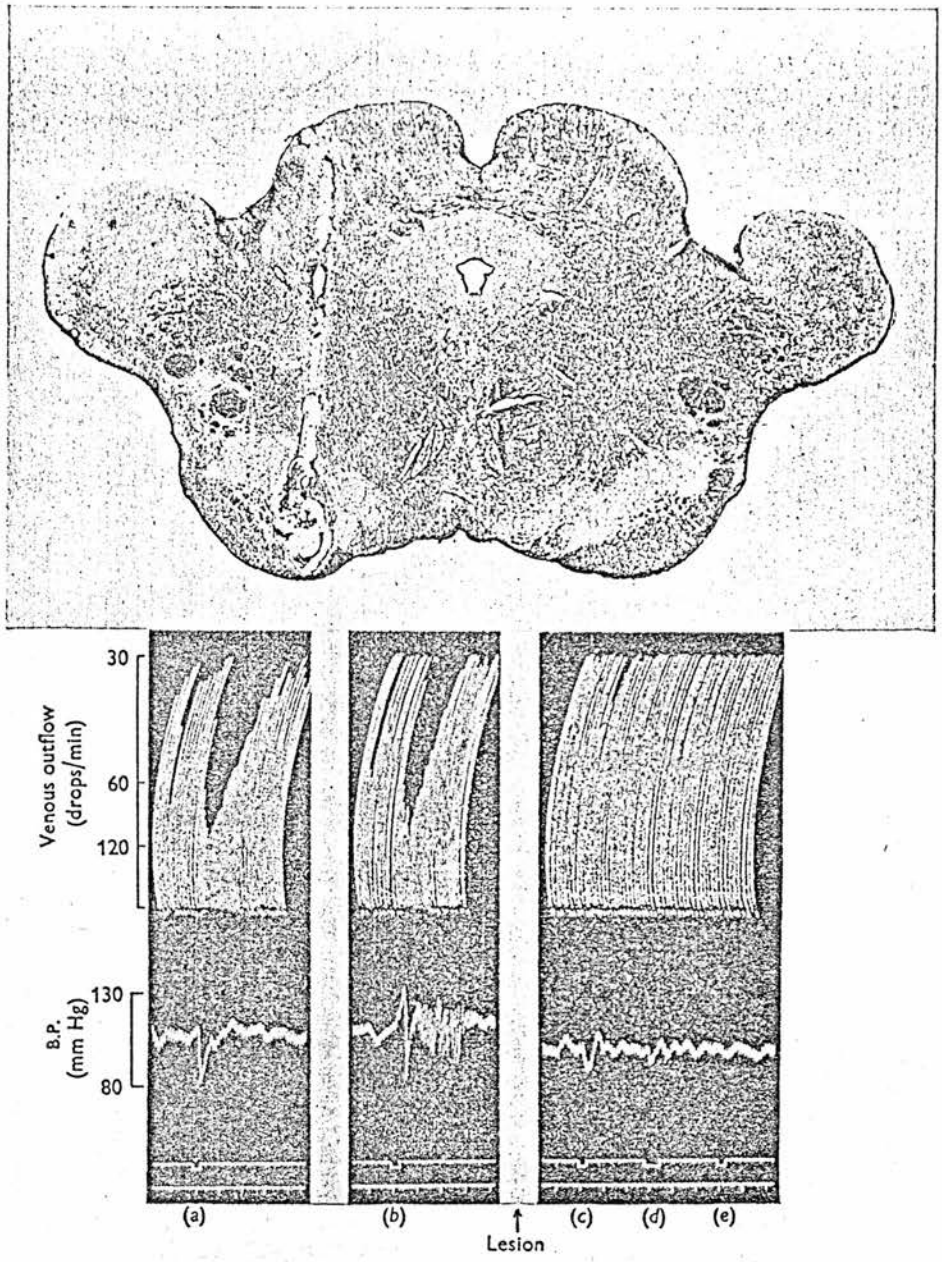

Fig. 7. Top: photomicrograph (coronal section) of cat's brain at level of superior colliculus, showing single electrolytic lesion in substantia nigra and cerebral peduncle. Bottom: records of venous outflow from skinned hind limb and of arterial blood pressure. At (*a*) and (*e*), stimulation of same point in peduncular region. At (*b*), (*c*) and (*d*), stimulation of hypothalamic point. Lesion made at peduncular point between (*b*) and (*c*). Time marker, 30 sec.

dilatation. When these animals were anaesthetized with chloralose and stimulation was repeated, large vasodilator responses were obtained.

The records shown in Fig. 8 are from such an experiment. A typical defence reaction had been obtained before anaesthesia when stimulating with the electrode in the pre-optic region, shown in the diagrammatic section. Stimulation under chloralose with the same parameters produced

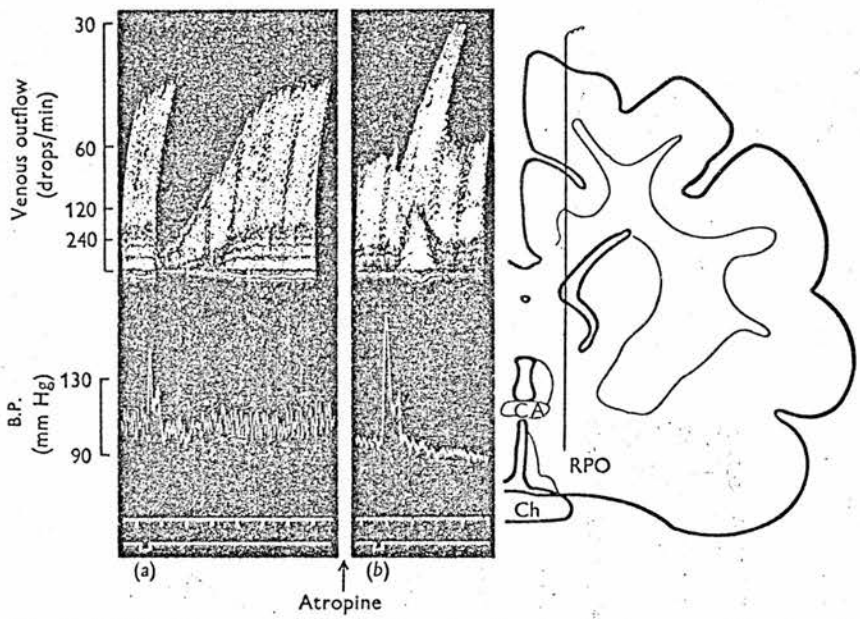

Fig. 8. Right: diagrammatic coronal section showing site in pre-optic region of cat's brain of implanted electrode. Left: records of venous outflow from skinned hind limb and of arterial blood pressure of same cat under chloralose anaesthesia. At signals, stimulation through implanted electrode before (*a*) and after (*b*) intravenous injection of atropine (0·5 mg/kg). CA, anterior commissure; Ch, optic chiasma; RPO, pre-optic area. Time marker, 30 sec.

the large muscle vasodilatation shown at (*a*) and, after an intravenous injection of atropine, the vasoconstriction shown at (*b*). With electrodes implanted in the hypothalamus, central grey matter and subcollicular tegmentum, the cardiovascular response and the behavioural reaction always went hand in hand.

From electrodes implanted in the peduncular region, however, the complex, co-ordinated, behavioural reaction was never obtained. The only responses seen in the conscious cat were either flattening of the homolateral ear, closure of the eye or rolling over on one side, whereas under anaesthesia the typical muscle vasodilatation was obtained.

It is concluded that the excitable regions on the hypothalamus and

central grey matter, and in the tegmentum ventral to the colliculi, function as integrative centres from which the component parts of the defence reaction, including muscle vasodilatation, are activated. These regions are shown as shaded areas in Fig. 9, which was constructed from our results. The areas in the hypothalamus and central grey matter are probably continuous. The posterior borders of the mid-brain areas have been left indefinite, since we have not explored further back in the brain stem, but from the reactions obtained by Keller (1932) and Bard & Macht (1958) with chronic decerebrate cats it appears that the areas might extend even into the pons. The vasodilator outflow from the integrative centres lies in the pathway just dorsal to the cerebral peduncles, indicated in Fig. 9 by the heavy interrupted line.

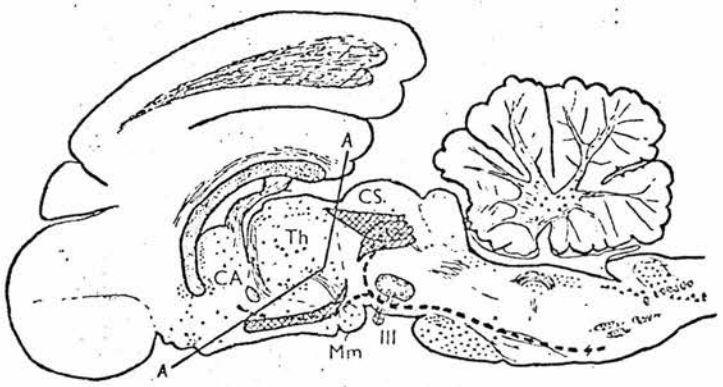

Fig. 9. Diagrammatic paramedian sagittal section of cat's brain. Cross-hatched areas represent regions in hypothalamus, central grey matter and mesencephalic tegmentum apparently functioning as reflex centres for the defence reaction. Heavy interrupted line represents vasodilator pathway connecting the centres with the periphery. *A–A* is the line of section used for decerebration. CA, anterior commissure; CS, superior colliculus; Mm, mammillary body; Th, thalamus; III, oculomotor nerve. Section reproduced by permission of Editor of *Brain*.

Reflex activation of the cholinergic vasodilator fibres to muscle in decerebrate preparations

In common with other reactions integrated at the diencephalic level, the defence reaction cannot be obtained as a reflex response in anaesthetized animals. Even under light chloralose anaesthesia, sciatic nerve stimulation will not produce any of the co-ordinated movements characteristic of the reaction. Of the autonomic responses, only the rise of arterial blood pressure and pupillary dilatation remain. We have never obtained an increase in muscle blood flow, and sometimes even saw a slight decrease.

The results in decerebrate preparations are different. Woodworth & Sherrington (1904) showed that sciatic nerve stimulation elicited a complex

reflex response in cats decerebrated at the pre-collicular level. This response, which they called the pseudaffective reflex, comprised pupillary dilatation, vocalization, snarling, movements of the limbs and a rise of blood pressure. As is seen from Fig. 9, decerebration at the pre-collicular level would leave intact the major part of the integrating region for the defence reaction.

We carried out experiments in which decerebration was started at the pre-collicular level and then extended rostrally to spare as much as possible of the hypothalamus, as indicated by the line on Fig. 9. In these preparations, electrical stimulation of the skin of one forelimb elicited, in addition to the effects described by Woodworth & Sherrington (1904),

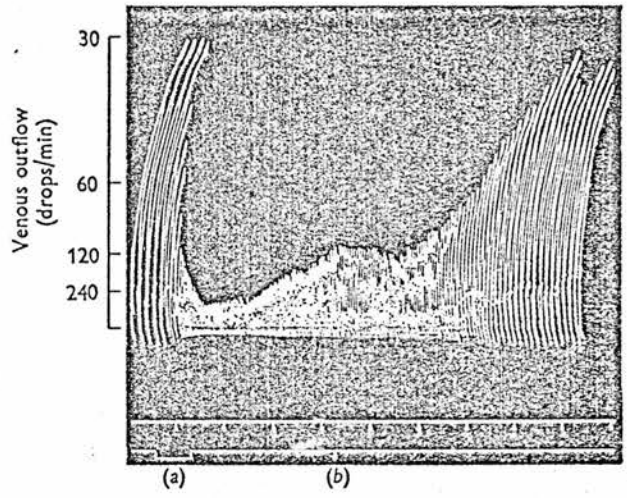

Fig. 10. High-decerebrate cat. Record of venous outflow from gastrocnemius muscle, perfused by donor cat. At (*a*) electrical stimulation of skin of forepaw. 'Spontaneous' reaction occurred at (*b*). Time marker, 30 sec.

arching of the back and pilo-erection. We did not always observe all parts of the reaction. This is in keeping with a similar finding of Cannon & Britton (1925) on partially decorticate cats. In 5 out of 9 such preparations skin stimulation also produced a large and prolonged increase in muscle blood flow. This is illustrated in Fig. 10, which is taken from an experiment in which the gastrocnemius muscle was perfused by a donor cat and its participation in the reflex movements was prevented by tying the spinal cord below L 6. Pseudaffective reactions, including muscle vasodilatation, frequently occurred in the absence of a specific stimulus: an effect on muscle blood flow of such a spontaneous reaction is also illustrated in Fig. 10. In three of the cats a simplified preparation was used in which changes of muscle blood flow were registered indirectly, using an external

venous thermal recorder. In these preparations consistent pseudaffective responses were obtained for an hour or two and the muscle vasodilatation could then be shown to be abolished by small doses of atropine (Fig. 11).

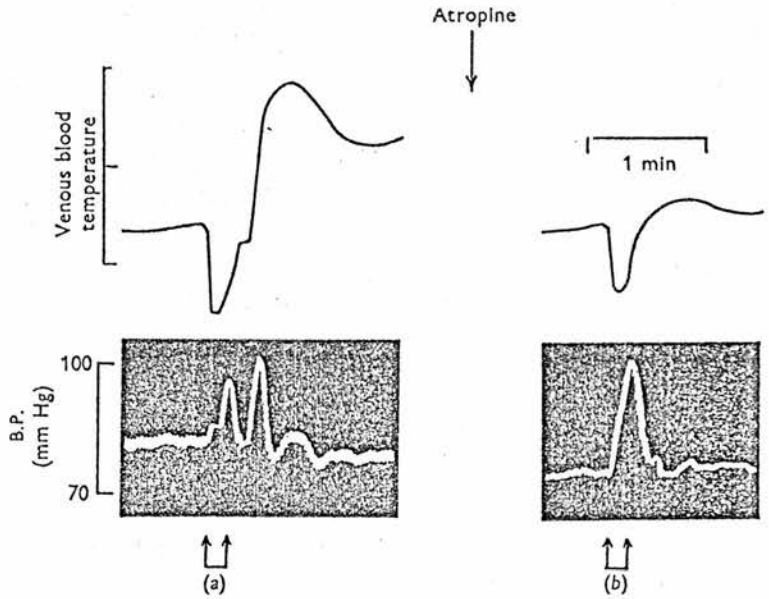

Fig. 11. High-decerebrate cat. Records of temperature of venous effluent from gastrocnemius muscle (upward deflexion signifies increase in venous outflow) and of arterial blood pressure. Each scale division on temperature record indicates 0·05° C. At signals, electrical stimulation of skin before (*a*) and after (*b*) intravenous injection of atropine (0·5 mg/kg).

DISCUSSION

Localization of the areas in the diencephalon and mesencephalon from which the cholinergic vasodilator fibres to skeletal muscle are activated has made it possible to draw several conclusions with regard to the organization of the integrated defence reaction in the brain stem. The hypothalamic area was seen to be strikingly similar to that defined by Hess, from which defence reactions are obtained in the conscious cat (Hess & Brügger, 1943). Indeed, stimulation by electrodes implanted in the 'vasodilator' area by means of the same parameters as those required for optimal vascular effects produced typical defence reactions ending in 'flight' or 'fight'. Even under an anaesthetic electrical stimulation produced all the features of the defence reaction which the preparation was capable of displaying. The pupillary dilatation, retraction of the nictitating membranes, pilo-erection and respiratory changes were almost always observed, and, if the anaesthesia was light, movements of the jaw, limbs

and tail were also seen. It thus appears that the muscle vasodilatation produced by stimulation of the hypothalamic area is really part of the whole defence reaction, which is integrated in this region of the brain stem.

Regions other than the hypothalamus were also found to possess this function. Active vasodilatation in muscle is produced by stimulating those parts of both the mid-brain tegmentum ventral to the colliculi and the central grey matter from which defence reactions are elicited in the conscious animal (Spiegel *et al.* 1954; Hunsperger, 1956). It appears from our results that these three brain-stem areas merge into one another, and we have not found any major functional distinction between them. Some small differences in one or another of the effects obtained from them on electrical stimulation were observed, the most consistent being the much larger rises of blood pressure obtained from the mid-brain tegmentum than from the hypothalamus or central grey matter. These large pressor reactions preceded the changes in regional blood flow, and therefore appear to result from an increase in cardiac output. Such an increased cardiac output, together with the vasodilatation in skeletal muscle and the vasoconstriction in the intestine, are well known components of the cardiovascular response observed during heavy muscular exercise, and can be understood as essential parts of the adjustments by which the circulatory system is prepared for the demands of sudden, severe muscular effort. Without this preparation, such exercise would rapidly entail a great increase in blood flow through all the muscles concerned, which would then have to be followed by widespread haemodynamic changes. It may therefore be of great advantage for the early period of such a response, if the vasodilatation, together with the circulatory adjustments necessary to maintain it, were to occur before the extensive muscular reactions have to take place. The pressor response obtained on stimulation of all these brain-stem areas (Kabat, Magoun & Ranson, 1935) would thus be seen, not as an event primarily concerned with regulation of arterial blood pressure, but as one feature of the defence reaction.

When Lindgren (1955) first showed that muscle vasodilatation could be elicited from points in the mid-brain tegmentum ventral to the colliculi, he concluded that he was stimulating the vasodilator outflow from the hypothalamus. This conclusion was based on an experiment in which the vasodilator response to hypothalamic stimulation was abolished by extirpating the inferior and superior colliculi on the same side. In another experiment undercutting the superior colliculus on the same side, and removing a large part of the subjacent tegmentum, reduced the vasodilator response to between a half and a third. In our experiments very small lesions in the mid-brain tegmentum often reduced the response to hypothalamic stimulation, when tested immediately after the lesion had been

made. However, a few minutes later the response was fully restored. This sensitivity to the acute effects of such lesions probably explains Lindgren's results (1955). By contrast, we found that small lesions restricted to the area above the cerebral peduncles from which vasodilatation could be elicited, regularly and irreversibly abolished the response to hypothalamic stimulation. We conclude that the true vasodilator pathway lies in this peduncular region. To support this conclusion were the additional findings that stimulation of the peduncular vasodilator region under an anaesthetic produced very little in the way of associated autonomic responses, sometimes only muscle vasodilatation with a small fall in blood pressure; and, even more striking, that in the conscious animal it never produced the co-ordinated defence reaction so characteristic of the other regions.

This peduncular vasodilator pathway, though most clearly seen as a separate entity at the mid-brain level, is also discernible in the posterior hypothalamus at the level of the mammillary bodies. As the excitable zones for the defence reaction in the hypothalamus, central grey matter and mid-brain tegmentum all connect with this peduncular pathway, there is every indication that this is the efferent pathway for the vasodilator component of the defence reaction. Thus our results support the hypothesis of a vasodilator pathway, although its course through the diencephalon and mesencephalon is not located as Lindgren *et al.* (1956) had thought. The more caudal extension of the pathway in the medulla was described by Lindgren & Uvnäs (1953) as a narrow longitudinal band lying 3–4 mm from the mid line and 1–2 mm above the ventral surface of the brain stem, and this is probably a direct continuation of the peduncular pathway in the mid-brain.

The pathway for the other cardiovascular effects and for the release of adrenaline and noradrenaline from the adrenals (Grant, Lindgren, Rosén & Uvnäs, 1958) which accompany the muscle vasodilatation, must be close to the vasodilator pathway; for all the cardiovascular effects of hypothalamic stimulation are abolished by the peduncular lesions. This result with such small lesions contrasts greatly with the finding of Magoun, Ranson & Hetherington (1938), who were unable to abolish the pressor responses to stimulation in the lateral hypothalamic area by extensive lesions in the mid-brain tegmentum. They concluded that the efferent pathway for these vasomotor responses is diffused through the whole mid-brain tegmentum. Our results with lesions restricted to the peduncular region show that specific and well-defined pathways do exist.

It is more than likely that the vasodilator points found in the anterior sigmoid gyrus and internal capsule (Eliasson *et al.* 1952) also connect directly with the peduncular pathway. This could provide the basis for activation of the same specific pattern of cardiovascular response in pre-

paration for any movement in which the sensori-motor cortex is involved, quite independently of the defence reaction. Indeed, Rushmer, Smith & Franklin (1959) have found that in conscious dogs electrical stimulation through an electrode implanted just dorsal to the peduncle, at the level of the mammillary bodies, produces a cardiovascular response similar to that of actual exercise without any external sign of distress.

It has been known since the classical descriptions of Goltz (1892) that the chronically decorticate animal readily displays defence reactions. In Goltz's dogs even quite trivial stimuli regularly and repeatedly produced a stereotyped pattern of aggressive behaviour, which was never modified as long as the animal lived. In other words, these behavioural reactions appeared as complex, co-ordinated reflexes. What Goltz (1892) could show in dogs, Bard & Rioch (1937) repeated in decorticate cats. 'Fear' and 'rage' reactions were regularly obtained in four such preparations, including one in which the thalamus and striatum had been removed in addition to the neocortex. Many years earlier, however, the pseudaffective reflex had been described by Woodworth & Sherrington (1904). This reflex response, which shows many features of the defence reaction, was obtained on electrical stimulation of the sciatic nerve in cats acutely decerebrated at the pre-collicular level. The stereotyped nature of this response to nociceptive stimulation enabled Woodworth & Sherrington (1904) to use it to trace the spinal pathways for pain. Their level of decerebration will have spared the mesencephalic section of the integrative region for the defence reaction and perhaps the posterior part of the diencephalic section. In our experiments, in the attempt to spare as much as possible of the whole brain-stem region, decerebration, though started at the same pre-collicular level, was extended further rostrally as the line of section approached the base of the brain. Sciatic nerve stimulation in such preparations produced more features of the defence reaction than were seen by Woodworth & Sherrington (1904). Moreover, we obtained evidence of active muscle vasodilatation which could be abolished by atropine. This is a direct demonstration that the muscle vasodilatation is an integral part of the whole co-ordinated defence reaction.

All these results lead us to conclude that the regions in the diencephalon and mesencephalon in which the response is integrated may be regarded, together, as the reflex centre for the reaction. The centre for the defence reflex would thus extend further caudally than was indicated by Bard's (1928) original decerebration experiments, which seemed to place it in the hypothalamus alone. It probably extends even into the pons; for Keller (1932) obtained typical 'rage' reactions in response to mild stimuli, such as pulling the skin of the back, in cats chronically decerebrated just

rostral to the beginning of the pons; and Bard & Macht (1958) obtained many features of the defence reaction in response to electrical stimulation of the tail, even when decerebration was carried out so far caudally as to remove the rostral part of the pons.

It is known that all the sensory systems have extensive connexions with the mesencephalic region in which we find part of the defence centre to be situated (Dell, 1952; French, Verzeano & Magoun, 1953). Evidence is accumulating that the undifferentiated grey matter of the hypothalamus should be regarded as a forward extension of this region. Of particular relevance is the recent demonstration by Nauta & Kuypers (1958) that fibres originating in the central grey matter and adjacent mid-brain tegmentum, which correspond to the more caudal part of the defence centre, project to the paramedian region of the hypothalamus and pre-optic area, which correspond to the more rostral part. It is therefore not surprising that electrophysiological techniques have shown sensory projections ascending not only to the sub-thalamic region of the diencephalon (Starzl, Taylor & Magoun, 1951; French, von Amerongen & Magoun, 1952), but also the hypothalamus itself (Ingvar & Hunter, 1955; Feldman, Van der Heide & Porter, 1959). Abrahams, Hilton & Malcolm (1959) have found that evoked potentials can be recorded in the cat in all parts of the defence centre in response to cutaneous stimulation. Responses to light flashes are almost as extensively found, and responses to clicks a little less so. In nature the full defence reaction is readily produced by nociceptive stimuli; but a sudden, strong stimulus of any modality will first evoke the alerting, or orientation reaction, which may develop into the full defence reaction if stimulation is sufficiently intense, and this is just the sequence of events seen on direct electrical stimulation of the defence centre. Hence the anatomical and electro-physiological evidence is in complete accord with the concepts put forward of a high-level centre for a complex behavioural reflex, elicited most readily by noxious stimuli but capable of reflex initiation by stimuli of all modalities. It is striking that the defence centre occupies such a large part of what has become known as the reticular activating system; for this emphasizes once again that, far from being merely a diffuse, non-specific system, a large part of this region of the mesencephalon and diencephalon has specific functions in the normal, unanaesthetized animal.

SUMMARY

1. In anaesthetized cats regions have been mapped in the hypothalamus, mesencephalic tegmentum and central grey matter from which active, atropine-sensitive muscle vasodilatation is elicited on electrical stimulation.

2. These three brain-stem regions are not merely 'vasodilator' areas, for the muscle vasodilatation is accompanied by other autonomic effects including a rise in arterial blood pressure, vasoconstriction in skin and intestine, pupil dilatation, retraction of the nictitating membranes and pilo-erection, by respiratory changes and movements of the jaw, limbs and tail.

3. Stimulation of all three brain-stem regions in the conscious cat through implanted electrodes produces co-ordinated defence reactions. It is concluded that the muscle vasodilatation is one component of the integrated response.

4. An additional, restricted region has been located just dorsal to the cerebral peduncles, stimulation of which produces active muscle vasodilatation in anaesthetized cats, and, in conscious cats, single or unorganized movements.

5. Lesions in the region dorsal to the cerebral peduncles abolish the vasodilator response to hypothalamic stimulation. It is concluded that the pathway for muscle vasodilatation runs in this peduncular region.

6. In the high-decerebrate cat, cutaneous stimulation reflexly produces many of the features of the defence reaction, including active muscle vasodilatation. It is concluded that the regions of the hypothalamus, mesencephalic tegmentum and central grey matter function as reflex centres for the co-ordinated autonomic and behavioural responses that comprise the defence reaction.

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Fifth International Congress of Electroencephalography and Clinical Neurophysiology

Rome - Italy, 7-13 Sept., 1961

78. HILTON S.M., ABRAHAMS V.C. and MALCOLM J.L. (London, Great Britain) The significance of afferent collaterals to the central grey matter, mid-brain tegmentum and hypothalamus in the reflex activation of the defence reaction

The defence reaction is a complex behavioural response which includes both visceral and somatic motor activity, and whose outward signs range from alerting to the fully fledged reactions of flight or attack. Regions of the hypothalamus and mid-brain have been defined from which the reaction is obtained on electrical stimulation (Hess and Brügger, 1943; Hunsperger, 1956), and these regions appear to be reflex centres for the response (Woodworth and Sherrington, 1904; Abrahams, Hilton and Zbrozyna, 1960a, b).

The afferent connections to these regions have recently been examined in cats anaesthetized with chloralose, by means of the evoked potential technique (Abrahams, Hilton and Malcolm, 1959). Using auditory, cutaneous and visual stimuli, evoked potentials were recorded in all parts of the centre for the defence reflex, and in surrounding regions of the brain-stem. The evoked potentials exhibited characteristics usually ascribed to activation of the collateral afferent, or extralemniscal, system (Starzl, Taylor and Magoun, 1951b; French, Verzeano and Magoun, 1953a, b; Ingvar and Hunter, 1955). These potentials have long latencies, they can survive the removal of the cerebral cortex in acute experiments, but are abolished by small doses of barbiturates. Potentials evoked by electrical stimulation of widely separated areas of skin and by illumination of separate, restricted areas of the retina can be recorded from a single recording site. Indeed, there is a remarkable degree of convergence in the afferent pathways to the defence centre, both within a single sensory modality and between different modalities.

Most of the regions in the hypothalamus and mid-brain that we have identified as being specifically concerned with the reflex mediation of the defence reaction (Abrahams, et al. 1960b) have been considered merely as part of the reticular activating system (Lindsley, Bowden and Magoun, 1949; Starzl, Taylor and Magoun, 1951a). This has led in the past to emphasis being placed on the role of these brain-stem regions in 'arousing' the cerebral cortex. Apart from such activating effects on the cortex of the 'collateral afferent system', it is important to emphasize the specific role of these regions in the brain-stem as the centres of high-order and complex reflexes such as the defence reaction. It has been well established that, in chronic decorticate cats and dogs, the defence reaction remains intact and is regularly obtained as a stereotyped reflex response (Goltz, 1892; Bard and Rioch, 1937). The same applies to alimentary and sexual reflexes which are also intact in such decorticate animals. As in the defence reaction, the afferent limb of these other high-order reflexes is almost certainly what has been called the collateral afferent system.

BLOCKING ACTION OF DECAMETHONIUM AT DIFFERENT SITES IN THE AUTONOMIC NERVOUS SYSTEM OF THE CAT

BY

V. C. ABRAHAMS and S. M. HILTON

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BLOCKING ACTION OF DECAMETHONIUM AT DIFFERENT SITES IN THE AUTONOMIC NERVOUS SYSTEM OF THE CAT

BY

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Doses of decamethonium sufficient to paralyse skeletal and respiratory muscles in the cat for 20 to 30 min can reversibly block transmission at several sites in the autonomic nervous system. The sympathetic vasodilator outflow to skeletal muscle was blocked at the post-ganglionic nerve endings, probably by preventing the release of acetylcholine. The effects of vagal stimulation on heart-rate and intestinal contraction were blocked in most experiments, possibly by an action on pre-ganglionic as well as post-ganglionic nerve endings. However, decamethonium did not block all cholinergic nerve endings—for example, it did not diminish either the effects of stimulation of the chorda tympani on the submandibular salivary gland or those of pelvic nerve stimulation on the bladder.

Decamethonium iodide is extensively used as a neuromuscular blocking agent in animal experiments. It was being used for this purpose, during experiments on the activation of cholinergic vasodilator nerves to skeletal muscle in decerebrate cats (Abrahams, Hilton & Zbrozyna, 1960), when it was found that the dose used to paralyse skeletal and respiratory muscles for 20 to 30 min also abolished the action of the vasodilator nerve fibres. Further experiments have shown that these doses of decamethonium affect transmission at a number of sites in the autonomic nervous system of the cat.

METHODS

The experiments were performed on cats anaesthetized with chloralose (70 mg/kg) given intravenously after the induction of anaesthesia with ethyl chloride and ether.

Regional blood flows were registered by the venous outflow technique using a transistorized drop recorder. The preparations for muscle, skin and submandibular salivary gland blood flow were those previously described (Hilton & Lewis, 1955; Abrahams, Hilton & Zbrozyna, 1960). Salivary secretion was recorded from the cannulated duct of one submandibular salivary gland: the gland was stimulated via a fluid electrode attached to the cut chordo-lingual nerve. The heart rate was recorded by means of a Statham strain gauge transducer connected to a cannula in one femoral artery, records being made with a high-speed pen recorder.

Records of intestinal activity were made *in situ* by introducing a small rubber bag into a loop of small intestine through a small slit. About 1 ml. of normal saline was introduced into the rubber bag, which was then connected by polythene tubing to a Statham strain gauge. One vagus nerve was dissected free in the neck, divided, and the peripheral stump placed on platinum stimulating electrodes and immersed in liquid paraffin. Stimuli were supramaximal square waves, 10/sec, with a pulse width of 1 msec.

To record bladder pressures, the urethra was exposed and cannulated with wide bore polythene tubing. A few ml. of normal saline were introduced into the bladder, and the cannula was connected to a Statham strain gauge. The pelvic nerve was dissected free on one side, and the cut distal end introduced into a fluid electrode for electrical stimulation.

Localized regions of the hypothalamus were stimulated through monopolar steel electrodes placed stereotactically, as previously described (Abrahams, Hilton & Zbrozyna, 1960).

In some cats the spinal roots L6, L7 and S1 were sectioned aseptically under pentobarbitone anaesthesia. In the final experiment carried out 10 to 14 days later, the sciatic nerve was stimulated via platinum electrodes mounted in a perspex cuff. The stimuli were supramaximal square waves at 32/sec, pulse width being 10 msec.

Decamethonium was given as the iodide, and gallamine as the triethiodide (Flaxedil). All doses are given as the salt.

RESULTS

In 5 cats the cholinergic vasodilator nerves to skeletal muscle were activated by localized electrical stimulation of brain stem regions concerned with the defence

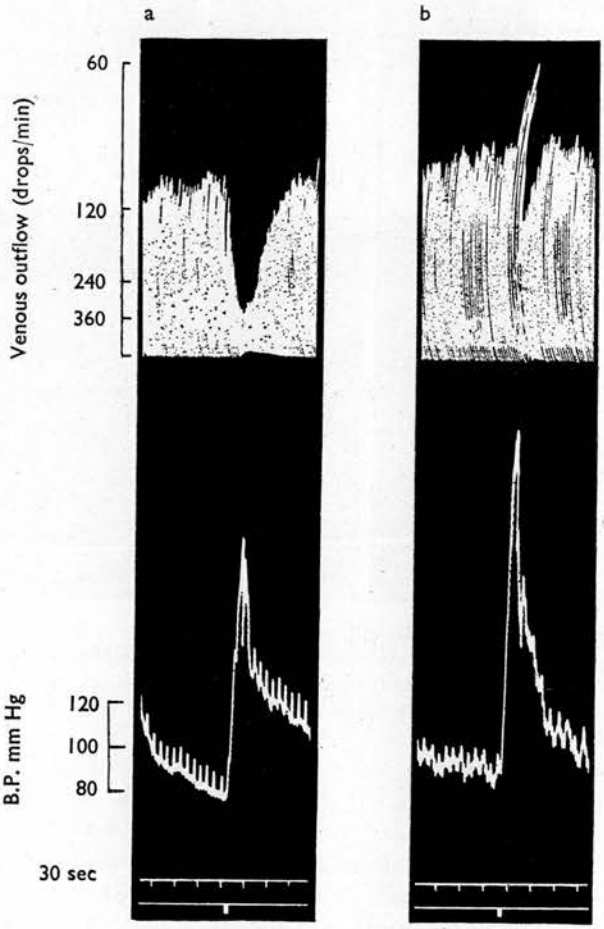

Fig. 1. Cat, 3 kg: records of venous outflow from skinned hind-limb (Gaddum drop-timer) and arterial blood pressure. Effect of electrical stimulation at a point in tegmentum ventral to superior colliculus before (*a*) and after (*b*) decamethonium (100 μg/kg) injected intravenously.

reaction (Abrahams, Hilton & Zbrozyna, 1960). The muscle vasodilatation was abolished in 3 cats by the intravenous injection of 100 μg/kg of decamethonium, as shown in Fig. 1. In the 2 other cats this dose reduced the vasodilatation, which, however, was abolished by administering further doses.

The initial injection of decamethonium itself caused a small dilatation of the muscle blood vessels. In the 2 cats in which a single dose of decamethonium did not abolish the vasodilatation produced by brain stem stimulation, it was found that subsequent injections of decamethonium still produced an increase in muscle blood flow. When a dose level had been reached at which further injection of decamethonium no longer had a vasodilator effect, brain stem stimulation also no longer produced a vasodilatation. In one cat this required a dose of 500 μg/kg.

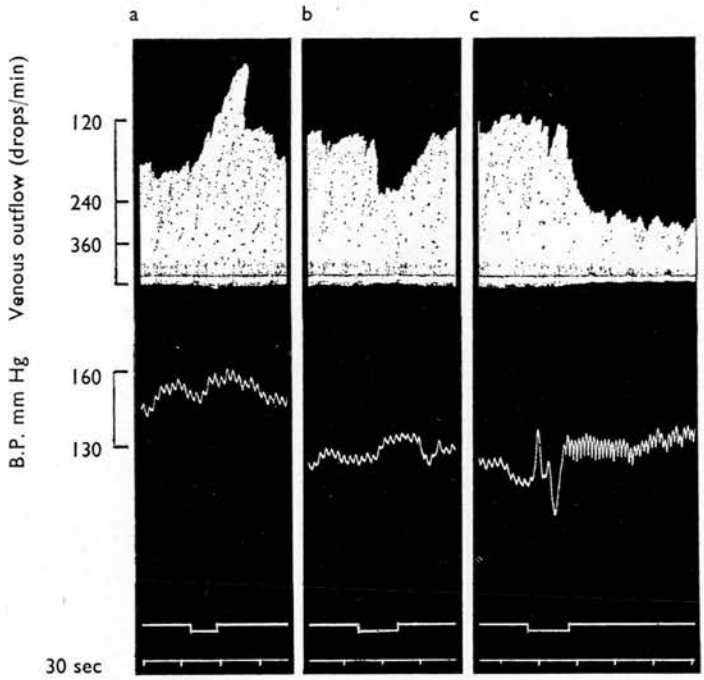

Fig. 2. Cat, 3.6 kg: records of venous outflow from gastrocnemius muscle, and of arterial blood pressure, 14 days after section of spinal roots L6–S1. Effect of sciatic nerve stimulation before (*a*) and after (*b*) 1(N-ethyl-N-2-bromoethylaminomethyl)naphthalene, SY/28 (100 μg), injected arterially. At (*c*) effect of decamethonium (100 μg/kg), injected intravenously.

Electrical stimulation of the regions of the brain stem which produce active muscle vasodilatation also elicits a number of other autonomic effects. These include vasoconstriction in skin, a rise in arterial blood pressure, pupil dilatation, retraction of the nictitating membrane, and pilo-erection. None of these responses was affected by the doses of decamethonium used. It therefore seemed likely that decamethonium was exerting its effect not on the muscle vasodilatation centrally, nor at the autonomic ganglia, but rather at the vasodilator nerve endings. This was confirmed by experiments in which the post-ganglionic vasodilator nerve fibres were stimulated

directly, along their course through the sciatic nerve. The spinal roots L6 to S1 had been sectioned 10 to 14 days previously, so that sciatic nerve stimulation only excited sympathetic nerve fibres to the muscles of the lower leg. The action of vasoconstrictor fibres as seen in Fig. 2*a* was blocked by two different drugs, in 5 experiments by guanethidine and in 2 experiments by 1(N-ethyl-N-2-bromo-ethylaminomethyl)naphthalene (SY-28). The latter was given by intra-arterial injection after which sciatic nerve stimulation then produced a vasodilatation as seen in Fig. 2*b*. Guanethidine was given either intravenously in a dose of 3 mg/kg, or by local intra-arterial injection in a dose of 30 μg/kg ; in either case, paralysis of the vasoconstrictor endings was complete after about 20 min.

When decamethonium was injected after one of these two drugs, it caused a prolonged vasodilatation (Fig. 2*c*). This vasodilatation gradually disappeared over a period of about 20 min. It was then possible to test again the vasodilator effect of sciatic nerve stimulation. This was usually blocked for a further period of 20 min (Fig. 3*b*).

There are similarities between some of the effects of decamethonium on the vasodilator fibres, and its effects on the neuromuscular junction. On intravenous

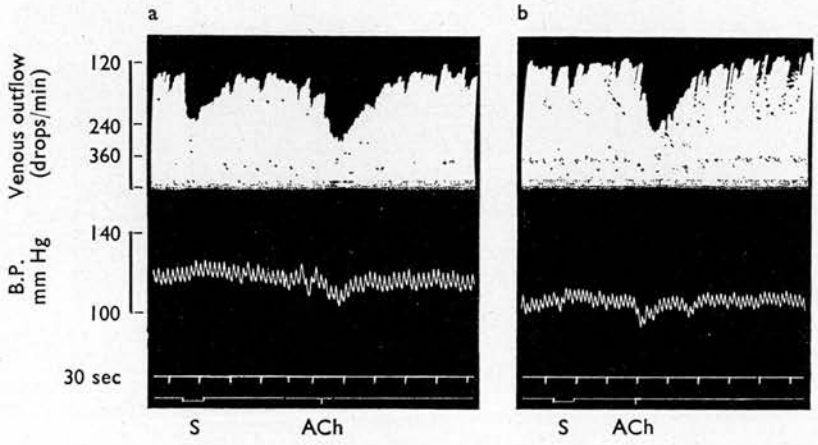

Fig. 3. Cat, 2.25 kg: records of venous outflow from gastrocnemius muscle and of arterial blood pressure, 14 days after section of spinal roots L6–S1. Guanethidine (100 μg) administered intra-arterially. Effects of arterial injection of 0.1 μg acetylcholine (ACh) and of sciatic nerve stimulation (S) before (*a*) and after (*b*) decamethonium 100 μg/kg injected intravenously.

injection, decamethonium causes muscular fasciculation, an excitatory action, before it produces neuromuscular block (Paton & Zaimis, 1949). This is paralleled by the vasodilatation that it produces on injection. Another similarity is found in the antagonism of other blocking agents towards decamethonium. Paton & Zaimis (1959) showed that both curare and gallamine could antagonize the neuromuscular blocking action of decamethonium. We have found that gallamine itself has no action on the vasodilator fibres, but that it can antagonize the action of decamethonium. If an injection of decamethonium is preceded by a paralytic dose of gallamine (2 mg/kg), then the decamethonium is without effect on muscle

vasodilatation (Fig. 4*c*). If time is allowed for the muscular paralysis following these injections to wear off, and a further dose of decamethonium is injected, the vasodilatation is markedly reduced (Fig. 4*d*).

Notwithstanding these findings, the mode of action of decamethonium at the vasodilator nerve endings is not identical with that at the neuromuscular junction,

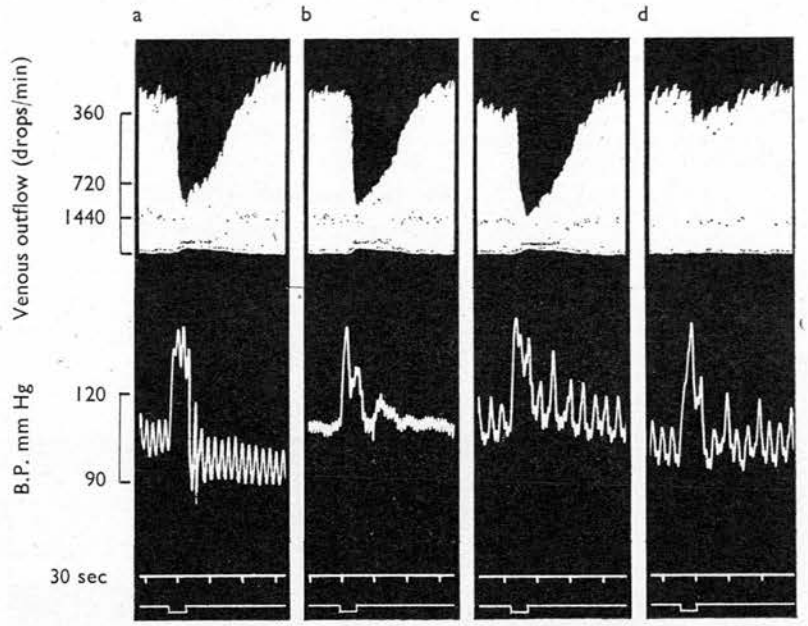

Fig. 4. Cat, 2.8 kg: records of venous outflow from skinned hind limb and arterial blood pressure. Effect of electrical stimulation at a point in hypothalamus. Gallamine (2 mg/kg) injected intravenously between (*a*) and (*b*); followed by decamethonium (100 μg/kg) a few min later between (*b*) and (*c*), and again after all drug effects had worn off, between (*c*) and (*d*).

for in 2 experiments in which the response to nerve stimulation was blocked by decamethonium, an intra-arterial injection of acetylcholine still produced a muscle vasodilatation (Fig. 3*b*).

Effects on the vagus innervation of the heart

Curare and gallamine, as well as blocking neuromuscular transmission, abolish the action of the vagus on the heart (Mautner & Luisada, 1941 ; Bovet, Depierre, Courvoisier & de Lestrange, 1947 ; Jacob & Depierre, 1950 ; Riker & Wescoe, 1951). We found decamethonium to have similar actions, although they were not regularly obtained. In 4 out of 9 cats a single dose of decamethonium of 100 μg/kg abolished the slowing of the heart normally produced by vagal stimulation (Fig. 5). Of the remaining cats, vagal slowing was much reduced in 3, but in 2 cats increasing the dose of decamethonium to as much as 500 μg/kg was without any effect.

Both curare and gallamine are thought to exert their effects on the heart in a manner similar to atropine, since, at the time the response to vagal stimulation is abolished, the slowing due to injected acetylcholine is also abolished.

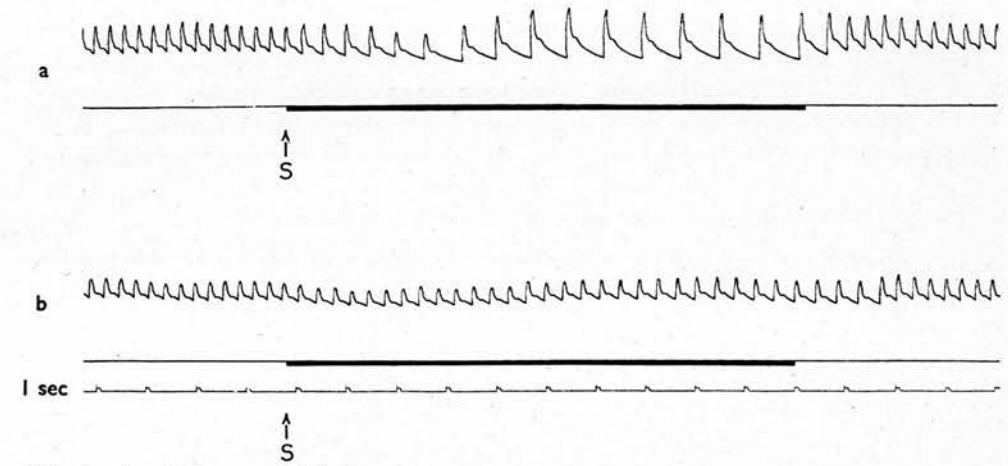

Fig. 5. Cat, 2.5 kg: records of arterial pulse pressure. Effect of vagal stimulation (bar marked S) before (*a*) and after (*b*) decamethonium (100 μg/kg) injected intravenously.

Decamethonium, however, has a variable effect on the cardiac slowing produced by injections of acetylcholine. This effect was examined in 6 cats. In 2 of the cats in which decamethonium had abolished the effect of vagal stimulation, acetylcholine was still effective in slowing the heart. In one cat where decamethonium had reduced the response to vagal stimulation, the effect of injected acetylcholine appeared to be enhanced. In the 3 remaining cats the effect of acetylcholine was much reduced by decamethonium, even though in one of these animals decamethonium had no effect on the response to vagal stimulation.

Effects on contractions of the small intestine in situ

Decamethonium had effects on intestinal activity in 5 out of 7 cats. An injection of 100 μg/kg caused a slowing and then cessation of the spontaneous activity of

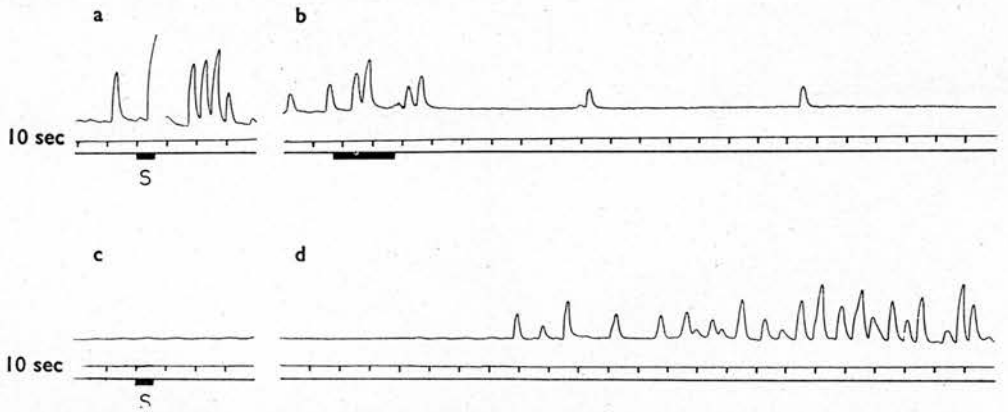

Fig. 6. Cat, 2.5 kg: records of contractions of small intestine *in situ*. Effect of decamethonium (100 μg/kg) injected intravenously at signal in (*b*) on spontaneous contractions and those evoked by vagal stimulation at signal marked S in *a* and *c*. An interval of 6 min elapsed between panel *b* and *c* and of 8 min between *c* and *d*.

the intestine (Fig. 6); and after a few minutes vagal stimulation became ineffective, although acetylcholine still produced contractions. These effects of decamethonium wore off in 4 cats several minutes after spontaneous respiration had reappeared. In the fifth cat spontaneous activity did not return during the remaining 2 hr of the experiment, neither was it possible to elicit contractions by vagal stimulation.

Effects at other sites of cholinergic transmission

The effects of decamethonium so far described are those at sites where transmission is thought to be mediated by acetylcholine. However, this drug does not exert a

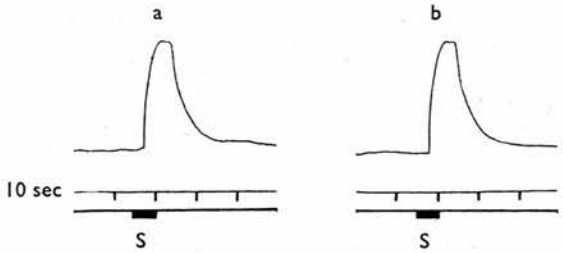

Fig. 7. Cat, 2.4 kg: records of bladder pressure. Pelvic nerve stimulated (S) before (*a*) and after (*b*); decamethonium (100 μg/kg) injected intravenously.

blocking action at all such sites. In 3 experiments we could find no effect of decamethonium on submandibular salivary gland secretion and the increase in blood flow elicited by chorda tympani stimulation. Also, in 4 experiments we were unable to demonstrate any effect of decamethonium on bladder contractions elicited by pelvic nerve stimulation (Fig. 7). In 2 of these experiments, spontaneous increases

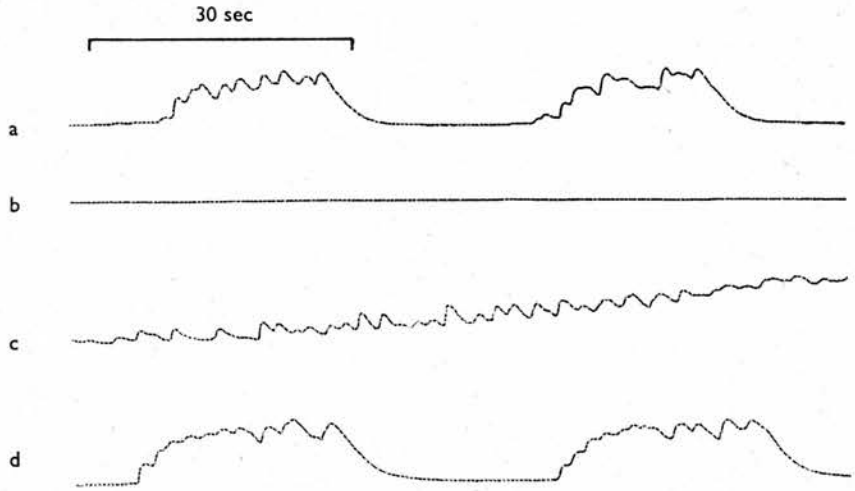

Fig. 8. Cat, 2.8 kg: records of bladder pressure. Effect of decamethonium (100 μg/kg), injected intravenously, on spontaneous contractions. Before (*a*) and immediately following injection (*b*); 17 min after injection (*c*) and 19 min after injection (*d*).

in bladder tone were observed. These were abolished in 1 or 2 min by decamethonium, reappearing 15 to 20 min after the return of spontaneous respiration (Fig. 8).

DISCUSSION

Since the extensive study of Paton & Zaimis (1949 ; 1951) on the polymethylene bistrimethylammonium salts, it has generally been assumed that, whereas those with a carbon chain length of 5 or 6 act predominantly at autonomic ganglia, the blocking action of decamethonium is exerted at the neuromuscular junction. In the cat this was the only action described in response to small doses, and in particular an atropine-like effect was denied (Paton & Zaimis, 1949). The present experiments show that the dose of decamethonium ordinarily used to paralyse skeletal and respiratory muscles of the cat for 20 to 30 min (100 μg/kg) commonly blocks at several sites in the autonomic nervous system.

The block of the sympathetic vasodilator outflow to skeletal muscle is exerted at the endings of the post-ganglionic nerve fibres ; for when these fibres are stimulated directly, their vasodilator action is regularly abolished by decamethonium. While we could not exclude, on the basis of our experiments, a concomitant block of the pre-ganglionic nerve endings, this seemed to be unlikely, for when the muscle vasodilatation was elicited by hypothalamic stimulation as part of a whole pattern of autonomic response, this single vasodilator component was abolished by decamethonium while the others were unimpaired.

The nature of the block of the post-ganglionic endings seems a curious one. The drug " stimulated " before paralysing, in that its initial effect was a vasodilatation. Its blocking action was antagonized by gallamine triethiodide. Thus far, the facts run parallel with those originally described at the neuromuscular junction (Paton & Zaimis, 1949) which led to the hypothesis that the block was due to a persistent depolarization of the muscle end-plates (Zaimis, 1951 ; Burns & Paton, 1951) ; but there are significant differences. We found gallamine triethiodide itself to have no blocking action on the vasodilator nerve endings ; and, secondly, during a decamethonium block, and when vascular tone had returned almost to its previous resting level, acetylcholine injected arterially exerted its usual vasodilator action. The latter finding also rules out competitive block by decamethonium, as was more recently proposed to explain later findings at the neuromuscular junction (Zaimis, 1953 ; Thesleff, 1955a & b). Since virtually nothing is known of the physiology of neuro-effector transmission at the sites of the autonomic system with which we have been dealing, it is particularly hazardous to guess at the implications of our results. The easiest explanation is that decamethonium had blocked the vasodilator nerve-endings by preventing release of acetylcholine.

When we turn to the action of decamethonium on the effect of vagal stimulation on heart-rate, the situation is still more complicated, for the electrical stimuli were being applied to pre-ganglionic fibres. Thus, the finding in some experiments of a block of the effect of nerve stimulation with a persistence of the effect of injected acetylcholine could be explained by an action interfering with transmission from pre- to post-ganglionic fibres. But since, in other experiments, the effect of injected acetylcholine was considerably reduced, some action on neuro-effector transmission

is definitely indicated. This could well be produced in more than one way, that is, by prevention of the release of acetylcholine and by prevention of the action of acetylcholine on the S-A node, one or the other of these two actions being more conspicuous in a particular experiment.

In the small intestine, not only the effect of vagal stimulation, but also the spontaneous activity of the gut was prevented, which may indicate an action on the myenteric plexus. The spontaneous activity of the bladder was also blocked, but without any action on transmission from the pelvic nerve.

Decamethonium can thus act on many different types of neurone: it has even been reported recently to interfere with transmission in the spinal cord of the cat, in a dose of 70 μg/kg, which is smaller than that we used (Fujimori & Eldred, 1961). The present findings show that decamethonium is not the drug of choice to prevent muscular movements in the cat, when studying centrally regulated adjustments of the cardiovascular system, and this consideration may also apply to other animal species. On the other hand, some use might be made of findings which otherwise seem to present unwanted complications; for we now have a means of selectively blocking the vasodilator fibres to skeletal muscle in the cat, leaving the other vasomotor fibres still functional. It would be useful if a similarly selective block could also be obtained in the human subject, in view of the recent interest in a similar nerve supply to muscle blood vessels in man (Blair, Glover, Greenfield and Roddie, 1959; Barcroft, Brod, Hejl, Hirsjarvi & Kitchin, 1960).

All in all, we are far from the simple statement of the mode of action of decamethonium that once seemed possible. The results of these experiments serve to show once more that it cannot be assumed that the effects of a drug are limited to its best-known and most commonly described action.

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SENSORY CONNEXIONS TO THE HYPOTHALAMUS AND MID-BRAIN, AND THEIR ROLE IN THE REFLEX ACTIVATION OF THE DEFENCE REACTION

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It has long been known that the behavioural and autonomic responses characteristic of flight or attack are integrated by structures in the hypothalamus and nearby mid-brain (Cannon & Britton, 1925; Bard, 1928). The responses appear in acutely decerebrated or decorticated cats without any deliberate stimulus being applied, and have been termed 'sham rage'. Bard (1928) mentioned the possibility that the responses might arise in such preparations from afferent impulses set up at wound edges and impinging on the brain-stem structures concerned; but both he and Cannon discussed their findings mainly in relation to Hughlings Jackson's (1898) concept that elimination of higher parts of the brain releases the activity of lower parts. They thus gave the impression that some spontaneous or tonic activity of the brain stem was being revealed in these experiments.

Hess, whose detailed work led to the location of precise brain-stem areas for integration of many basic behavioural patterns, also seemed to regard responses such as flight or attack—which he called the defence reaction—as automatic activities of the hypothalamus which could be inhibited or facilitated from higher parts of the brain (Hess & Brügger, 1943). Yet Woodworth & Sherrington (1904) had observed long before that several components of the whole reaction to nociceptive stimulation in the cat could be obtained as a stereotyped response to stimulation of the sciatic nerve shortly after pre-collicular decerebration. They characterized this as a reflex response and called it the pseudaffective reflex. Recently it has been shown that if section of the brain stem is made at a slightly higher level, so as to spare the hypothalamus, the reflex response then obtained, even in an acute preparation, comprises most of the features of the defence reaction (Abrahams, Hilton & Zbrozyna, 1960*b*). In the chronic decorticate preparation, as first exemplified by Goltz's (1892) dogs, such reactions persist as stereotyped responses to given stimuli, quite unchanged so long

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as the animal survives. Thus, there is every reason to consider these complex, autonomic and behavioural patterns of response as reflexes.

It is surprising, therefore, that, following the discovery of sensory pathways which impinge upon the brain stem (Starzl, Taylor & Magoun, 1951*b*), the role of such pathways has been considered mainly in relation to the concept of the reticular activating system; and their possible significance as the afferent pathways of specific brain-stem reflexes has never yet been seriously considered. This was the hypothesis, however, which led us to carry out experiments to see whether the brain-stem regions which constitute the integrative centre for the defence reaction have the sensory input necessary for them to function as a reflex centre, in the usually accepted sense of this term.

It was clear from previous work that potentials could be evoked in part of the mid-brain structures involved, in response to nociceptive stimulation (French, Verzeano & Magoun, 1953*a*) and flashes of light (Ingvar & Hunter, 1955), and in part of the hypothalamic structures in response to nociceptive stimulation (Dell, 1952; Feldman, van der Heide & Porter, 1959). But a large part of the centre for the defence reaction remained to be explored systematically for responses evoked by different sensory modalities.

In the present experiments, we have found that responses can be evoked in all parts of the integrative centre for the defence reaction to brief electrical pulses applied to the superficial radial nerve or to the skin itself, to single flashes of light to the eye and to single clicks. Thus, the afferent connexions do exist which would enable these brain-stem regions to function as a reflex centre for the defence reaction.

METHODS

Most of the experiments were performed on cats anaesthetized with chloralose in a single intravenous dose of 60 mg/kg, after preliminary induction with ethyl chloride and ether. In two cats pentobarbitone (30 mg/kg) was used instead. The electrodes used for recording were placed by conventional stereotactic methods. The exact electrode positions were determined after fixation of the brain *in situ* by examination of frozen sections of the brain, stained by the Luxol Fast Blue technique of Klüver & Barrera (1953).

Metal micro-electrodes were used, with tip diameters ranging from 1 to 10 μ. They were made from tungsten wire or stainless-steel surgical needles by electrolytic erosion (Hubel, 1957; Green, 1958). The conventional techniques of amplification and recording were used. Auditory stimuli consisted of clicks, generated by passing a square wave through a rochelle-salt crystal, or in later experiments, through a crystal microphone insert. Two sources of visual stimuli were used, either a miniature neon lamp, or a miniature incandescent lamp (L.E.S. 12 V, 0·75 W, Radiospares) driven directly from a stimulator. When the incandescent bulb was used, the flash produced was monitored by a phototransistor placed in close apposition to the bulb. The output from the phototransistor was displayed on one beam of the double-beam oscilloscope. In most experiments the light source was mounted close to one eye, the pupil of which had previously been dilated by the local instillation of a 1 %

solution of atropine sulphate. When small, restricted areas of the retina were to be stimulated, the light source was mounted on a perimeter, and by the use of apertures of varying diameter the angle subtended by the light source could be controlled. The eye being illuminated was secured to a ring by a few stitches in the sclera, and the other eye was occluded by a tin-foil cup.

For cutaneous stimulation single shocks were delivered either to the superficial radial nerve, or through a pair of needles inserted subcutaneously. In some experiments the action potential generated in the superficial radial nerve was monitored by a pair of electrodes placed proximal to the site of stimulation. When cutaneous stimulation led to small reflex muscular movements, either decamethonium iodide (100 μg/kg) or gallamine triethiodide (Flaxedil, 3 mg/kg) was injected and the animal maintained on artificial respiration.

In some experiments the cerebral cortex was removed under chloralose anaesthesia, either by undercutting with a scalpel or by means of a suction apparatus. In all these experiments, arterial blood pressure was continuously monitored by a Statham strain-gauge manometer connected to one femoral artery.

RESULTS

Distribution of evoked potentials

In a previous investigation it had been concluded that certain regions of the hypothalamus, central grey matter and mid-brain tegmentum function as a reflex centre for the defence reaction (Abrahams *et al.* 1960*b*). These regions have been explored in cats anaesthetized with chloralose. All regions were explored in eight cats, the hypothalamus alone in nine, and the mid-brain alone in three.

Electrical potentials were evoked in all these regions by cutaneous, auditory and visual stimuli. This has been demonstrated under other experimental conditions for the posterior hypothalamus, central grey matter and mid-brain tegmentum (Starzl *et al.* 1951*b*; Dell, 1952; Ingvar & Hunter, 1953; Feldman *et al.* 1959). We recorded, in addition, responses from more anterior regions of the hypothalamus, extending into the pre-optic region of the brain stem. Their distribution is illustrated in Fig. 1, in which they are plotted on diagrammatic sections of the pre-optic region (*A*) and at the chiasmatic (*B*) and tuberal (*C*) levels of the hypothalamus. The sections on the right show, for comparison, the region at each level from which the defence reaction is elicited on electrical stimulation. It can be seen that the centre for the defence reaction occupies only a small part of the region from which evoked responses can be obtained. This was found to be true also for the posterior hypothalamus, the central grey matter and mid-brain tegmentum.

Potentials were consistently elicited in all regions explored in response to cutaneous stimulation. In the experiments in which the superficial radial nerve was stimulated directly, the action potential was monitored and it was found that the responses resulted from conduction centrally by nerve fibres of the $A\delta$ group.

Responses to auditory stimuli were only seen in 8 of the 20 experiments,

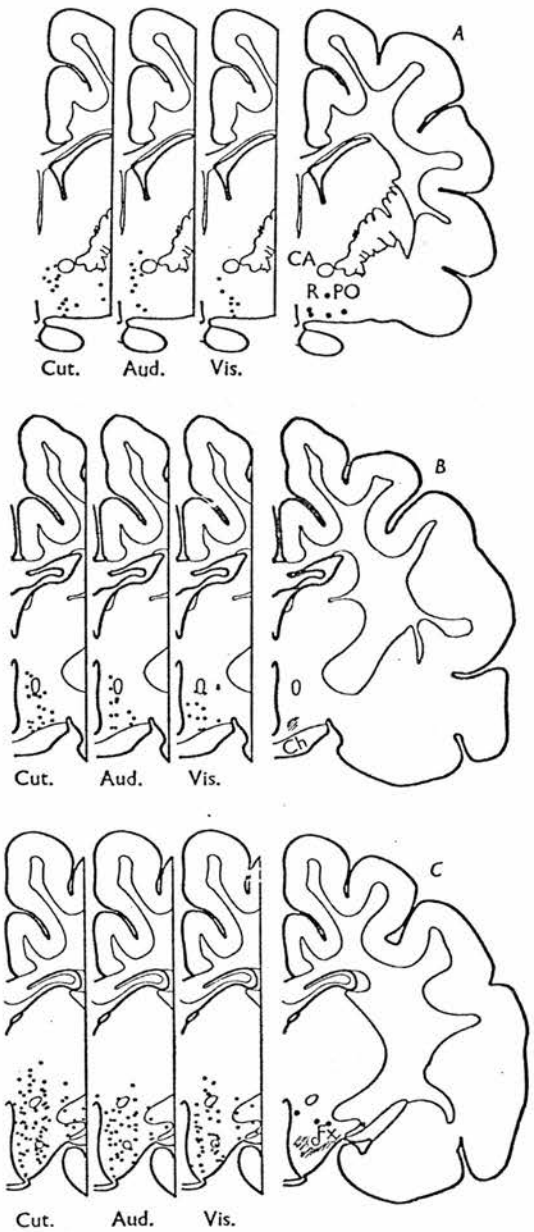

Fig. 1. Diagrammatic coronal sections at three levels of cat's hypothalamus to show points at which evoked potentials have been recorded. *A*, pre-optic region; *B*, level of optic chiasma; *C*, tuberal region of hypothalamus. Extreme right-hand section shows regions concerned with defence reaction (from Abrahams *et al.* 1960*b*), hatched region being that from which responses are regularly obtained and dots indicating sites of responses obtained in individual experiments. The three panels to the left of each semisection show distribution of evoked potentials in response to cutaneous (Cut.), auditory (Aud.) and visual stimulation (Vis.). *CA*, anterior commissure; Ch, optic chiasma; Fx, fornix; RPO, pre-optic area.

but in these 8 experiments the responses were found in all the regions explored. This suggests that the absence of responses in the remaining experiments was due to damage of the middle ear by the ear bars used to fix the head.

Responses were evoked by visual stimuli in all but one experiment. In any single experiment responses were not always seen in all the regions explored, but as shown in Fig. 1, when the results from all 20 experiments were put together, the responses were distributed throughout the centre

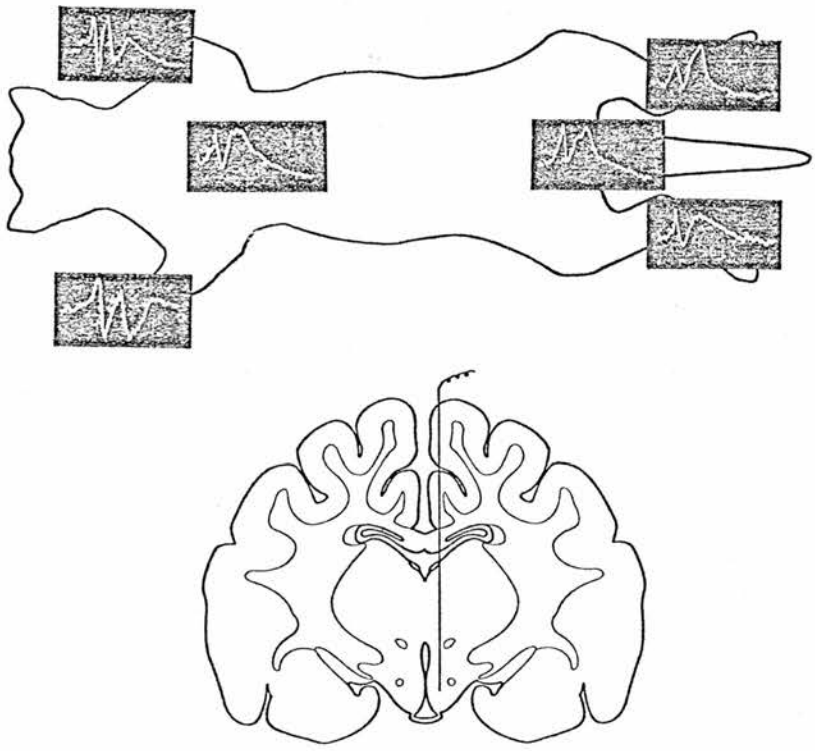

Fig. 2. Potentials evoked by electrical stimulation of different areas of skin. Above: diagram indicating site of cat's skin stimulated, with the potential evoked. Below: diagrammatic coronal section of cat's brain through tuberal region of hypothalamus showing position of recording electrode tip, medial to fornix.

for the defence reaction. The amplitude of these potentials depended on the area of retina illuminated. An increase of the illuminated area from 1 to 10° was sufficient to increase the amplitude of the evoked potential.

The electrical responses obtained by recording from any one point in the brain stem were little altered by changing the position of the source of stimulation. Figure 2 shows the responses recorded at a single point in the hypothalamus on electrical stimulation of six widely separated regions of

the skin, on each of the extremities, at the root of the tail and between the scapulae. Similarly, when small restricted regions (1°) of the retina of one eye were illuminated, as shown on the perimeter diagram in Fig. 3, similar evoked responses were seen at a single recording site whichever retinal region was illuminated.

This evidence of convergence within a single sensory system was complemented by evidence of convergence between systems. The experiments

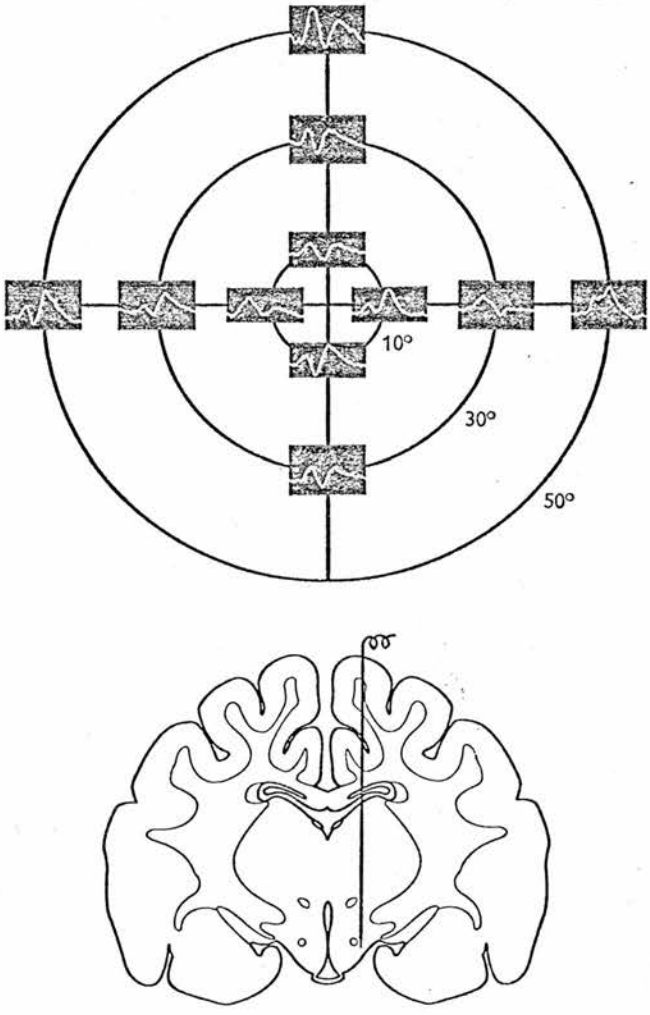

Fig. 3. Potentials evoked by flashes of light, illuminating 1° of retina. Above: perimeter diagram with evoked potentials superimposed on the position of the light source. Below: diagrammatic coronal section of cats' brain through tuberal region of hypothalamus showing position of recording electrode tip, lateral to fornix.

of Starzl *et al.* (1951*b*), Scheibel, Scheibel, Mollica & Moruzzi (1955) and Amassian & Waller (1959) had shown considerable convergence of the sensory pathways which relay into the mesencephalon and diencephalon, and this was readily demonstrated in the present experiments. In four cats the effect was examined of a response evoked by one sensory system upon the response evoked shortly after by a different system. Evidence of interaction between responses was obtained in tests carried out in the

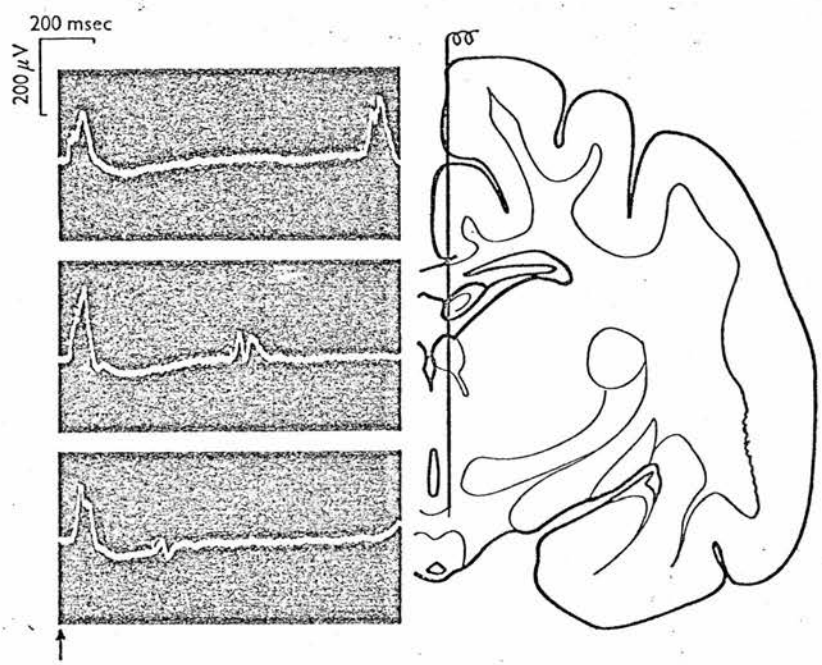

Fig. 4. Interaction of potentials evoked by different stimulus modalities. Potentials recorded dorsal to mammillary body (as shown in diagrammatic coronal section on right), in response to click (at arrow) followed by electrical stimulation of forepaw skin (stimulus shown by artifact).

hypothalamus, superior colliculus, central grey matter and adjacent tegmentum in that the second response was reduced or abolished when the interval between the stimuli was sufficiently short. In Fig. 4 this is shown for a response to cutaneous stimulation following one to a click. When skin stimulation follows the click with an interval of 340 msec between click and cutaneous stimulation, the response to the latter is just distinguishable above the background. In several interaction experiments, when the second response was reduced to this extent, a brief spike of short latency still remained, possibly due to impulses in fibres running near the tip of the electrode.

Characteristics of the evoked potentials

The evoked potentials were complex, of long duration and were considerably affected by the fluctuations of potential which occurred in the absence of deliberate stimulation. The potentials evoked by cutaneous and auditory stimulation were not consistent in form from one animal to another, neither were they related to the location of the electrode tip. Potentials evoked by visual stimulation were more consistent, frequently appearing as a double-peaked negative wave.

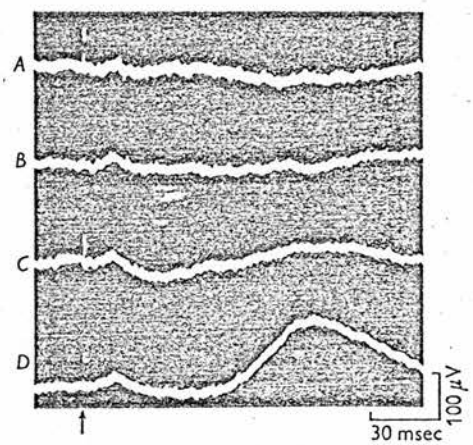

Fig. 5. Appearance of evoked potentials after administration of small doses of chloralose to a cat anaesthetized with pentobarbitone (30 mg/kg). Potentials recorded at single site in hypothalamus in response to shocks delivered to forepaw skin (at arrow). Records taken before (*A*) and after I.V. administration of chloralose, 3·5 mg/kg (*B*), 7 mg/kg (*C*), and 10·5 mg/kg (*D*).

Whereas potentials in lemniscal pathways in the brain stem are relatively resistant to anaesthetics, those in extralemniscal pathways are readily reduced or abolished by agents such as nitrous oxide, ether, pentobarbitone and cyclopropane (French, Verzeano & Magoun, 1953*b*; Randt, Collins, Davis & Dillon, 1958). In two cats anaesthetized with pentobarbitone (30 mg/kg) extensive explorations revealed only occasional, small evoked potentials. Further, in cats under chloralose anaesthesia the potentials were considerably reduced by intravenous injection of 3–4 mg/kg of pentobarbitone. In cats anaesthetized with pentobarbitone, chloralose was found to antagonize the depression of the activity of the extralemniscal system produced by the barbiturate. The successive sweeps of Fig. 5 show the gradual appearance of a response to cutaneous stimulation as small doses of chloralose (3·5 mg/kg) were given intravenously to a cat anaesthetized with pentobarbitone.

This is evidence of a facilitatory effect of chloralose on the neurones of the afferent pathway, but, in addition, these experiments show one of the sites where chloralose exerts a blocking action. Electrical stimulation via electrodes stereotactically placed in the same brain-stem regions readily elicits all the autonomic components of the defence reaction in cats under chloralose. Nevertheless, even following intense cutaneous stimulation in such cats it is not possible to obtain the autonomic components of the defence reaction reflexly (Abrahams *et al.* 1960*b*). It therefore appears that one of the sites of action of chloralose is at the synapses between afferent and efferent pathways in the brain-stem centre for the defence reaction.

Latencies of evoked potentials

In individual animals differences were recorded in the latencies of the responses, according to the site of the recording electrode and the sensory system being activated. The latencies also depended on the polarity of the initial component, being shorter when this was negative-going. In 19 experiments, in which latencies were measured of responses to electrical stimulation of the skin of the fore or hind limbs, the values lay between 6 and 16·5 msec. In most experiments the latencies tended to be shorter in the central grey matter than in the hypothalamus, and so in two cats a large number of observations were made of latencies in both brain-stem regions. Only responses with an initial negative-going component were compared. The mean value for the latency in the central grey matter was shorter than that in the hypothalamus by 3·9 msec ($t = 8{\cdot}696$, $P < 0{\cdot}001$) in one experiment and 6·1 msec ($t = 11{\cdot}05$, $P < 0{\cdot}001$) in the other. The latencies of the responses evoked in the hypothalamus were, of course, longer than those recorded in the somatic sensory cortex. In 2 experiments potentials were recorded simultaneously at a point in the hypothalamus and at a site on the precruciate region of the cerebral cortex where the shortest latencies were found. The latency of the cortical responses was always less than that of the corresponding hypothalamic response, the mean difference being 4 msec in one experiment and 5·1 msec in the other.

The latencies of the responses to auditory stimulation ranged from 10 to 22 msec, there being no consistent difference in the values recorded in the various brain-stem regions. The responses to visual stimulation appeared with longer latencies—from 40 to 50 msec in all regions—except for the hypothalamus where they were as short as 30 msec in one experiment and as long as 68 msec in another.

Evoked potentials after removal of the cerebral cortex and basal ganglia

In four cats under chloralose anaesthesia decerebration was performed at a high level with the intention of removing most of the brain lying

dorsal and anterior to the hypothalamus. The recording electrode was then inserted into the various regions of the brain stem. In all experiments cutaneous stimulation evoked responses in the relevant mid-brain regions: that is, in the central grey matter and the adjacent tegmentum. This is illustrated by the results shown in Fig. 6. Auditory stimulation evoked responses in these regions in only one cat, but in no experiment were responses obtained to visual stimulation. In 3 of the experiments the line of section encroached upon the hypothalamus, and evoked potentials were

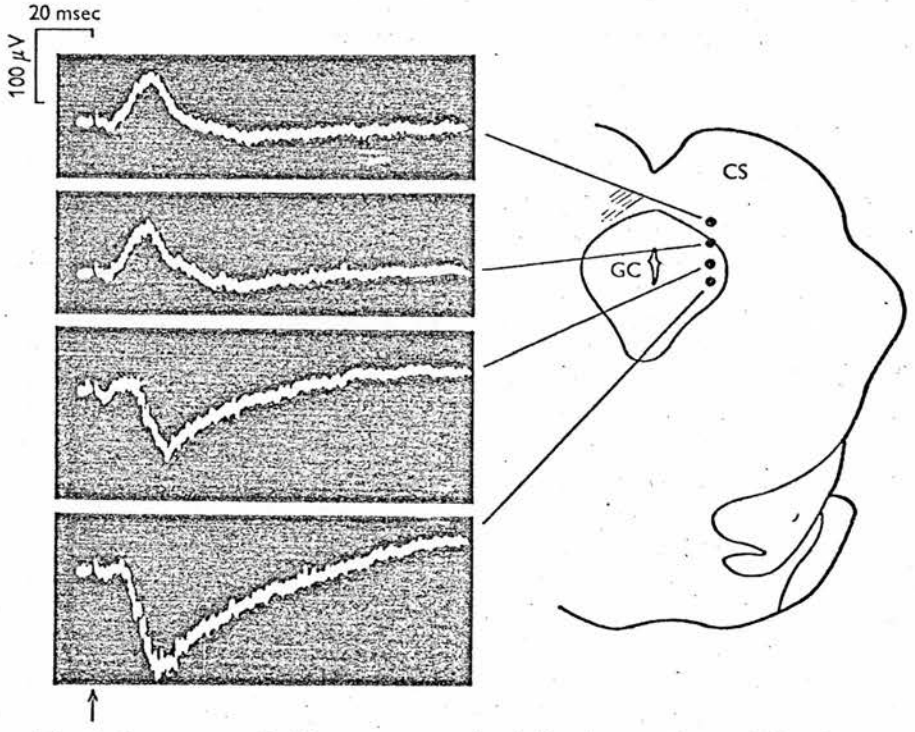

Fig. 6. Responses evoked by cutaneous stimulation (at arrow), recorded at four points in the mid-brain of a cat decerebrated under chloralose anaesthesia. GC, central grey matter; CS, superior colliculus.

not recorded in this region. In the remaining cat the hypothalamus was intact and potentials were evoked in this part of the brain stem by cutaneous stimulation, but not by auditory or visual stimuli. The responses to cutaneous stimulation were recorded from sites both in the tuberal and pre-optic regions, an example from the tuberal region being shown in Fig. 7.

In five cats under chloralose anaesthesia the cerebral cortex was removed in stages by suction. A recording electrode had previously been introduced into the hypothalamic region and evoked potentials obtained. This

electrode was left *in situ* during removal of the cortex. The advantage of this method is that it allows the potential to be followed through successive stages of the decortication, although it is difficult to avoid damage to the brain stem by movements against the electrode, particularly when the cortex in the vicinity of the electrode is being removed. Nevertheless, in every experiment the cutaneous evoked responses survived removal of the pericruciate area and the orbitofrontal cortex anterior to it, regions which include the primary and secondary cortical receiving areas. Similarly,

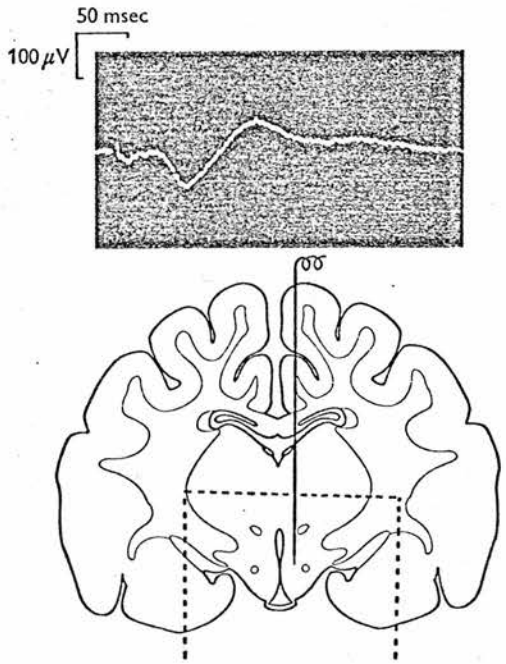

Fig. 7. Above: potential evoked by cutaneous stimulation in hypothalamus of cat decerebrated under chloralose anaesthesia. Below: diagrammatic coronal section through tuberal region of hypothalamus showing position of recording electrode, medial to fornix. The brain dorsal and lateral to the dotted line had been removed before insertion of the recording electrode.

visual responses survived removal of the striate region and a large area of surrounding cortex, including the whole of the occipital lobe. In 2 out of the 5 experiments the response to cutaneous stimulation survived the removal of nearly the whole of the cerebral cortex. In one of these the visual response also survived (Fig. 8): in this experiment only the superficial layers of the cerebral cortex had been removed.

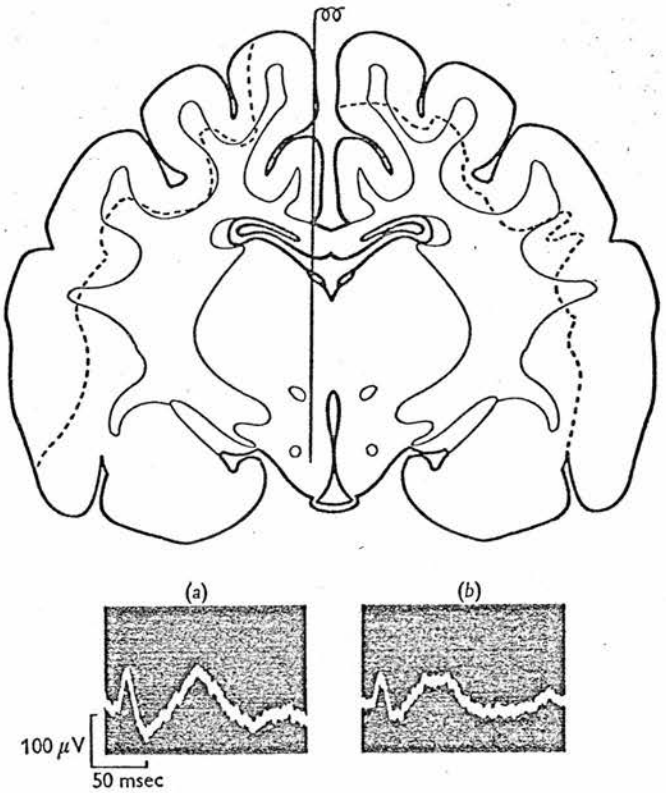

Fig. 8. Potentials evoked by flash of light before and after almost complete removal of superficial layers of cerebral cortex of cat under chloralose anaesthesia. Above: diagrammatic coronal section through tuberal region of hypothalamus showing position of recording electrode, medial to fornix, and extent of removal of cerebral cortex (shown by dotted line) at this level. Below: potentials evoked (*a*) before and (*b*) after decortication.

DISCUSSION

This investigation has been primarily concerned with the afferent pathways by which the defence reaction can be elicited reflexly. The term 'defence reaction' is here used to include not only the fully developed responses of flight or attack, but also the alerting response; for the evidence suggests that the regions in the hypothalamus, central grey matter and mid-brain tegmentum concerned in the integration of all these responses are the same; the responses simply represent different stages in a graded reaction. Hess & Brügger (1943) showed that the response to threshold electrical stimulation of these regions of the brain is alerting, and that if the stimulus is prolonged or its intensity increased the reaction progresses to flight or attack. When the visceral signs of the defence reaction are taken into account, most of them are seen to be fully developed during the

early stages of the response, when the only outward sign is alerting, no matter whether the alerting is produced by direct electrical stimulation of the brain stem or by a stimulus from the external environment such as a loud noise. This applies not only to such features of the response as pupillary dilatation and the rise in arterial blood pressure, which are not specific to the defence reaction; but also to the atropine-sensitive muscle vasodilatation that Abrahams, Hilton & Zbrozyna (1960*a*, *b*) have shown to be a characteristic and invariable component, which is well developed during alerting.

The appearance of evoked potentials in all these regions in response to cutaneous, auditory and visual stimuli provides direct evidence of relays from all three systems converging on the appropriate brain-stem regions. It might be questioned whether the widespread distribution of the evoked potentials arose in part from an action of chloralose. However, when explorations have been made in similar regions of the cat's brain without the use of any anaesthetic, the same distribution of evoked potentials has been observed (e.g. Starzl *et al.* 1951*b*; Feldman *et al.* 1959). Our results, therefore, may be taken to indicate that the afferent connexions exist which would enable the brain-stem regions concerned to act not simply as an integrative centre but, indeed, as a reflex centre for the defence reaction.

The characteristic features of the evoked potentials were their long latencies, sensitivity to barbiturate anaesthesia and persistence after acute removal of the cerebral cortex. Potentials with these features have been reported previously in the mid-brain of the cat and monkey (Starzl *et al.* 1951*b*; French *et al.* 1953*a*, *b*). Starzl *et al.* (1951*b*), who coined the term 'afferent collateral system' for the multineurone pathway giving rise to evoked potentials of this kind, believed this to be an afferent system which activates large regions of the brain stem, so that these in turn can maintain the cerebral cortex in a state of 'alertness'. Thus the function of these parts of the brain stem has come to be considered mainly in relation to the concept of the ascending reticular activating system (Lindsley, Bowden & Magoun, 1949; Starzl, Taylor & Magoun, 1951*a*). It was recognized that these parts of the brain stem can influence motor, autonomic and endocrine activity (Magoun, 1958), but attention has been concentrated on one single manifestation of their activity. Yet there is evidence from several sources, established over many years, which points to the role of these regions as centres of complex, co-ordinated reflex responses, integrating the autonomic and behavioural patterns of alimentary and sexual reflexes, as well as of the defence reaction (Goltz, 1892; Woodworth & Sherrington, 1904; Bard & Rioch, 1937; Bard, 1940). The reflex centre for the defence reaction itself occupies a major part of the

defence reaction, including the collateral afferent pathway, is intact, but the projections of the classical sensory pathways are removed, defence reflexes are continually produced, as stereotyped responses, even to hardly noxious stimuli. A characteristic feature of the decorticate preparation, therefore, is the absence of inhibition as normally manifested in the phenomenon of habituation. In the normal cat the alerting produced by any sudden stimulus, such as the sound of a buzzer, soon disappears with repetition of the stimulus. This is true also for the defence reactions in response to weak noxious stimuli. Here we can see a possible significance of the slowness of conduction in the collateral afferent system, reflected in the long latencies of the evoked responses, as compared with the much shorter latencies of the cortical evoked responses. The inhibitory pathway which underlies the phenomenon of habituation involves the cerebral cortex. Nevertheless, impulses set up in this inhibitory pathway must arrive at the reflex centre, and so depress its excitability, *before* impulses travelling in the multineurone pathway impinge on the centre. It is possible that transmission is inhibited in the multi-neurone pathway itself.

SUMMARY

1. In cats anaesthetized with chloralose, cutaneous, auditory and visual stimuli evoke potentials in widespread regions of the hypothalamus, central grey matter and mid-brain tegmentum.

2. These potentials appear after relatively long latencies. They are reduced or abolished by small amounts of pentobarbitone, but survive removal of the cerebral cortex.

3. Convergence of the afferent pathways is indicated by considerable interactions obtained between potentials evoked by different sensory systems. There is also convergence within the afferent pathways of a single system.

4. The multineurone pathway giving rise to these potentials is apparently identical with that originally termed the afferent collateral system, which is usually considered in relation to the concept of the ascending reticular activating system. It is suggested, however, that the significance of this pathway lies chiefly in connexion with complex reflexes organized at the level of the hypothalamus and mid-brain, such as the defence reaction, and that it constitutes the afferent limb of the unconditioned reflex.

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Electrical activity in the brain stem of cats during eserine perfusion of the cerebral ventricles

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In cats anaesthetized with chloralose the cerebral ventricles have been perfused from one lateral ventricle to the cisterna with a fluid containing eserine sulphate 10 μg/ml. Within a few minutes of commencing perfusion unit discharges appear in the central grey matter and neighbouring tegmentum. The rate of discharge steadily increases for about 1 hr, then slows and ceases.

No equivalent discharge of units occurs in either the hypothalamus or caudate nucleus during the eserine perfusion. Unit discharges already present in the hypothalamus before commencing the eserine perfusion persist unaltered for several hours. In one experiment a pattern of unit discharge resembling that observed in the central grey matter was recorded from the amygdala, commencing in the 80th minute of eserine perfusion.

During the eserine perfusion there occurs, however, an increase in background activity in the caudate nucleus, hypothalamus and the central grey matter. This effect occurs after 60–90 min perfusion. An earlier effect is seen on the potentials evoked in these structures on electrical stimulation of the skin. These potentials are prolonged within 15 min of the eserine perfusion and this effect persists for long periods until the potentials suddenly collapse. In the amygdala the evoked potentials are not affected even if the eserine perfusion is continued for several hours, but there is an increase in background activity coincident with that appearing in the other subcortical structures.

Perfusion of the cerebral ventricles with eserine is known to result in the appearance of acetylcholine (ACh) in the effluent fluid (Adam, McKail, Obrador & Wilson, 1938; Bhattacharya & Feldberg, 1958). In the present experiments a correlation between the electrical activity of the brain and the output of ACh has been found. When pentobarbitone sodium is injected intravenously during the course of perfusion a sharp reduction in low-voltage background activity is seen, which is accompanied by a fall of 30–60 % in the output of ACh. These results thus support the conclusion of Bhattacharya & Feldberg (1958) that ACh appears as a result of central neuronal effects. Initially the ACh might arise from neurones discharging in the central grey matter; in later stages of perfusion it might also arise from the low-voltage background activity.

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Histochemical localization of cholinesterases in some brain stem regions of the cat

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The thiocholine method for histochemical localization of cholinesterases (Koelle & Friedenwald, 1949) as modified by Holmstedt (1957) was used to examine the distribution of true and pseudocholinesterases in regions of the cat's brain stem anterior to the inferior colliculi. In these regions cholinesterases are found in association with both neuronal and glial elements.

True cholinesterase is not only present in neuronal cytoplasm and on neuronal membranes, but is also present in high concentrations in some glia, particularly the hippocampal astrocytes, protoplasmic astrocytes in the dorsal hypothalamus and in the subependymal cell plate.

Pseudocholinesterase is present in high concentrations in some nuclear groups, particularly in the lateral nucleus of the substantia nigra, the 3rd nerve nucleus, the red nucleus, the deep layers of the superior colliculi and in nucleus centramedianus, nucleus paracentralis and nucleus subthalamicus. In these thalamic nuclei pseudocholinesterase is confined to the cytoplasm of the cells. It is also present in fibres of the optic nerve, optic chiasma, mamillo-thalamic tract, descending columns of the fornix and the cerebral peduncles. Pseudocholinesterase is also present in the periventricular glia, but only in small amounts. In the cuboidal ependyma of the hypothalamus it is present on the medial aspect of the cells. Elsewhere in the periventricular glia pseudocholinesterase is seen as scattered granules.

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Interactions between evoked potentials and background electrical activity in the hypothalamus of the cat

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Visual, auditory and cutaneous stimuli may lead to the appearance of evoked potentials in the hypothalamus of cats anaesthetized with chloralose. The form of these evoked potentials is affected by fluctuations in background electrical activity (Abrahams, Hilton & Malcolm, 1962). We have now found that a fixed relation exists between background electrical activity and evoked potentials. The timing of a stimulus in relation to background activity determines whether or not an evoked potential appears and, to a considerable extent, the form of the evoked potential.

The background electrical activity recorded in the hypothalamus of the cat anaesthetized with chloralose consists of high-amplitude spikes distributed amongst bursts of low-amplitude ($< 50\ \mu$V) waves. The spikes usually consist of a negative-positive complex with a negative excursion in excess of 100 μV, lasting about 50 msec, followed by a positive deflexion of some 50 μV which decay in 200–300 msec or longer.

Electrical stimulation of the skin during the presence of bursts of low-amplitude waves did not usually lead to an evoked potential, but barely recognizable potentials were occasionally seen. The interaction between an evoked potential and the high-amplitude spikes took one of two forms. Less commonly the pattern resembled the interaction between two evoked potentials, for stimulation of the skin during or immediately following a spike led only to the appearance of a minute negative-going potential. When the interval between the spikes and the stimulus lengthened, then an evoked potential appeared.

More commonly, the effect of a stimulus arriving during the spike complex was to initiate a potential change. In the negative portion of the complex this consisted of a sharp positive deflexion. In the positive part of the complex the positive deflexion was reduced and followed by a negative wave whose amplitude increased as stimuli arrived later in the positive complex. Stimuli arriving at a similar point in the negative-positive complex always resulted in the same potential change.

Using the discriminator described by McDonald (1963) we examined the result of applying stimuli during periods of minimal background activity. Evoked potentials then showed great constancy in their latency and form, but their amplitude was slightly variable; on occasion an evoked potential failed to appear.

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Interaction between evoked potentials and background electrical activity in the brain stem of the cat. Its effect on 'computer averaging'

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We have recently described a pattern of interaction between background electrical activity in the hypothalamus and potentials evoked there by electrical stimulation of the skin. Stimuli applied during periods of minimal background electrical activity lead to evoked potentials of relatively consistent form, amplitude and latency, but during appreciable background activity most stimuli either do not lead to evoked potentials, or they lead to potentials of reduced amplitude which show considerable but predictable variation (Abrahams & Langworth, 1963).

A similar pattern of interaction between evoked potentials and background activity has now been found to occur in other brain-stem regions, and is present whether potentials are evoked by cutaneous stimuli or by a light flash. This pattern of interaction may thus be a general phenomenon. It is of note that Raab & Kiang (1955) describe a negative correlation between background electrical activity and the amplitude of evoked potentials in the auditory cortex of cats anaesthetized with Dial. The functional significance of the interaction between background activity and evoked potentials is at present uncertain.

There are, however, immediate practical consequences of this interaction. Recently attempts have been made to overcome variation in evoked potentials by 'computer averaging' (Goldstein, Peake, Geisler, Kiang, Sandel & Barlow, 1959; Brazier, 1960; Goldstein, 1961), a technique in which evoked potentials are arithmetically summed. The average, when computed, gives a stable wave form which is used as a base line, and changes in these averaged potentials have provided a means, for example, of assessing drug actions. However, when alterations in background activity are not taken into consideration, changes in evoked potentials demonstrated by the averaging technique may be invalid.

This is readily demonstrated by an experiment in which 3 mg pentobarbitone sodium/kg is injected intravenously into a cat anaesthetized with chloralose. The background activity recorded from the hypothalamus is then substantially reduced, and the effect on averaged evoked potentials, recorded at 5 sec intervals, is an increase in amplitude (cf. Brazier, 1960). The present experiments show that this increase is misleading. It is

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Brain Stem Electrical Activity and the Release of Acetylcholine

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When the cerebral ventricles of the cat or dog are perfused with an anticholinesterase, acetylcholine appears in the effluent (Adam, McKail, Obrador and Wilson, 1938; Bhattacharya and Feldberg, 1958). By analogy with other sites it seems likely that this acetylcholine arises from the activity of cholinergic neurones. This being so, we would expect the output of acetylcholine to be related to activity, for the output of acetylcholine from a perfused ganglion is increased by preganglionic stimulation (Feldberg and Gaddum, 1934; Feldberg and Vertiainen, 1934; Brown and Feldberg, 1936; Perry, 1953; Emmelin and MacIntosh, 1956), and when acetylcholine is collected from the exposed surface of the cerebral cortex there is a definite relationship between the amounts of acetylcholine appearing and the cortical electrical activity (MacIntosh and Oborin, 1953; Mitchell, 1961). Attempts to relate the output of acetylcholine from perfused cerebral ventricles with activity have been less successful. Adam *et al.* (1938) electrically stimulated the hypothalamus during perfusion of the cerebral ventricles of the dog with eserine, and found this to increase the acetylcholine output in half the animals, but the increase was attributed to thermal damage caused by the stimulus. Hilton and Schain (1961) who performed similar experiments in cats were unable to demonstrate any increase in acetylcholine output even to extreme electrical stimulation of the brain.

The effect of cholinesterase inhibition on the electrical activity of a cholinergic site can be manifested in two ways, it can initiate unit activity (Eccles, Fatt and Koketsu, 1954; Eccles, Eccles and Fatt, 1956; Curtis and Eccles, 1958; Curtis and Koizumi, 1961; Curtis, Phyllis and Watkins, 1961; Krnjevic and Phyllis, 1961) or it may potentiate existing unit discharge or evoked potentials (Eccles, 1952; Eccles, Fatt and Koketsu, 1954; Taverner, 1954; Desmedt and Schlag, 1957). In order to find whether the acetylcholine present in ventricular effluents is neuronal in origin we examined the electrical activity of periventricular brain regions during perfusion of the cerebral ventricles with eserine. We recorded background activity and unit discharges as well as potentials evoked by electrical stimulation of the skin of one forepaw.

All experiments were performed on cats lightly anaesthetized with chloralose (60

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mg/kg). In each animal up to 4 stainless steel micro-electrodes (Green, 1958) were implanted into brain structures bordering the ventricular system. The electrodes were sealed in position with dental cement and the brain was perfused from one lateral ventricle to the cisterna magna (Bhattacharya and Feldberg, 1958). Although the whole ventricular system was perfused, the recording electrodes were positioned in structures close to the lateral and third ventricles and the anterior portion of the aqueduct of Sylvius, for Bhattacharya and Feldberg (1958) had shown these regions to be the probable source of most of the acetylcholine.

Perfusion of the ventricles with an artificial CSF solution had no effect on electrical activity of brain structures surrounding the cerebral ventricles, but when the perfusion fluid included one part in 50,000 of eserine sulphate, then, within a few minutes an intense discharge of units began in the central grey matter (Fig. 1). After about half

Fig. 1. Eserine perfusion initiates unit discharge in the central grey matter. The diagram on the right indicates the position of the recording micro-electrode in the central grey matter. No unit activity was present before commencing eserine perfusion. The three panels on the left show unit activity initiated by eserine, its reduction and disappearance during prolonged eserine perfusion. The time on the left of each panel indicates the duration of eserine perfusion. Time constant: 2msec; S.C. = superior colliculus; C.G. = central grey matter.

an hour of rapid firing the rate of unit discharge slowed and after another half hour it ceased. Such eserine-induced activity restricted to the central grey matter was found in no other region except in one experiment when a similar discharge was recorded from a single unit in the anterior amygdala commencing in the 130th minute of perfusion.

In other brain regions such as the hypothalamus, unit activity was commonly recorded in the control period before eserine perfusion but was not affected by perfusion of the cerebral ventricles with eserine even for prolonged periods (Fig. 2).

References p. 34/35

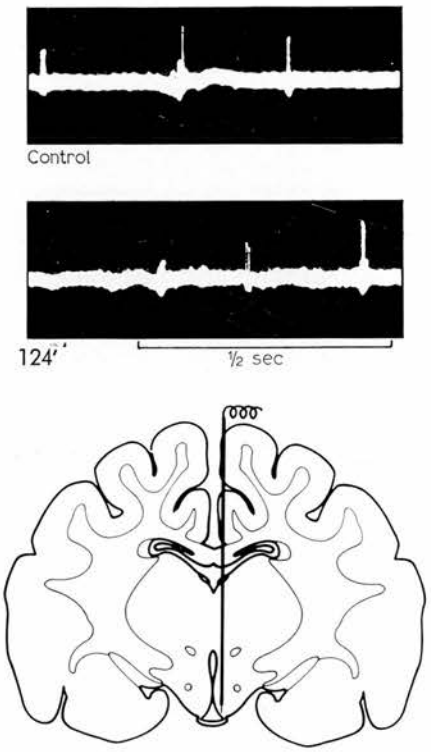

Fig. 2. Unit activity in the hypothalamus is unaffected by eserine perfusion. The lower diagram shows the position of the recording micro-electrode in the tuberal region of the hypothalamus. The top panels show the pattern of unit discharge before eserine perfusion and after 2 h and 4 min of eserine perfusion. Time constant: 2 msec.

Despite the absence of effect on unit activity in these regions, eserine perfusion caused a considerable increase of their slow wave electrical activity. In the hypothalamus, caudate nucleus and in amygdaloid structures this activity became apparent within an hour (Fig. 3). In the central grey matter such changes were present much earlier,

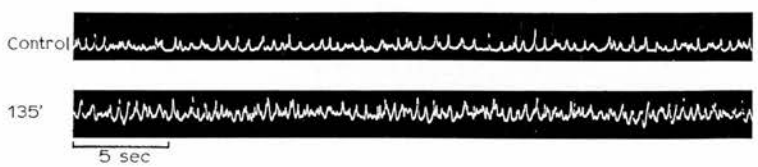

Fig. 3. Increase in hypothalamic slow wave activity after eserine perfusion of the cerebral ventricles. Time constant: 200 msec.

appearing within 15 minutes or so of perfusion at a time when the unit discharge was at its peak.

Eserine perfusion also greatly changed the evoked potentials in the central grey matter, hypothalamus and caudate nucleus, but caused only minor changes in the

References p. 34/35

evoked potentials recorded from amygdaloid structures (Fig. 4). In the central grey matter eserine perfusion led to a prolongation of the evoked potentials together with an increase in amplitude. The changes in evoked potentials recorded from the caudate nucleus and the hypothalamus were more complex. The early small negative wave

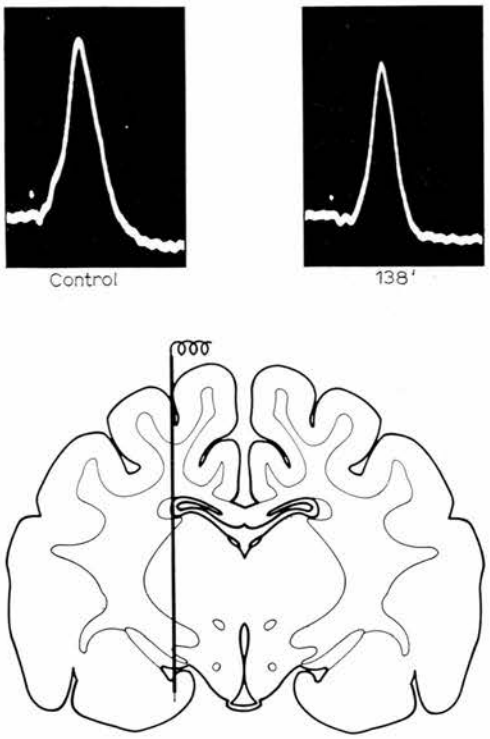

Fig. 4. Potentials evoked in the hippocampus by electrical stimulation of the skin of one forepaw before and after eserine perfusion. The lower diagram shows the position of the recording microelectrode in the hippocampus. The top left panel shows an evoked potential recorded before eserine perfusion, and the top right hand panel shows a potential recorded after 2 h and 18 min of eserine perfusion. Time constant: 200 msec.

associated with the afferent volley (Abrahams, Hilton and Malcolm, 1962) is enhanced, but as Fig. 5 shows, the main late wave due to local activity fractionates into an early and late peak, and then becomes reduced and delayed. Thus eserine potentiates the afferent volley but its local effect on the hypothalamus and caudate nucleus is quite different.

The recording experiments show that eserine perfusion of the cerebral ventricles can lead to substantial alterations in the electrical activity of the brain stem, although the only change typical of a cholinergic site was the unit discharge observed in the central grey matter. The effects on slow wave activity and on evoked potentials suggest complex actions, both direct and indirect, on brain stem structures.

Evidence has been obtained that the increase in slow wave electrical activity is itself responsible for some of the acetylcholine which appears in the ventricular effluent for

References p. 34/35

there is an association between the electrical activity and the output of acetylcholine. Slow intravenous infusion of 10 μg/kg pentobarbitone sodium (Nembutal, Abbott) which reduced or abolished slow wave electrical activity in all regions reduced the acetylcholine output by 30–60%. But even when all the electrical activity was abolished, some acetylcholine was still being released, thus raising the possibility that some of the acetylcholine appearing may not necessarily be directly related with any neuronal event.

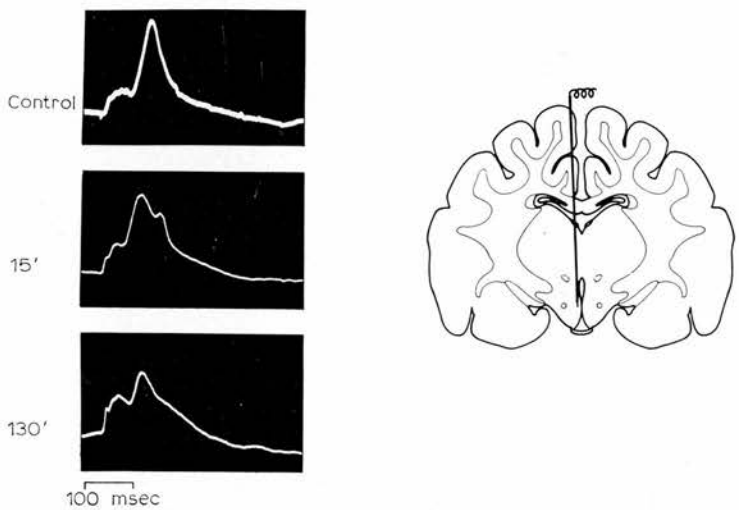

Fig. 5. Potentials evoked in the hypothalamus by electrical stimulation of the skin of one forepaw. The right hand diagram shows the position of the recording micro-electrode in the hypothalamus. The top left hand panel shows an evoked potential recorded during perfusion of the cerebral ventricles with a CSF solution, and after 15 and 130 min of eserine perfusion. Time constant: 200 msec.

Our other experiments were therefore concerned with the histochemical examination of cholinesterase distribution in the cat brain stem, for apart from one account of the regional distribution of cholinesterases (Snell, 1961) little is known of their detailed

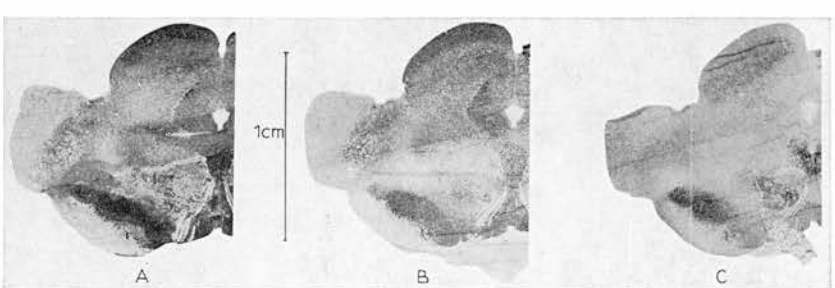

Fig. 6. Serial coronal sections of the cat brain at the level of the superior colliculus to show the distribution of cholinesterases. (A) All cholinesterase. Incubated for 3 h in acetylthiocholine. No inhibitors present. (B) True cholinesterase. Incubated for 3 h in acetylthiocholine. Mipafox as inhibitor of pseudocholinesterase. (C) Pseudocholinesterase. Incubated for 3 h in acetylthiocholine. B.W. 284c51 as inhibitor of true cholinesterase.

location. We used Holmstedt's (1957b) modification of the thiocholine technique of Koelle and Friedenwald (1949). The advantage of this modification is the reliability of the discrimination between true (acetylcholinesterase) and pseudocholinesterase (non-specific cholinesterase), and the ability to demonstrate cholinesterase at sites of low activity (Holmstedt, 1957a; Holmstedt and Sjöqvist, 1961). The incubations were performed on batches of serial 10-μ sections cut from unfixed brain.

It has been shown in rat, sheep, cat, monkey and man that there are wide variations in the concentrations of cholinesterases from one brain region to another (Koelle, 1954; Pokrovskii and Ponomareva, 1961; Snell, 1961; Palmer and Ellerker, 1962). Fig. 6 shows the differences we found in local concentrations of both true and pseudocholinesterases.

In sections incubated in acetylthiocholine we found high concentrations of cholinesterases in the caudate nucleus, the interpeduncular nucleus, the substantia nigra, the red nucleus, the 3rd nerve nucleus, the superior colliculi, the central grey matter and in three thalamic nuclei (N. centremedianum, N. submedianum and N. reticularis).

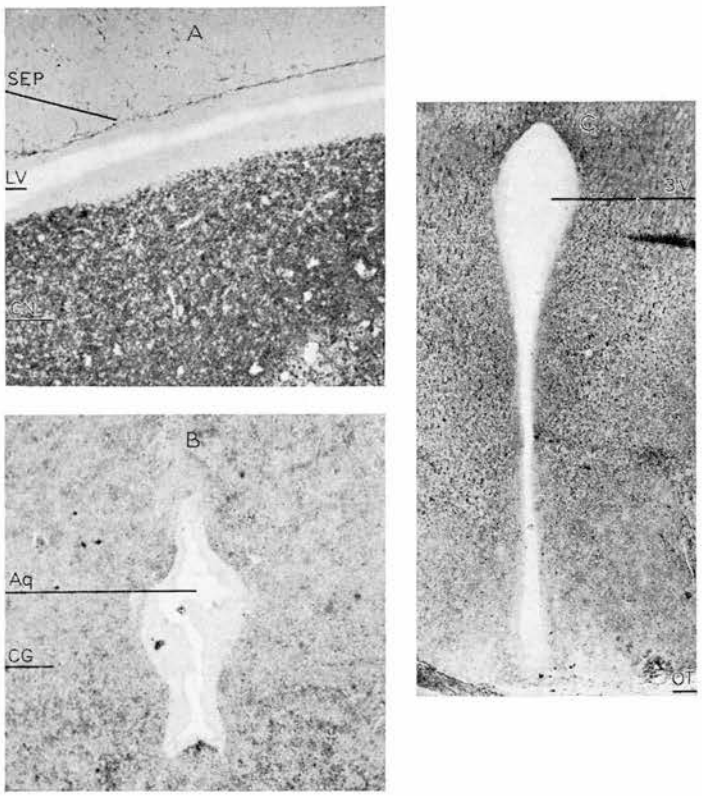

Fig. 7. Sections of periventricular regions of the cat brain stem showing the absence of true cholinesterase in the immediate periventricular regions. (A) Coronal section through the anterior portion of the lateral ventricle. SEP = sub-ependymal cell plate; LV = lateral ventricle; CN = caudate nucleus. (B) Coronal section through the aqueduct of Sylvius. Aq = aqueduct; CG = central grey matter. (C) Coronal section through the 3rd ventricle. 3V = third ventricle; OT = optic tract.

References p. 34/35

Lesser concentrations were found in many regions including the hypothalamus, the habenulus and in cellular layers of the hippocampus and Ammon's horn. Control incubations using selective inhibitors as well as butyrylthiocholine as substrate showed that although much of this cholinesterase was true cholinesterase there was a substantial proportion of pseudocholinesterase present, particularly in the lateral nucleus of the substantia nigra, the 3rd nerve nucleus, the red nucleus and in the thalamic nuclei previously referred to.

Of particular interest in view of the electrical changes found in the present experiments was the finding (Fig. 7) that the immediate periventricular regions of the lateral and third ventricles and of the aqueduct of Sylvius are devoid of true cholinesterase. Such cholinesterase as is present is pseudocholinesterase, which appears as scattered deposits, except in the dorsal part of the third ventricle where it is evenly distributed on the medial aspect of the cuboidal ependyma (Fig. 8B).

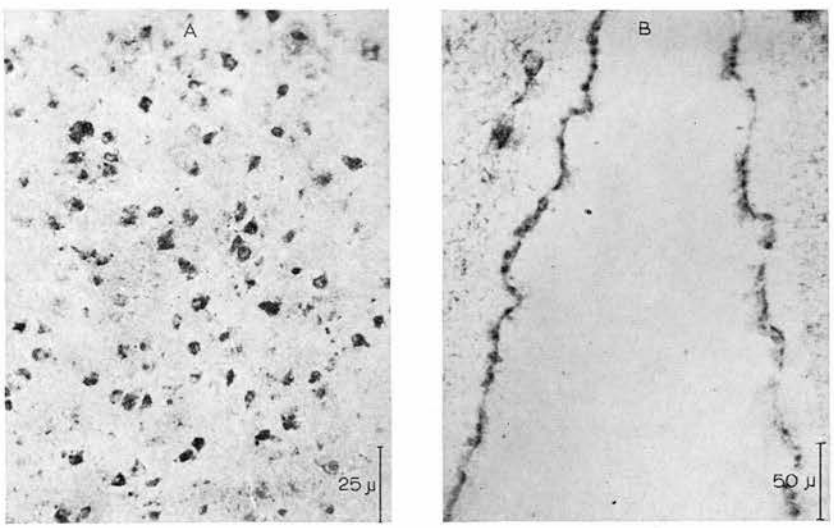

Fig. 8. Pseudocholinesterase distribution at two sites in the cat brain stem. (A) Section of N. centre-medianum of the thalamus showing pseudocholinesterase in the cytoplasm of neurones. (B) Coronal section through the dorsal portion of the 3rd ventricle showing pseudocholinesterase on the medial aspect of ependymal cells.

The details of the cellular arrangements of both true and pseudocholinesterase do not permit any single assumption to be made concerning the role of these enzymes in the brain. It has frequently been stated that true cholinesterase is predominantly found in neurones and pseudocholinesterase in glia (Bülbring, Philpot and Bosanquet, 1953; Cavanagh, Thompson and Webster, 1954; Desmedt, 1956; Desmedt and La Grutta, 1957). Examination of our sections does not support such a clear cut distinction, for substantial concentrations of true cholinesterase were found associated with glial elements and substantial concentrations of pseudocholinesterase with neurones. As Fig. 9 shows protoplasmic astrocytes in both the dorsal hypothalamus and the central grey matter have a high concentration of true cholinesterase on their

processes and scattered on their cell bodies, and the astrocytes of the hippocampus were also found to be rich in the enzyme. The fibre ramifications in the superficial layers of the superior colliculi, axonal and glial, are also rich in true cholinesterase, and the enzyme is present on the sub-ependymal cell plate.

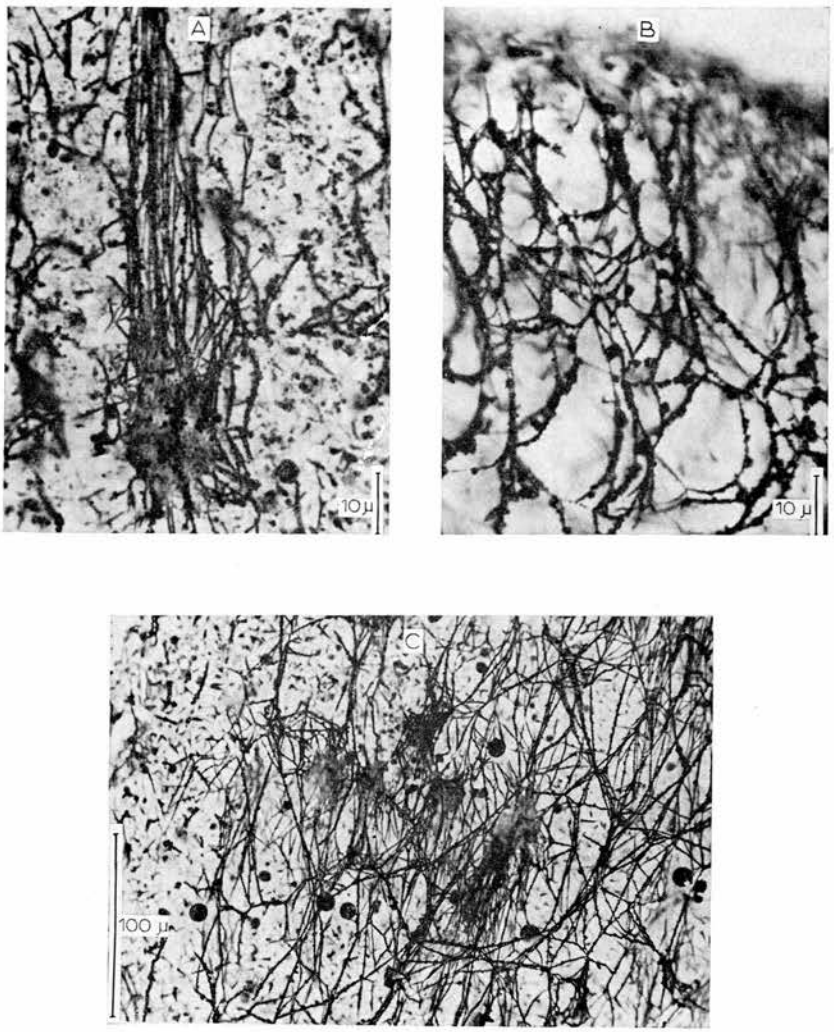

Fig. 9. Distribution of true cholinesterases. (A) Protoplasmic astrocyte of dorsal hypothalamus. A low power view of these cells can be seen in Fig. 7C. (B) Astrocytic and axonal fibres in the superficial layers of the superior colliculus. (C) Fibrous astrocytes of the hippocampus.

The association of pseudocholinesterase with neurones is illustrated in Fig. 8A. Neurones in the thalamic nuclei which are rich in pseudocholinesterase contain the enzyme in the cytoplasm of the cell bodies. In other regions pseudocholinesterase appeared to be localised on the membranes of nerve cells whose cytoplasm contains no cholinesterase at all. Pseudocholinesterase was also found in the large fibre tracts

References p. 34/35

of the brainstem, including the cerebral peduncles, the mamillothalamic tract, the optic nerve and the descending columns of the fornix. However, we cannot say whether the fibres which stain are axons or glial processes.

Despite the various anomalies found in these experiments, a considerable proportion of the true cholinesterase of the brain stem is associated with neurones, sometimes in appreciable concentrations on the cell boundary and sometimes within the cell cytoplasm.

To what extent is it possible to reconcile the histochemical findings with the changes in electrical activity resulting from perfusion of the cerebral ventricles with eserine? The absence of true cholinesterase from the regions lining the ventricles suggests that eserine must penetrate the brain substance with great rapidity, unless of course significant effects can arise as a result of pseudocholinesterase inhibition of the regions immediately adjacent to the ventricles. This explanation is not likely, for the behaviour of units in the central grey matter is typical of a cholinoceptive site treated with an anticholinesterase, so that local eserine penetration must be assumed. The other effects of eserine perfusion, the increase in the slow wave background activity and the various changes in evoked potentials do not permit of any simple explanation. It is possible that such changes could be due to inhibition of neuronal cholinesterase, but equally the effects may be entirely secondary to the inhibition of cholinesterases at a non-neuronal site. At the present time these experiments demonstrate the possibility that cholinesterase may serve many functions within the brain and that it is not sufficient to assume that the results of anticholinesterase poisoning are explicable solely in terms of effects on cholinergic neurones.

SUMMARY

The electrical activity of brain structures surrounding the lateral and third ventricles and the aqueduct of Sylvius were recorded during eserine perfusion of the ventricular system. Such perfusion led to an intense transient unit discharge in the central grey matter and to an increase in the slow wave background electrical activity at many brain stem sites. Histochemical examination of the same regions of the brain stem showed a high proportion of the cholinesterase to be present at non-neuronal sites. Some of the observed electrical effects of cholinesterase inhibition may thus be secondary to inhibition of the enzyme at non-neuronal sites.

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DISCUSSION

GEREBTZOFF: Concerning the subependymal localization of true cholinesterase I would suggest the possibility of an artefact, since the technique is conducted on unfixed preparations.

ABRAHAMS: The possibility of staining not being due to true cholinesterase is unlikely. The identification of true or pseudocholinesterase is based on an examination of seven serial sections which between them embrace the full range of inhibitor and substrate combinations proposed by Holmstedt. Thus there are ample controls. In any case I would expect unfixed preparations to be less prone to artefact than fixed preparations.

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THE ROLE OF ACTIVE MUSCLE VASODILATATION IN THE ALERTING STAGE OF THE DEFENCE REACTION

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Muscle vasodilatation, produced by cholinergic nerve fibres of the sympathetic system, appears to be an integral feature of the defence reaction which an animal displays as a reflex response to any sudden stimulus (Abrahams, Hilton & Zbrożyna, 1960*b*). This vasodilatation is itself only one component of a complex pattern of cardiovascular change in which a greatly increased cardiac output is directed chiefly through the skeletal musculature. As previously emphasized, the defence reaction is a graded response whose behavioural expression is manifested initially as alerting. With sufficiently intense stimulation it will culminate in flight or attack, and the complex cardiovascular reaction seems best regarded as a preparatory adjustment of the animal for the muscular exertion inseparable from these activities (Abrahams *et al.* 1960*b*; Abrahams, Hilton & Malcolm, 1962). Studies of the changes of muscle blood flow in the conscious cat have reinforced this view (Abrahams, Hilton & Zbrożyna, 1960*a*).

The experimental results which are described here show that atropine-sensitive muscle vasodilatation occurs when the earliest behavioural signs of alerting are seen, whether such alerting has been produced by direct electrical stimulation of the appropriate brain-stem regions, or by a sudden environmental stimulus such as a flash of light or the sound of a buzzer. Further, the vasodilatation is readily obtained as a conditioned-reflex response.

In all these experiments use has been made of temperature changes of the whole-limb venous effluent as an index of muscle blood flow in the conscious animal (Abrahams, 1959). The results of experiments on anaesthetized cats which demonstrate the validity of this technique are included in this paper.

METHODS

Anaesthetized cats. Cats were anaesthetized with chloralose, 70 mg/kg, injected intravenously after induction of anaesthesia with ethyl chloride and ether. One hind limb was

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prepared for registration of changes of muscle blood flow, as previously described (Abrahams *et al.* 1960*b*) and the other for registration of changes of whole-limb flow by the same method, except that the skin was not separated from the underlying tissues. On each side the femoral venous outflow was passed via a polyethylene cannula through a photo-electric drop recorder and returned to the femoral vein more centrally. Localized stimulation in the region of the hypothalamus integrating the defence reaction was effected by the use of stereotactically placed steel micro-electrodes, also as previously described (Abrahams *et al.* 1960*b*).

Conscious cats. The cats were in a sound-proofed observation chamber with a double glass panel in the front wall. They were observed through a half-silvered mirror mounted outside and attached to the base of this wall at an angle of 45°. With good illumination in the chamber and a dim light outside, the animal could not see out of the chamber, neither could it see a reflexion of itself. A loudspeaker mounted on one inner wall and a 500 W projection lamp behind a protective wire-netting screen were used for delivering acoustic and visual stimuli respectively, and a microphone for monitoring sounds. Sockets in the wall were available for connexion to a multi-way lead which could be fixed to a connector on a thick polyethylene jacket worn by the animal. The lead was held by a light spring attached to the roof of the chamber, so that the animal could move freely.

Stimulation of brain-stem and skin. For these experiments electrodes were implanted stereotactically into the brain-stem in sterile operations under pentobarbitone anaesthesia, as previously described (Abrahams *et al.* 1960*b*). The leads from the electrodes were fixed to the connector on the polyethylene jacket. The skin of the footpads was stimulated by passing current through a metal grid on which the animal was standing. Square waves of 1·5 msec duration at 40/sec were applied, at whatever voltage was found necessary to produce behavioural alerting. In the conditioning experiments in which a more precise control of the stimulus was required, two sterile Michel clips, with leads attached, were fixed to the skin of the back about 2 in. (5 cm) apart. The intensity of the electrical stimulation was then 10 V, the other parameters being the same. The clips were protected by the polyethylene jacket, and the leads were fixed to a connector on it. These clips appeared to cause the animal no discomfort and could be left *in situ* for about a week without any untoward reaction.

Registration of venous-effluent temperature. In experiments lasting a single day changes of muscle blood flow were indicated by a venous-temperature method (Abrahams, 1959), in which a small chamber bearing a thermistor bead is inserted between the cut ends of the femoral vein. The operation was performed under ether anaesthesia, and heparin (1000 u./kg) was injected intravenously before the vein was opened. The leads were sutured to the fascia on the ventral aspect of the thigh at several points, then led under the skin and brought out between the scapulae at a site protected by the polyethylene jacket, to be fixed to a connector on the jacket. The actual experiment was carried out several hours after recovery from the anaesthetic and the animal was killed at the end of the day with an overdose of pentobarbitone, given intraperitoneally.

When the cat was to be observed over a period of days or weeks the method was modified as previously described (Abrahams *et al.* 1960*b*), the thermistor bead being mounted in a cuff placed round the unopened vessel.

RESULTS

Experiments on anaesthetized cats

In a previous investigation we showed that the atropine-sensitive vasodilatation in skeletal muscle, which appears to be a characteristic component of the defence reaction, can be obtained as a feature of the pseudaffective reflex response to stimulation of a peripheral nerve in the high-

decerebrate cat (Abrahams *et al.* 1960*b*). We also showed that this increase in muscle blood flow could be registered indirectly with a venous blood temperature recorder. Before we could extend the use of this simple recording technique to a study of the occurrence of similar vascular changes

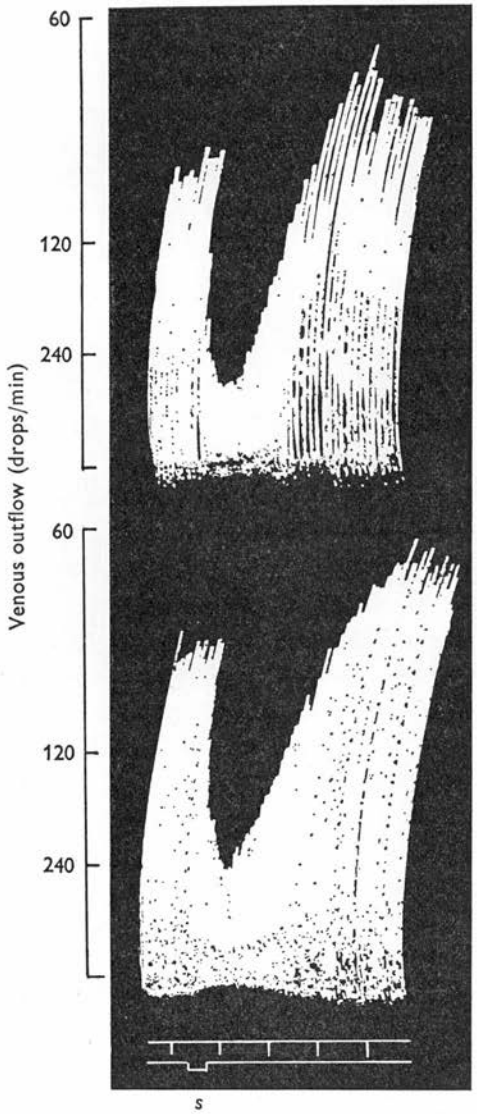

Fig. 1. Cat, chloralose. Records of venous outflow from skinned hind limb (upper record) and whole hind limb (lower record), showing similarity of increases in flow on stimulation (at *s*) in hypothalamic region for the defence reaction (cf. Abrahams *et al.* 1960*b*). Time marker, 30 sec.

in the normal, conscious animal without any operation to separate skin and muscle blood flow, it was necessary to carry out experiments on the changes in the flow of mixed venous blood through the femoral vein, and to see how these compared with the changes of muscle blood flow, recorded simultaneously. The experiments were performed on three cats, and it was found (Fig. 1) that localized stimulation, within the region of the hypothalamus

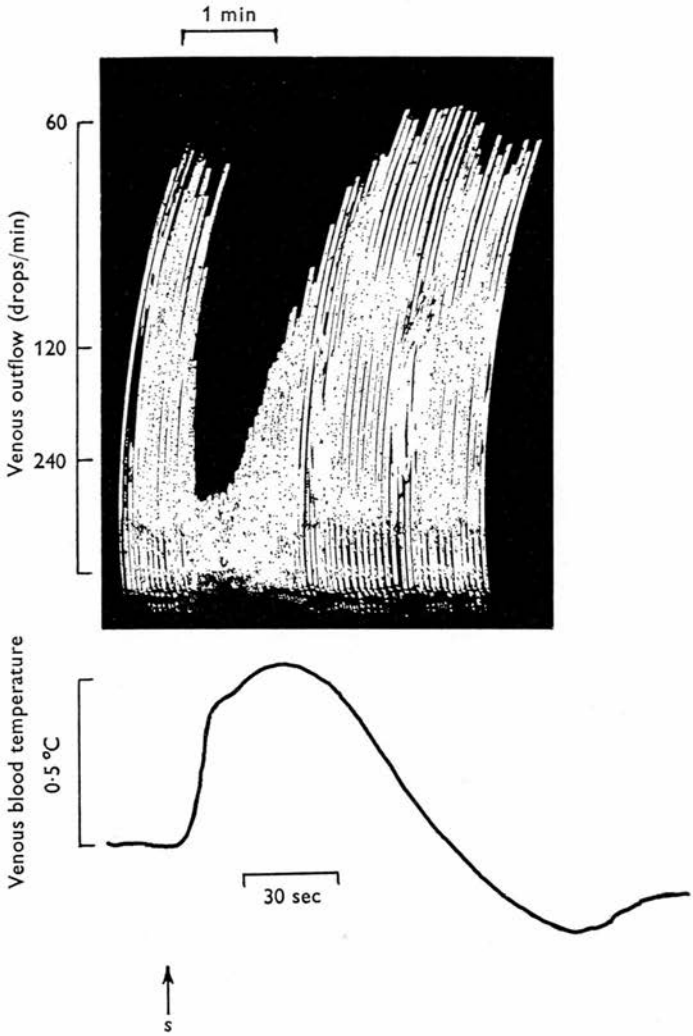

Fig. 2. Cat, chloralose. Upper record, venous outflow from whole hind limb. Lower record, venous effluent temperature, recorded simultaneously but with different time scale (upward deflexion = increase in temperature). At *s*, electrical stimulation for 10 sec in hypothalamic region for the defence reaction (cf. Abrahams *et al.* 1960*b*).

integrating the defence reaction, produces an increase in blood flow through the whole hind limb, which is practically as great as that recorded simultaneously in the muscles of the other hind limb. The contribution of the skin blood flow to the whole-limb flow appears to be so small that the skin vasoconstriction, which occurs as part of the complex circulatory response, is almost completely obscured by the increase in muscle blood flow. Thus, during this reaction the change in whole femoral venous flow is a reliable index of the increase in flow through the muscles of the hind limb. The femoral venous temperature changes associated with these increases in whole-limb flow were recorded in experiments on four cats. As is illustrated in Fig. 2, with an approximately fourfold increase in blood flow, which was the usual effect of hypothalamic stimulation, the venous blood temperature increased by 0·5° C or more. With greater increases in blood flow the venous effluent temperature could increase by as much as 1° C. When the increase in flow was only threefold, the temperature increase was about 0·3° C.

Experiments on conscious cats

Evidence for muscle vasodilatation as an early component of the defence reaction

Hypothalamic stimulation. The same parameters of stimulation of the same specific region of the hypothalamus elicit defence reactions in the conscious cat and atropine-sensitive muscle vasodilatation in the anaesthetized cat (Abrahams *et al.* 1960*b*). When the venous temperature recorder employed in the experiments described above is used in experiments on the whole femoral venous flow in conscious cats, and the specific hypothalamic region is stimulated by means of implanted electrodes, similar increases in venous blood temperature are recorded. This was established in experiments carried out on five cats. To elicit the vascular response seen in Fig. 3*a* stimulation was only at threshold intensity for alerting; the pupils were dilated, the head raised, the ears pricked and respiratory rate increased. That the vascular response itself is evidence for activation of the same cholinergic fibres to the muscle vessels is confirmed by its abolition after atropine 1 mg/kg (Fig. 3*b*). This was injected subcutaneously and the response was found to be abolished 25–30 min later. This dose of atropine, which represented a smaller effective dose than that used in our earlier investigation of the vasodilator innervation in anaesthetized cats, did not appear to be blocking the vascular response by an action on the central nervous system, since though the pupils were already dilated all the other signs of alerting were elicited as before; and increasing the stimulation intensity led, as usual, to the full reactions of flight or attack.

There was no evidence of habituation of the response elicited by hypothalamic stimulation, i.e. the response did not diminish when stimulation was repeated daily over a period of days or weeks. If anything, both the vascular response and the signs of alerting were elicited a little more readily with such repetition.

Auditory, visual and cutaneous stimulation evoked similar vascular responses. Indeed, the ease with which the response was elicited by

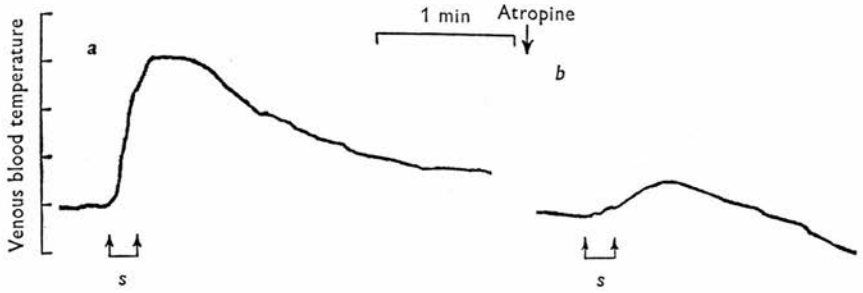

Fig. 3. Conscious cat. Records of femoral venous blood temperature (upward deflexion = increase in temperature, indicating increase in blood flow). Each scale division on temperature record indicates 0·1° C. Effect of electrical stimulation (at *s*) by means of an electrode implanted in hypothalamic region for the defence reaction, *a* before and *b* 30 min after subcutaneous injection of atropine (1 mg/kg).

natural stimuli made it essential that the animals should be familiar with the experimental surroundings before any of these tests were carried out. Otherwise, when introduced into the observation chamber the animal would show not only the behavioural signs of alerting, but also a prolonged and persistent muscle vasodilatation, as indicated by a steep rise of femoral venous temperature. No response could then be elicited by any of the stimuli, but the steady, high level of venous temperature was reduced by subcutaneous injection of atropine.

Figure 4 illustrates a response, in an animal familiar with its surroundings, to the 1000 c/s tone used as an auditory stimulus. In three out of five cats the responses to the auditory stimulus were smaller, while in the remaining cat the tone elicited no response (Fig. 5*a*). These responses were abolished by atropine, as is shown in the experiment illustrated in Fig. 4. Similar vasodilator responses were obtained to flashes of light, and to stimulation of the skin, and like those elicited by the auditory stimulus, these responses were accompanied by the same behavioural signs of alerting as are seen on threshold hypothalamic stimulation. This would be expected since these are well-known features of the reaction to a sudden stimulus.

When testing the effects of cutaneous stimuli, particular care had to be taken to produce alerting alone and to avoid movements of the limbs, for muscular contraction produces changes in limb blood flow independently of the vasodilator innervation. The skin stimulation used was therefore very mild, but nevertheless it was usually more effective than auditory

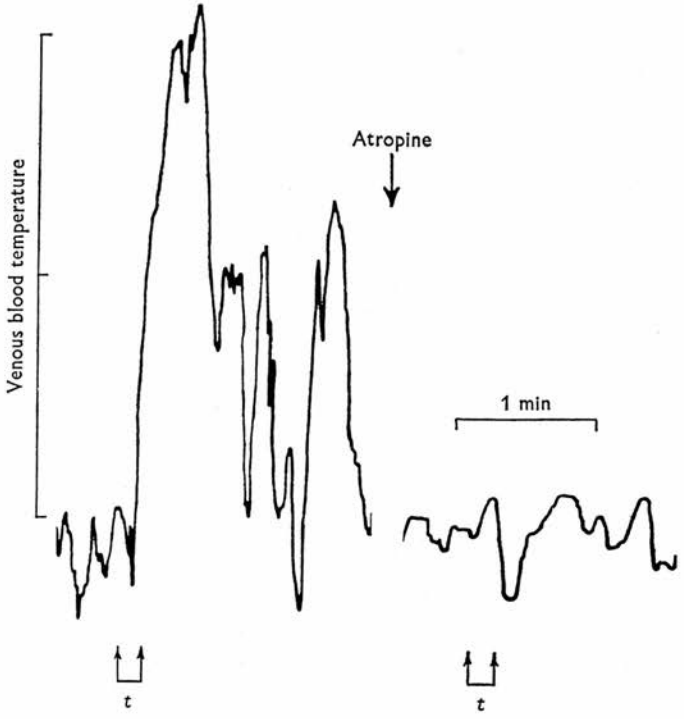

Fig. 4. Conscious cat. Records of femoral venous blood temperature (upward deflexion = increase in temperature, indicating increase in blood flow). Each scale division on temperature record indicates 0·5° C. Effect of 1000 c/s tone (at *t*) before and 30 min after subcutaneous injection of atropine (1 mg/kg).

or visual stimuli in eliciting a vasodilator response. Such responses were obtained in all five cats and one is illustrated in Fig. 5*b*.

In the cats giving vasodilator responses at the initial trial, repetition of the auditory, visual or cutaneous stimuli led to gradual diminution of the response. This habituation proceeded at different rates in the different animals, and habituation to cutaneous stimulation was the least marked.

Evidence for muscle vasodilatation as a conditioned reflex reaction

It is known that conditioned defence reactions are readily established and that once established they are relatively stable, in that they can be

obtained after long intervals of no reinforcement. It was therefore to be expected that the vascular response could be obtained as a conditioned reflex.

The experiments were performed on three of the five cats whose responses to natural stimuli had been tested. In the cat whose responses are illustrated in Fig. 5, the tone itself did not elicit vasodilatation. Conditioning experiments were carried out in the following way: the tone was

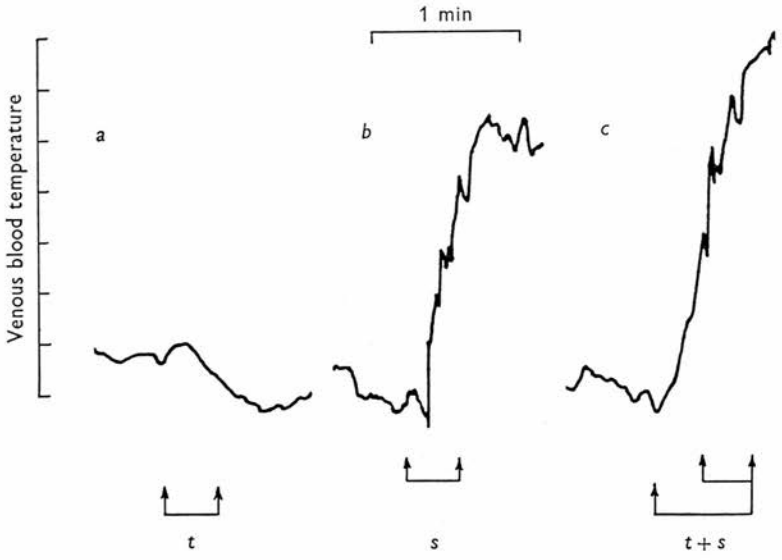

Fig. 5. Conscious cat. Records of femoral venous blood temperature (upward deflexion = increase in temperature, indicating increase in blood flow). Each scale division on temperature record indicates 0·1° C. Effects of *a* 1000 c/s tone, at signal *t*; *b* stimulation of foot pads, at signal *s*; *c* 1000 c/s tone, initially alone, then combined with stimulation of foot pads (4th trial of combination) at signals *t*+*s*.

sounded for 7 sec, skin stimulation commenced 2 sec after the tone had been turned on, and both stimuli were turned off together. After this combination of stimuli had been applied three times, the response illustrated in Fig. 5*c* was obtained. Even at this early stage of the experiment the response was developing during the tone alone. Such early conditioning was observed in each cat during the first training session. The action of atropine on these responses was not tested, because the procedure of injection would itself have a disturbing effect. However, there seems little reason to doubt that the conditioned and unconditioned vascular responses were the same.

In one of the cats training was continued over a period of several weeks. This cat initially gave a small response to the tone, as shown in Fig. 6*a*. It was brought into the experimental chamber several times a week and the buzzer sounded five or six times at each session. At the end of a month, habituation was firmly established and the cat was giving no response at all to the tone. Conditioning experiments were started a week later. As before, the tone was sounded for 7 sec, and combined with skin stimulation for the last 5 sec. On the first day the conditioning combination was applied three times, and on the second day five times. On the third day the tone was tested at the beginning of the experiment, and was

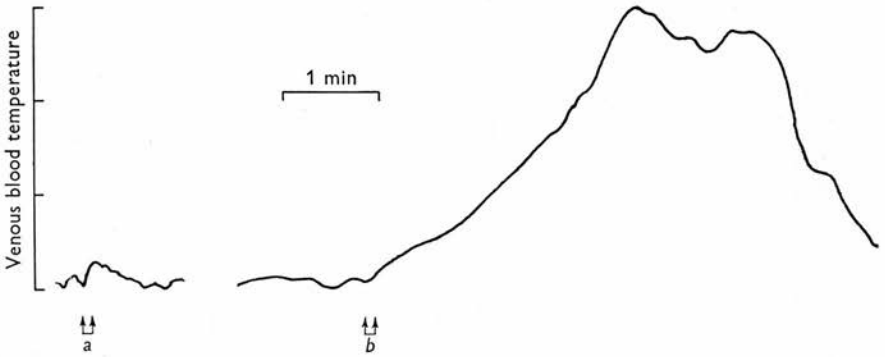

Fig. 6. Conscious cat. Records of femoral venous blood temperature (upward deflexion = increase in temperature, indicating increase in blood flow). Each scale division on temperature record indicates 0·5° C. Effects of 1000 c/s tone before habituation, at signal *a*. Response to same tone at signal *b* after prolonged habituation, followed by combination with electrical stimulation of the skin of the back on 4 days, and 5 subsequent days without experiment.

found to cause some ear movements and turning of the head. The reinforcing combination was then applied three times. At the beginning of the experiment on the fourth day, the tone alone caused behavioural alerting, together with vasodilatation in the hind limb: the reinforcing combination was applied once. No experiments were carried out during the following 5 days. On the next day, the tone alone produced the full reaction, including the large vascular response shown in Fig. 6*b*. Reinforcement was applied once. Two days later the tone produced a strong behavioural reaction in which the cat appeared to be trying to escape from the cage. No reliability could therefore be placed on the venous-temperature record. Skin stimulation was not attempted. Two days later the tone produced a strong alerting reaction and vasodilatation. At this stage the cat was beginning to give a reaction to being placed in the experimental chamber and experiments were discontinued.

DISCUSSION

The initial behavioural responses to any sudden stimulus have in the past been variously termed. They include alerting and arousal, the Pavlovian orientation reflex and the startle reaction. Although there are small differences in these reactions, there is no reason to doubt that they are basically one response arising from near-threshold activation of specific regions in the hypothalamus and mid-brain. When the same brain-stem regions are activated more intensely, the fully-fledged defence reactions of flight or attack are elicited. It is not surprising, therefore, that autonomic changes characteristic of the defence reaction occur during the orientation reflex (Birjukov, Korneva, Šljafer & Jakovleva, 1962).

The conclusion that muscle vasodilatation, produced by cholinergic fibres of the sympathetic outflow, is an integral part of the defence reaction has so far been based on the following evidence: (1) When the hypothalamus, central grey matter or mid-brain tegmentum are stimulated with micro-electrodes, the regions from which active muscle vasodilatation is elicited under anaesthesia are identical with those from which alerting, flight and attack are obtained in the same animals when conscious. (2) Active muscle vasodilatation is obtained as part of the pseudaffective reflex response in the high-decerebrate cat (Abrahams *et al.* 1960*b*).

The opportunity to obtain information about changes in muscle blood flow in the conscious cat has now enabled us to show that atropine-sensitive vasodilatation occurs at the initial stage of the defence reaction, no matter whether elicited by threshold electrical stimulation of the brain stem or by an environmental stimulus. At this stage of the reaction the only outward signs are pricking of the ears, pupillary dilatation and an increase in the rate of respiration. The muscle vasodilatation therefore occurs at a sufficiently early stage of the graded reaction for it to fulfil the role of a preparatory response.

When the muscle vasodilatation is elicited by electrical stimulation of the brain stem of anaesthetized animals, it appears as part of a complex pattern of cardiovascular change. Uvnäs and his colleagues (Uvnäs, 1954) noted concomitant vasoconstriction in skin and intestine, and tachycardia, which we could readily confirm (Abrahams *et al.* 1960*b*). There is also evidence of increased contractile force of the heart and increased cardiac output (Rosén, 1961), while simultaneous venoconstriction has been suggested by Folkow, Mellander & Öberg (1961) and Hilton (1963). When all these changes are taken together it appears that the cardiovascular system is being adjusted in such a way that it is fully prepared for the demands of sudden widespread muscular activity, characteristic of flight or attack. Since in all these experiments the atropine-sensitive vasodilatation has

appeared together with the other cardiovascular changes, it may be assumed that the vasodilatation observed in the present experiments is part of this same pattern of cardiovascular response.

Cutaneous, visual and auditory pathways converge on the brain-stem regions integrating the defence reaction; and the active muscle vasodilatation is a component of the pseudaffective reflex elicited in the high-decerebrate cat when these regions of the brain stem have been spared (Abrahams *et al.* 1960*b*; Abrahams *et al.* 1962). We therefore regard the integrative centre for the defence reaction as a reflex centre in the classical sense, and hence we would class this pattern of cardiovascular response as a preparatory reflex reaction. The well-known cardiovascular reflexes are usually categorized as homoeostatic, in that they appear to function in a way that will maintain or restore the *status quo*. The new state of equilibrium produced by a preparatory reflex is appropriate in relation to long-term homoeostasis; but in the short term it may lead to a radical departure from the *status quo*. It was the finding of Uvnäs (1960), that O_2-uptake in skeletal muscle is reduced during activation of the vasodilator nerve fibres, that led him to disclaim any belief that these nerve fibres could be activated as part of a response in preparation for muscular exertion. Since, however, our present findings show that they are so activated, the pattern of cardiovascular adjustment appears to be of over-riding importance. Contracting skeletal muscles can no doubt provide for their own metabolic requirements, through the widespread capillary dilatation that so rapidly develops within them, so long as the circulation is adjusted to provide the necessary blood flow. This still leaves open the question whether such an over-all pattern of circulatory adjustment is maintained by the same regulatory mechanism *during* muscular exertion (Barcroft, 1960).

It is possible to incorporate, within the framework of this concept, other findings of similar cardiovascular changes produced by sudden or intense stimuli in conscious animals and men. For instance, when conscious dogs were subjected to a sudden shower of water, cardiac output was almost doubled, with an increase in both stroke volume and heart rate, and mean arterial blood pressure increased while total peripheral resistance was unchanged or fell (Charlier, Guz, Keatinge & Wilcken, 1962). This seems to be evidence for the same preparatory reflex in the conscious dog, particularly when it is recalled that the whole pattern of cardiovascular response, including the active muscle vasodilatation, has been found in anaesthetized dogs on stimulation of the same brain-stem regions from which the response is elicited in the cat (Eliasson, Lindgren & Uvnäs, 1952; Lindgren & Uvnäs, 1953; Lindgren, 1955). The cardiac responses characteristic of muscular exertion have been obtained on electrical stimulation at some points in the posterior hypothalamus of conscious

dogs (Smith, Jabbur, Rushmer & Lasher, 1960). Moreover, the same changes of heart rate and left ventricular performance were obtained in dogs exercising on a treadmill and in well-trained dogs when the experimenter held the treadmill switch (Rushmer, Smith & Lasher, 1960). The latter observation demonstrates once again the preparatory or anticipatory nature of the circulatory response, and also how readily it can be produced as a conditioned reflex.

A considerable amount of work has been carried out on dogs by Gantt and his colleagues (Gantt, 1960) showing that the tachycardia and hypertension which are produced by a painful stimulus can be readily conditioned and that the conditioned reflex thus obtained is remarkably persistent, surviving up to 13 months without reinforcement, even when the motor components of the reaction have long been extinguished. In our experiments, the muscle vasodilatation was also readily conditioned. As in the corresponding experiments of Gantt (1960) and his colleagues, we found that the conditioned vasodilator response was easily developed, and once established was remarkably stable.

A similar cardiovascular response occurs in man, when in situations causing 'anxiety'. In addition to the well-known rise of arterial blood pressure and heart rate, the cardiac output is usually increased, and the total peripheral resistance reduced (Stead, Warren, Merrill & Brannon, 1945; Hickam, Cargill & Golden, 1948). The muscle blood flow increases (Wilkins & Eichna, 1941; Golenhofen & Hildebrandt, 1957). Brod, Fencl, Hejl & Jirka (1959) measured arterial blood pressure, cardiac output, forearm blood flow and renal plasma flow simultaneously in subjects given a severe test in mental arithmetic, and thereby revealed a general pattern of response which is the same as that found in anaesthetized and conscious animals. It is of particular interest that the increase in muscle blood flow was shown to result mainly from a decrease of flow resistance, since activation of the vasodilator nerve fibres to skeletal muscle is characteristic of the defence reaction in experimental animals (Abrahams *et al.* 1960*b*). Atropine-sensitive vasodilator nerve fibres of the sympathetic outflow have since been shown to be involved in the response to a frightening situation in man (Blair, Glover, Greenfield & Roddie, 1959), although in some subjects circulating adrenaline may make a significant contribution to the muscle vasodilatation (Barcroft, Brod, Hejl, Hirsjärvi & Kitchin, 1960).

There is every reason to believe that the pattern of cardiovascular response underlying defence reactions in man, as in animals, will result from stimuli of different modalities, and if this response is as readily conditioned in man as it is in animals it might be expected to play an important part in pathological as well as physiological reactions. Indeed,

muscle blood flow has been found to be significantly higher in hypertensive than in normotensive human subjects (Brod, Fencl, Hejl, Jirka & Ulrych, 1962). The increase in blood flow is too great to be explained by the increase in perfusion pressure. Apparently the resistance vessels in skeletal muscle do not usually participate in the general increase in vascular tone observed in hypertension, and they may even be dilated. Brod *et al.* (1962) therefore suggest that the pattern of cardiovascular response characteristic of the defence reaction may participate in the pathogenesis of essential hypertension in man.

SUMMARY

1. In cats anaesthetized with chloralose, active vasodilatation in the muscles of the hind limb, elicited by electrical stimulation of the hypothalamus, leads to an increase in temperature of the femoral venous effluent.

2. This change of temperature has been used in experiments on conscious cats as an index of the onset and course of the atropine-sensitive muscle vasodilatation occurring during defence reactions.

3. Atropine-sensitive vasodilatation in the muscles is invariably found to accompany the early, alerting stage of the defence reaction produced by near-threshold electrical stimulation of the hypothalamus or by environmental stimuli.

4. It is proposed that the pattern of circulatory change, of which this vasodilatation is a characteristic component, should be regarded as a preparatory cardiovascular reflex.

5. The muscle vasodilatation is readily obtained as a conditioned-reflex response which appears to be stable, whereas the unconditioned response to environmental stimuli shows rapid habituation.

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Potentials evoked in the Hypothalamus and Cerebral Cortex by Electrical Stimulation of the Uterus

AFTER the 36th week of pregnancy, labour is readily initiated by such procedures as dilating the cervix, passing uterine bougies and, most effectively, by amniotomy. This ability to initiate labour by mechanical interference suggests the existence of nervous pathways from uterus to hypothalamus. The presence of appropriate receptors in the rabbit uterus has been demonstrated by Ferguson[1] and by Bower[2], but little is known of the central pathways involved. In the experiments recorded here on cats anæsthetized with chloralose we used the evoked potential technique to detect uterine afferent relays to the hypothalamus. Pairs of stainless steel stimulating electrodes were implanted into the myometrium, one pair high in one uterine horn and another pair below the level of fusion of the two horns, but above the vagina. The uterus was then subjected to direct electrical stimulation through either pair of electrodes by square wave pulses of 1–3 msec duration.

Evoked potentials recorded from the hypothalamus of cats anæsthetized with chloralose show irregularities[3] due largely to an interaction with background activity[4]. The background activity at the recording site was therefore monitored and stimulation was deferred until a period

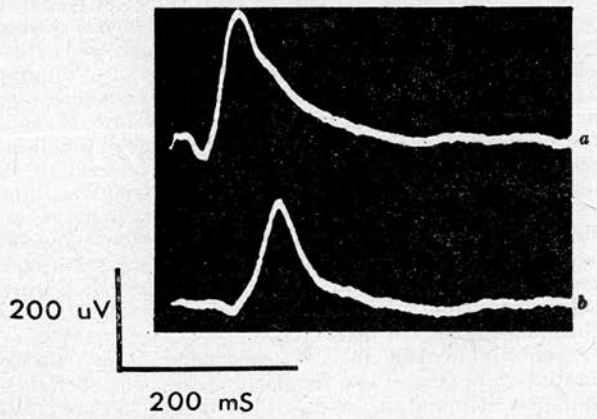

Fig. 1. Responses obtained at the same hypothalamic recording site in response to (*a*) electrical stimulation of the skin of one forepaw and (*b*) electrical stimulation of the myometrium of one uterine horn. Note that uterine stimulation is followed by an evoked potential of small amplitude and long latency

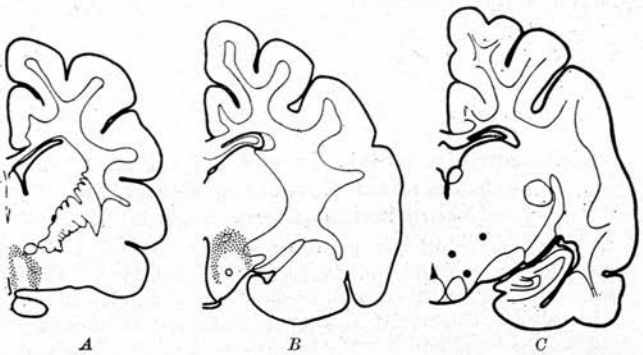

Fig. 2. Diagrammatic coronal sections of the cat brain to show the distribution of potentials evoked in the hypothalamus by electrical stimulation of the uterus. The stippled regions in sections *A* and *B* are those from which responses could consistently be elicited. The three large dots in section *C* indicate where responses were obtained on one occasion

of 80 msec had elapsed with no such activity in excess of 15 μV negative or positive[5]. The minimum interval between stimuli was 3 sec. The hypothalamus was explored millimetre by millimetre. At each recording site the skin of one forepaw was first stimulated, and stimuli were then applied to each pair of uterine electrodes in turn. Whereas cutaneous stimulation evoked potentials in all hypothalamic regions[3] potentials evoked by uterine stimulation were largely limited to two hypothalamic regions. Fig. 1 compares the potentials evoked by cutaneous and uterine stimulation at the same recording site. In Fig. 2 the distribution of responses to uterine stimulation from 13 experiments is plotted on to three diagrammatic sections of the hypothalamus. It can be seen that the responses are virtually confined to the pre-optic and tuberal regions. The distribution of evoked potentials in any one experiment is more restricted than the composite diagram suggests. In two experiments the electrode passed through the supra-optic nucleus. The results of one of these experiments are shown in Fig. 3 in which evoked potentials resulting from uterine stimulation were confined to the supra-optic nucleus and the region immediately surrounding it. The reason that the area from which evoked potentials are recorded appears larger in the composite diagram than in a single experiment may be attributed to variation in the location and size of the supra-optic nucleus from animal to animal, and the possibility that additional nuclei are activated.

Potentials evoked in the hypothalamus by uterine stimulation appeared less regularly than those elicited by cutaneous stimulation, and with a longer latency. Responses evoked by uterine stimulation had a latency of 30–50 msec, whereas responses to cutaneous stimulation had a latency of 15–17·5 msec. In some experiments responses were obtained from stimulation of the electrodes high in the uterine horn, but not from those in the

Fig. 3. Localization of potentials evoked by uterine stimulation in the supra-optic nucleus. The photomicrograph shows an electrode track passing through the supra-optic nucleus into the optic tract. The evoked potential illustrated in *A* was recorded just dorsal to the supra-optic nucleus, that in *B* in the ventral portion of the supra-optic nucleus, and that in *C* in the optic tract. *son*, supra-optic nucleus ; *od*, optic tract

fused portion of the uterus; in others the opposite was true. In all experiments responses were most consistent when using stimulating pulses of long duration, that is, 3 msec. In a single experiment the cerebral cortex in the

region of the sigmoid gyrus was explored with a platinum ball recording electrode, and uterine stimulation was found readily to evoke potentials in the somato-sensory region. Presumably such potentials arise from the activation of receptors or their nerve fibres in the myometrium[2].

Our experiments thus demonstrate that neural pathways exist from the uterus to the hypothalamus and apparently also to the cerebral cortex, but doubt must obtain as to the specificity of these relays. Barraclough and Cross[6] found that units in the hypothalamus of the rat responding to pressure on the os cervicis also responded to chilling or pinching the tail. Their recordings were, however, confined to a region of the lateral hypothalamus from which, in the cat, we did not usually observe a response to uterine stimulation. It is also known[7] that many forms of visceral stimulation can evoke potentials in the cerebral cortex.

It is increasingly recognized[8–11] that the onset of labour in the human being is due to an increase in the sensitivity of the myometrium to oxytocin rather than to an increase in the amounts of oxytocin released into the blood. Since the hypothalamus is functionally connected with both neurohypophysis and adenohypophysis it is tempting to speculate that the relay we have demonstrated between uterus and hypothalamus may be concerned not only with the release of oxytocin but also with effecting increased myometrial sensitivity to it, factors which together underly the mechanism of normal parturition.

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A NOTE ON THE USE OF COMPUTER AVERAGEING IN THE ASSESSMENT OF CENTRALLY ACTING DRUGS

SEPARATUM

III. CONFERENTIA HUNGARICA PRO THERAPIA ET INVESTIGATIONE IN PHARMACOLOGIA

CONTENTS СОДЕРЖАНИЕ INHALTSVERZEICHNIS

A NOTE ON THE USE OF COMPUTER AVERAGING IN THE ASSESSMENT OF CENTRALLY ACTING DRUGS

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Long after the necessary compromise has been reached which will resolve the moral and ethical dilemma of clinical pharmacology, we shall still be faced by the difficulties inherent in identifying the mode of action of a drug. Nowhere is this more difficult than when we are considering the action of drugs on the human central nervous system where, of necessity, testing is largely based on indirect procedures. Recently, it seemed that a small advance had been made in this direction, and that a new method was available offering objective, quantitative, and direct methods for the testing of drugs which act upon the human central nervous system. Specifically, I refer to the technique which is now known as "computer averaging of evoked potentials".

In order to understand this technique, we have to remind ourselves of some elementary facts of physiology. When a nerve is excited by a sudden stimulus, it is very easy to detect the passage of a compound action potential along that nerve. This is most commonly done with the recording electrodes applied directly to the nerve, for example, with a frog nerve dissected free and laid onto recording electrodes, but such potentials can be also recorded in situ in man with cutaneous electrodes placed over a superficial nerve. Once the conducting fibres enter the spinal cord, the potential change associated with conduction can only be recorded by electrodes thrust into the substance of the cord. The electrical change then recorded is termed an evoked potential. The evoked potential in the appropriate receiving area of the cerebral cortex can be recorded from electrodes placed on the scalp, but the amplitude of the signal is small and, being of the same order of magnitude of the spontaneous electroencephalogram, is very difficult to distinguish.

Dawson (1951, 1954) showed that these cortical evoked potentials could be made more apparent by borrowing a technique first used by oceanographers in studying wave movements and referred to as "averaging". This technique, as applied in neurophysiology is actually a summation technique and consists of adding a number of successive electrical signals following stimulation. If a consistent potential change follows the stimulus, it rapidly summates, whereas randomly disposed signals summate less rapidly and merge into a baseline. Thus, by repeating a stimulus 50 or 100 times and utilizing the averaging process, we can readily distinguish the presence of evoked potentials in a recording from the scalp. Such summations may be performed arithmetically,

but are tedious and time consuming. The advent of computer technology has enabled this type of averaging to be performed virtually instantaneously and a number of suitable machines are available commercially. Hence, the name of the technique, "computer averaging of evoked potentials".

The importance of this technique to pharmacology is that such potentials are constant when recorded under standardized conditions, as are evoked potentials from many brain sites, and the technique provides a stable background against which the effect of a drug on a sensory system can be investigated and, furthermore, can be examined in man with the minimum of inconvenience. In Figure 1, we have used records made from the brain stem of a conscious cat to illustrate the use of the technique. The two records on the left are the averaged responses recorded in the hypothalamus and reticular formation following 100 flashes of light. When the animal is anaesthetized with 60 mg/Kg. of chloralose there are quite different effects on the potentials recorded in the two areas. By applying this type of experiment to cortical potentials, the computer averaging technique would seem able to detect whether a drug is exerting an effect on sensory pathways in man.

However, early experiments utilizing this technique have blunted our faith, for some of the results appear to be at variance with well-established ideas. For example, BRAZIER (1960) reports that Pentobarbitone sodium (Nembutal) increases the amplitude of evoked potentials recorded in the sensory cortex, but Nembutal is generally regarded as having a depressing action on sensory systems. Do these results mean that our previous ideas were in error, or do they point to some fundamental flaw in the averaging technique?

Wherever recorded, evoked potentials are influenced by the spontaneous background activity of the recording site. First noted by BARTLEY and BISHOP (1933) when recording from the visual cortex, the phenomenon has been described by RAAB and KIANG (1955) in the auditory cortex, and by FORBES and MORISON (1939) in the somato-sensory cortex, and LANGWORTH and I (1963) have found these interactions also to occur in the brain stem. Figure 2 shows such an interaction, in which the response to stimulation is large when there is little background electrical activity and is reduced, or grossly altered, when stimulation falls in periods of background electrical activity. Bearing this in mind, let us examine the brain stem electrical activity following the injection of Nembutal (Figure 3). The animal was already anaesthetized with chloralose, but Nembutal quite clearly reduces the amount of background electrical activity and, in so doing, must increase the probability of stimulation occurring during a quiescent period and in turn, this increases the probability of a large, evoked potential following sensory stimulation. That this may be the explanation of BRAZIER's results can be seen in Figure 4. This figure shows averages of responses to stimulation given regularly at 4-second intervals, prior to Nembutal, and also shows the average of responses in which

the 4-second minimal interval is preserved, but stimulation is further delayed until there has been a period of 80 msecs with little or no background activity (McDonald, 1963). When stimulation occurs at rigidly-timed intervals, the effect of Nembutal is to increase slightly the amplitude of the evoked potential

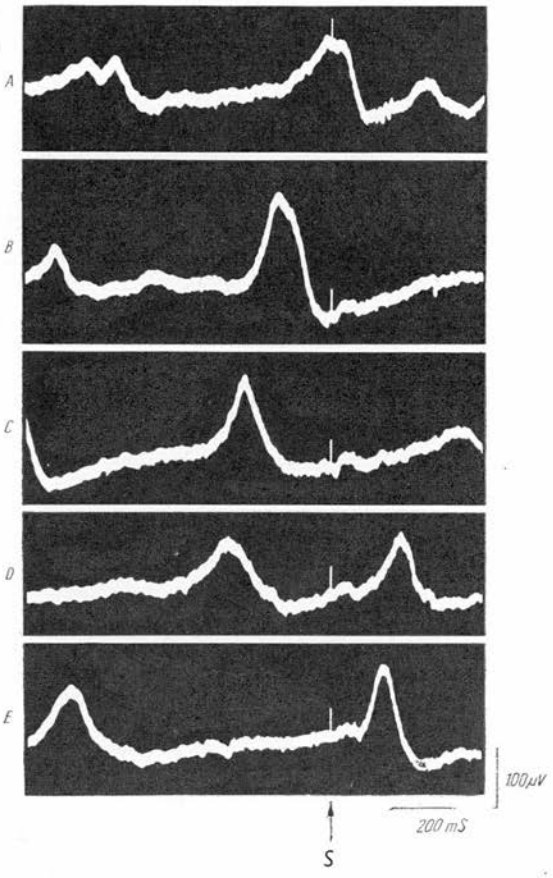


Fig. 1. Cat. Chloralose nteraction patterns between evoked potentials and background electrical activity. Recording electrode in hypothalamus. At S, electric shock delivered to the skin of one forepaw.

A. Stimulus during spontaneous wave leads to small evoked potential.
B. & C. Stimuli immediately following spontaneous wave lead to small evoked potentials.
D. Stimuli 80 msec. after spontaneous activity leads to reduced and delayed evoked potential.
E. Large evoked potential recorded in the absence of spontaneous background activity.

and to delay its peak. However, when the stimulus is delayed and the technique compensates for alterations in background activity by restricting stimulation to periods of equivalent background activity, then we can see that Nembutal quite clearly reduces the amplitude of the evoked potential. Thus, before adopting the technique of computer averaging of evoked potentials in a whole-

Averaged evoked potentials from hypothalamus and mesencephalic reticular formation

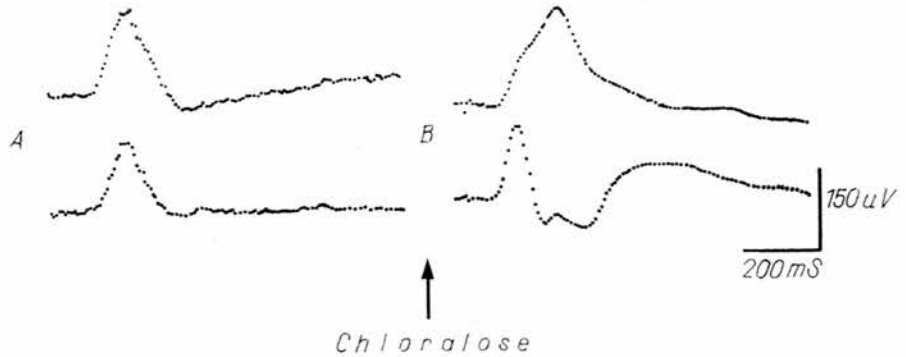


Fig. 2. Cat. Effect of chloralose on computer averaged evoked potentials recorded simultaneously from hypothalamus (Top) and mesencephalic reticular formation (Bottom). Stimulus is a flash of light.

A. Potentials recorded from conscious animal.
B. Potentials recorded after anaesthetic dose of chloralose (60 mg/Kg) i. v.

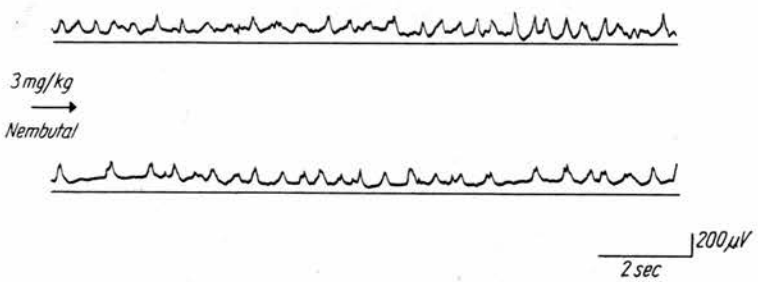
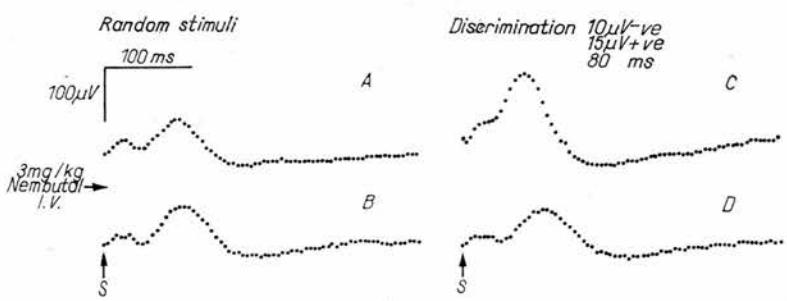


Fig. 3. Cat. Chloralose. Reduction of spontaneous electrical activity of the hypothalamus by the intravenous injection of 3 mg/Kg Nembutal.

Effect of discrimination on the form of averaged evoked potentials



Cat. Chloralose 60mg/kg. Electrical stimulation of contralateral forelimb.

Fig. 4. Cat. Chloralose. Effect of Nembutal on arithmetically computed evoked potentials recorded from hypothalamus. At S, electric shock delivered to the skin of one forepaw.
A. & B. Stimuli delivered at regular 4-second intervals. Nembutal between A & B.
C. & D. Minimum interval of 4 seconds between shocks, but stimuli further delayed until 80 msecs. elapses with no activity in excess of 10 μV. negative and 15 μV. positive

sale fashion, the clinical pharmacologists would well be advised to understand that there is still much to be learned about evoked potentials, and where such uncertainty exists, the interpretation of changes in evoked potentials must be on an empirical basis and, although capable of numerical quantification, it is largely devoid of rationale.

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(50) R. L. WILLIAMS* and V. C. ABRAHAMS

Department of Physiology, Queen's University.

Patterns of circulatory change elicited by electrical stimulation of the medulla in chloralosed cats.

Higher brain centres with a circulatory function have long been thought to bring about their effects by the activation of centres in the medulla. There has been little attempt to examine the nature of the intrinsic patterns of circulatory change which the medulla can initiate, yet if the medulla is to subserve such a function these patterns must be numerous. In the present experiments we have examined the patterns of circulatory change that direct electrical stimulation of the medulla can elicit and have attempted to localize anatomical points associated with specific circulatory changes. The medulla was systematically explored whilst stimulating through a monopolar electrode and simultaneous measurements made of the venous outflow from skin and muscle, blood pressure, heart rate and respiration. A wide range of circulatory patterns were found which were largely consistent from experiment to experiment and each pattern was associated with stimulation in the same general anatomical area. Circulatory changes elicited in this way can include relatively independent alterations in one parameter and evidence has been obtained which suggests that some of these patterns include a temporary suspension of baroreceptor reflexes.

This work was made possible by an M.R.C. grant and gifts of equipment by Pfizer Ltd., and by Smith, Kline and French Ltd.

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Department of Physiology, Queen's University.

Synchronous potentials evoked in widespread brain stem regions of conscious cats by visual, auditory and cutaneous stimuli.

We previously showed that visual, auditory and cutaneous stimuli evoke potentials of long latency and uncertain form in the hypothalamus and neighboring brain stem regions of the chloralosed cat. When these evoked potentials were examined utilizing a technique which reduced their variability it seemed that they might arise from a potential change occurring synchronously in widespread brain stem regions. The present experiments were performed to see if similar potential changes occur in the conscious cat. Stainless steel electrodes were implanted into the candate nucleus, septal nuclei, hypothalamus, mesencephalic reticular formation and tectum of cats trained to sit quietly in a restraining box. Visual, auditory and cutaneous stimuli applied to these animals evoked potentials from all areas and in response to all stimuli. The evoked potentials were highly variable, but use of the averaging technique showed that they were largely synchronous in widespread regions, and although of smaller amplitude, bore great similarity to the responses previously observed in the choralosed cat.

This work was supported by a grant from the M.R.C. and by an equipment grant from the Wellcome Trust.

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Department of Physiology, Queen's University, Kingston.

The effect of stimulus modality and position on the discharge of units in the superior colliculus.

For many years the superior colliculi were thought to be concerned with the reflex organization of visually directed head and eye movements, but lesions of the superior colliculi lead to perceptive defects which also involve the auditory and cutaneous systems. In experiments on chloralosed cats we have commonly found single units of the superior colliculi activated by visual, auditory, cutaneous and muscle afferent stimuli. Using the post-stimulus histogram technique to compare firing patterns, several types of units have been found. In one group the firing patterns show a general similarity regardless of the stimulus modality employed. In another group, the firing pattern is unique and characteristic for each sensory modality. When the response to skin stimulation was examined stimulus position was found to have a great influence in determining firing patterns; but it was possible to duplicate these firing patterns by appropriate stimulation of a neck muscle nerve. The explanation of two cell types may lie in the existence of two functional roles, one concerned with the spatial location of a stimulus and the directional component of reflexes following that stimulus, and a second involving a response to sensory stimuli in which the stimulus modality may have some significance.

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(121) V. C. ABRAHAMS

Department of Physiology, Queen's University, Kingston.

The form of averaged evoked potentials in the chloralosed cat and the effect of background activity.

Experience in the physical sciences have shown that if the waveform of a signal extracted by the averaging technique is to be meaningful, then it is necessary to remove from the original data large variations not related to the functions being studied. When introducing the averaging technique into physiology, Dawson stressed this principle, but it has rarely been applied. If the waveform of an averaged evoked potential is to be meaningful and subject to physiological analysis it would seem essential that the effects of possible interfering factors be investigated. In experiments in chloralosed cats where the effects of background electrical activity on evoked potentials is considerable, it is clear that this activity may substantially alter the form of an averaged evoked potential. Small regular waves in individual responses may seem to be of considerable amplitude in the averaged record, whereas large less regular components may be minimized or contribute to an averaged waveform which differs from any individual evoked potential.

[*From the Proceedings of the Physiological Society*, 22–23 *April* 1966]
Journal of Physiology, 185, 60–61*P*

Some factors affecting the form of averaged evoked potentials

By V. C. Abrahams. *Department of Physiology, Queen's University, Kingston, Ontario*

When introducing the averaging technique into physiology, Dawson (1954), drawing on the lessons gained from the use of the technique in other disciplines, pointed out that: 'Wherever possible any large variations either irregular or systematic which are not related to the functions being studied should be removed from the data before averaging is carried out. ...In the electroencephalogram any variation such as those due to spontaneous brain potentials or instrumental causes must likewise be reduced to a minimum.' In the cat anaesthetized with chloralose a large part of the observed variation in evoked potentials is due to the existence of complex interactions with background electrical activity (Abrahams & Langworth, 1963). Using a technique which timed stimuli to minimize the effects of interaction with background activity (McDonald, 1964) experiments were performed which supported Dawson's (1954) suggestions and showed that minimizing interactions with background activity led to substantial changes in computed averages (Abrahams, Langworth & McDonald, 1963).

More recent experiments utilizing an averaging computer have demonstrated the existence of another factor which can affect the computed average, namely the existence of prolonged recovery periods (in excess of 5 sec) in chloralosed cats. The technique used to minimize interactions with background activity introduces an increase in stimulus interval averaging 1·8 sec and thus the possibility exists that the previous results stemmed from this increase in stimulus interval. In examining this possibility it was found that the changes resulting from an increase in stimulus intervals were relatively small and never of the same order of magnitude as those resulting from minimizing the effects of background activity. In these experiments it was also possible to examine the form of individual evoked potentials which when summed constituted the average. It was found that the amplitude of individual components constituting the average reflected mostly the stability of that component. Small components which are stable in individual potentials appear grossly exaggerated in computed averages, and the less stable larger components are not so prominent. The effect of minimizing background activity is to produce an averaged potential in which less stable components contribute significantly to the form.

Chloralose anaesthesia exaggerates background activity, evoked potentials, and their mutual interactions, but such interactions occur in many

[P.T.O.

sites and situations and can be seen when recording from man (Bechtereva & Zontov, 1962; Callaway & Layne, 1964). Since the averaging technique is being adopted by some as an objective technique for the measurement of neural processes (Uttal & Cook, 1964) it would seem essential to ensure that Dawson's (1954) advice be followed to avoid the recording of spurious and misleading signals.

The research was aided by grants from the Wellcome Foundation and the Medical Research Council of Canada.

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PATTERNS OF UNIT RESPONSE IN THE CAT SUPERIOR COLLICULI ELICITED BY NON-VISUAL STIMULI. V. C. Abrahams and S. Falchetto*. Department of Physiology, Queen's University, Kingston, Ontario, Canada.

Although the superior colliculus of the cat is usually regarded as part of the central visual pathway, 95% of the units from which recordings were made in the chloralose anaesthetized cat responded to one or more non-visual stimuli. These stimuli include electric shocks applied to the skin, clicks, and electrical stimulation of the central end of a cut muscle nerve. Units responding to skin stimulation respond when stimuli are applied over a wide area of the skin. In such experiments examination of post-stimulus histograms has shown that the pattern of cell discharge is usually related to the area being stimulated. Commonly a brisk, short latency (6 m.sec. or so) discharge may be obtained from a single unit when a restricted area of skin is stimulated, whereas the same unit may discharge weakly after a much longer latency when other skin areas are stimulated. Similarly units responding to muscle afferent stimulation may show a brisk early discharge to stimulation of a particular muscle nerve, but respond weakly and at a longer latency to stimulation of a number of other muscle nerves. (Supported by the Defence Research Board and Medical Research Council of Canada)

[*From the Proceedings of the Physiological Society*, 14 *January* 1967]
Journal of Physiology, **189**, 58–59*P*

Blood flow, heart rate, and blood pressure alterations elicited by direct electrical stimulation of the lower brain stem

By V. C. Abrahams and R. L. Williams.*† *Department of Physiology, Queen's University, Kingston, Ontario*

The lower brain stem has long been thought to exert a dominant role in the regulation of the circulation. This role was believed to be exercised through reciprocally acting vasopressor-cardioaccelerator and vasodepressor-cardioinhibitor systems whose anatomical locations were determined by recording the blood pressure changes which accompany stimulation of the lower brain stem (Wang & Ranson, 1939; Alexander, 1946). In the present experiments these same anatomical locations were stimulated and peripheral blood flow was recorded, as well as blood pressure and heart rate. It was found that blood pressure alterations in a given direction may be accompanied by a variety of patterns of peripheral blood flow.

Experiments were performed on chloralose-anaesthetized cats with intact buffer nerves. Blood flow from muscle and skin were measured with the venous outflow technique, and rigid criteria were adopted so that results were considered only from experiments in which the cats were judged to be in a stable satisfactory condition. In these experiments it was found that ten distinct patterns of circulatory change regularly accompany brain stem stimulation, three with a fall in blood pressure, five with a rise in blood pressure and two without any blood pressure alteration. Table 1 summarizes the details of the ten patterns of circulatory change.

Table 1. Patterns of circulatory change consistently elicited from threshold electrical stimulation of the lower brain stem. ↑ rise; ↓ fall; ↓ ↓ pronounced fall; → no change.

	1	2	3	4	5	6	7	8	9	10
Blood pressure	↓	↓	↓	→	→	↑	↑	↑	↑	↑
Heart rate	↓	↓↓	↓	↓	→	↑	↓	↑	↓	↑
Muscle blood flow	↓	↓	↑	→	→	↓	↓	↓	↓	↑
Skin blood flow	↓	↓	↓	→	↓	↓	↓	↑	↑	↓

Patterns 1 and 6 were most frequently seen, and each of the remaining patterns occurred in at least 4 % of the stimulations. It is concluded that the functional characterization of the lower brain stem areas into discrete pressor and depressor regions is a gross over-simplification and masks the ability of the structure to generate a wide complex of cardiovascular reactions.

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EXPERIMENTAL NEUROLOGY **18**, 253-266 (1967)

The Contribution of Background Electrical Activity to the Form of Averaged Evoked Potentials in Chloralose Anesthetized Cats

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An examination of potentials recorded from the hypothalamus of the chloralose-anesthetized cat following cutaneous or visual stimuli revealed the existence of complex interactions between evoked potentials and background electrical activity. These interactions led to alterations in the form of evoked potentials or to their nonappearance. The evoked potentials could be stabilized to a large extent by timing stimuli in relation to background activity so that stimuli were delivered in periods of little or no activity. Further, it was found that the effects of one evoked potential on subsequent evoked potentials lasted for periods of up to 6 sec. When averages of evoked potentials were computed it was found that the form of the average was markedly affected by background activity, which exaggerated stable wave-form components and distorted others. Averages computed from stimuli delivered in periods of little or no background activity showed clear differences from averages computed from stimuli delivered at the same time intervals, but without regard to background electrical activity.

Introduction

The averaged evoked potential which can be computed by summing the brain electrical activity following repeated applications of a sensory stimulus is affected by a number of factors including spontaneous activity at the recording site (14, 15). Thus the form of an averaged signal may depart substantially from the form of the individual signals contributing to the average (13). We have sought some quantitative assessment of the degree to which background activity can affect averaged evoked potentials in one particular preparation, the chloralose anesthetized cat. Recordings were largely confined to hypothalamic sites, for potentials recorded here show considerable variability due to an interaction with background activity (1, 2), but are generally large enough for the individual signals contributing to the average to be recognized.

[1] The authors gratefully acknowledge the financial assistance of the Wellcome Trust and the Medical Research Council of Canada.

Methods

All experiments were performed on cats anesthetized with chloralose (60-80 mg/kg) given intravenously in a single dose. Evoked potentials were recorded with tungsten microelectrodes insulated with epoxy resin and Insl-X (18) and with a tip diameter small enough to record unit activity. Signals were amplified by a type 2A61 Tektronix amplifier mounted in a Tektronix type 565 oscilloscope. The output from the 565 oscilloscope was fed to an interface unit and then to one input of a computer of average transients (Mnemotron Corporation, North Haven) and simultaneously to an FM tape recorder (Ampex SP-300).

The same signal was fed via an attenuator to the discriminator circuit described by McDonald (21) for timing stimuli on the basis of the background activity of the recording site. This circuit controlled a Digitimer (Devices Ltd., Welwyn Garden City, England) which generated trigger pulses for the oscilloscope time base, the computer of average transients (CAT) and a stimulator. The discriminator operated by monitoring the ongoing electrical activity at the electrode site, and allowed four criteria to be specified before a trigger pulse was generated. These were, minimum interval between stimuli, an interval thereafter which must meet fixed limits of electrical quietness, and the positive and negative voltage limits of that electrical quietness. The trigger pulse generated by the discriminator was recorded by the tape recorder, so that patterns of stimulus sequence generated on the basis of brain electrical activity could be re-presented in control experiments.

Cutaneous stimulation consisted of shocks delivered to the skin of one forepaw by subcutaneous needles, and visual stimuli were flashes generated by applying a 4-msec pulse to an incandescent bulb. Averages of responses to fifty stimuli were computed and printed out using an X-Y plotter (Moseley, California). In experiments in which differences in average were examined the computer mode was changed from "Add" to "Subtract" between samples.

In experiments in which the effect of background activity on individual evoked potential was examined, stimuli were delivered regularly at 5-sec intervals, and single oscilloscope sweeps were photographed, showing background electrical activity preceding the application of stimulus, at the moment of stimulus, and the evoked potential resulting from the stimulus. At least 100 records were made at each recording site and on occasions 200 were taken. Prints were made of individual records and classified according to the background electrical activity at the moment of stimulation. In this way five or six records were obtained from each recording site with background activity at the moment of stimulation apparently identical.

The condition of the animal was monitored and recordings made of blood pressure, heart rate, respiration and body temperature. In experiments where

the effect of changing intravesicular pressure was investigated the urethra was cannulated with a large bore polyethylene tube. The tube was connected to a Y piece, one arm of which went to a Statham gauge for the measurement of pressure, and the other to a system for filling the bladder.

The position of the recording electrodes was verified histologically by the technique of Klüver and Barrera (20).

Results

Intracranial hemorrhage following electrode penetration of the brain cannot be eliminated and is inevitable in a small proportion of experiments. To avoid errors due to damage sustained by the animal during the preparation, results were considered only from experiments in which animals maintained a regular pulse and a stable level of arterial blood pressure (usually about 130 mm systolic) for the duration of the experiment.

Relationship Between Background Electrical Activity and Evoked Potentials Recorded from the Hypothalamus of a Cat Anesthetized with Chloralose. In fourteen experiments, interactions between background electrical activity and potentials evoked by cutaneous stimuli were examined and in

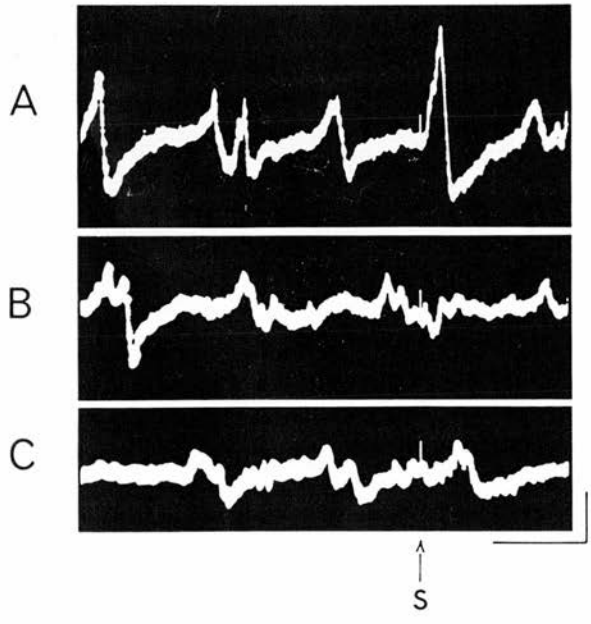

FIG. 1. Cat, chloralose 60 mg/kg. Effect of low amplitude wave activity at recording site on potentials evoked in the hypothalamus by electrical stimulation of the skin of the contralateral forepaw. A. Stimulation in a period of little background activity. B. Stimulation in period of low amplitude wave activity. C. Stimulation in period of low amplitude wave activity. S. Time of shock. Calibrations: 100 μv and 200 msec.

six of these experiments we also examined interactions with potentials evoked by a brief flash of light. In most experiments responses were recorded from the hypothalamus but, responses were also recorded on occasion from the caudate nucleus, the central gray matter, the mesencephalic reticular forma-

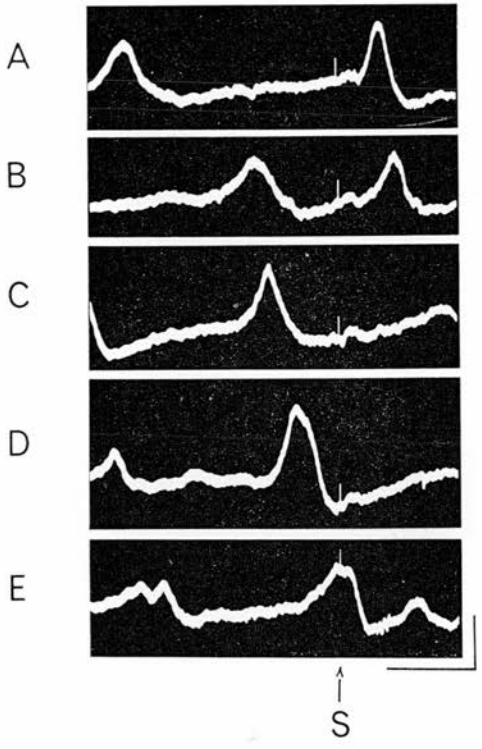

FIG. 2. Cat, chloralose 60 mg/kg. Effect of "spikes" in background activity on potentials evoked in the hypothalamus by electrical stimulation of the skin of the contralateral forepaw. A. Double negative wave evoked in a period of little background activity. B. Reduction and slowing of second negative wave of evoked potential by preceding spike. C. Abolition of second negative wave by preceding spike. D. Initial negative wave of evoked potential present immediately following spike. E. Initial negative wave of evoked potential still present during spike. S. Time of shock. Calibrations: 100 μv and 200 msec.

tion and the thalamic nuclei. The background electrical activity recorded in all these areas was similar to that found in the cerebral cortex (19, 26) with prominent negative going high amplitude excursions, which we term spikes, occurring once or twice a second. In addition, there were bursts of low-amplitude irregular waves, seldom exceeding 50 μv in amplitude and usually lasting about a second.

Both the wave and the spike activity had characteristic effects on evoked potentials. Stimuli applied during periods of wave activity either led to a small evoked potential or none at all (Fig. 1). The interaction between spikes and evoked potentials was usually occlusive (Fig. 2). The evoked potential recorded in the absence of background electrical activity usually consisted of a low-amplitude, initial negative wave, followed by a second negative wave

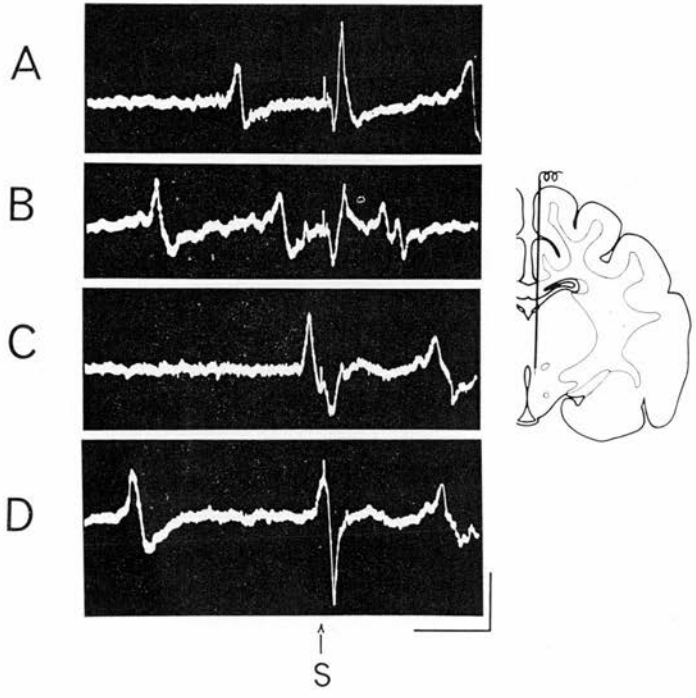

FIG. 3. Cat, chloralose 60 mg/kg. Effect of "spikes" on triphasic evoked potentials evoked in the hypothalamus by electrical stimulation of the skin of the contralateral forepaw. A. Evoked potential recorded in the absence of prior background activity. B. Reduction in negativity and enhancement of positive wave following spontaneous spike. C. Abolition of second negative wave, prolongation and enhancement of positive wave following spike. D. Exaggerated positive wave resulting from stimulation during spike. Inset. Diagram to illustrate electrode placement in dorsal hypothalamus. S. Time of shock. Calibrations: 100 μv and 200 msec.

of much greater amplitude and long duration (Fig. 2A). The occurrence of a spontaneous spike shortly before an evoked potential prolonged and reduced the large, negative wave (Fig. 2B) and eventually abolished it (Fig. 2C,D). The initial negative wave of the evoked potential was relatively stable and could still be seen even when stimulation occurred at the peak of the spike (Fig. 2E).

A second, less common pattern of interaction between spikes and evoked potentials was seen when the evoked potential had a positive deflection separating the negative waves (Fig. 3A). The reduction in the large, negative potential here was accompanied by a progressive increase in the amplitude

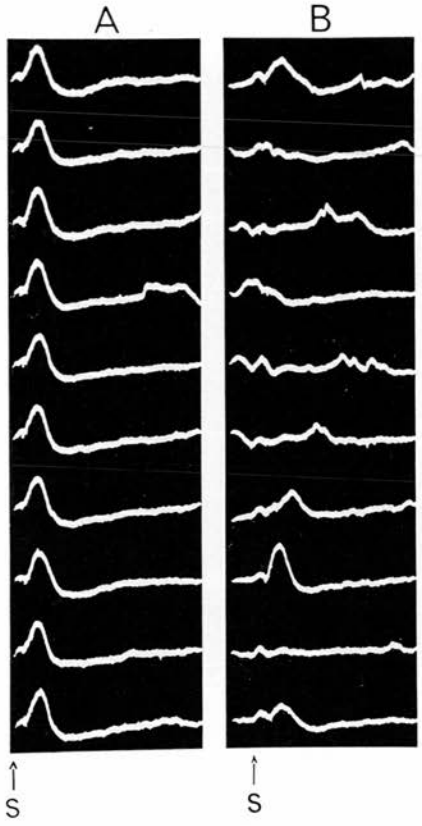

FIG. 4. Cat, chloralose 60 mg/kg. Effect of timing contralateral forepaw stimulation in relation to background activity on the form of evoked potentials recorded in the hypothalamus. A. Responses recorded to ten consecutive shocks timed in relation to background activity. Minimum interval 4 sec. Shock delayed until 80 msec elapsed with no activity in excess of 15 μv negative or positive. B. Next ten shocks delivered at regular 4-sec intervals. Calibrations: 200 μv and 200 msec.

of the early positive wave which could reach several hundred microvolts (Fig. 3B,C,D).

Patterns of interaction of these three types were observed in all recording sites following both visual and cutaneous stimulus. In a few experiments potentials evoked by a flash were of considerable amplitude, usually in

excess of 300 μv, and there was then a reduction of the interval following a spontaneously generated spike during which an interaction pattern occurred.

Effect on Evoked Potentials of Restricting Stimulation to Periods of Minimal Background Electrical Activity. The effects of interactions with background electrical activity and the evoked potential were examined using the circuit devised by McDonald (21). A minimum interval between stimuli of 5 sec was set, but stimuli were then withheld until the background electri-

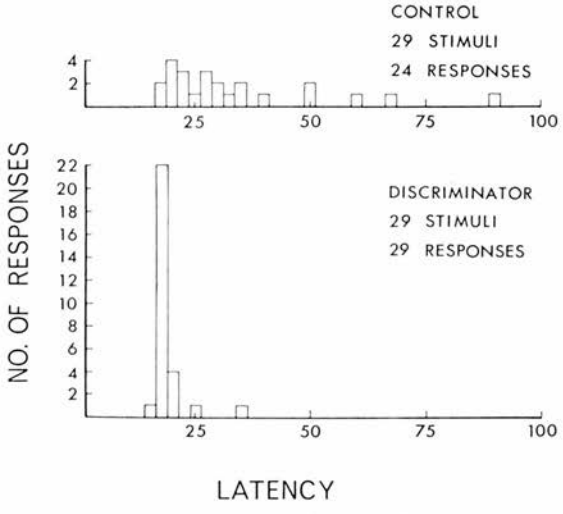

FIG. 5. Cat, chloralose 60 mg/kg. Effect of restricting cutaneous stimulation to periods of little or no background activity on latency of response. Minimum interval between shocks, 4 sec; discriminator setting, 100 sec ± 15 μv. Upper: latencies of twenty-four responses seen following the application of twenty-nine shocks at regular intervals. Lower: latencies (msec) of twenty-nine responses to twenty-nine shocks with timing controlled by discriminator.

cal activity was below specified voltage levels for a fixed interval. The use of this device led to a substantial increase in both the stability form of the evoked potential (Fig. 4), and its latency (Fig. 5), but variations were still present and on occasion a stimulus still failed to evoke a potential. Fluctuations in blood pressure, intravesicular pressure and respiration are known to affect electrical responses recorded from the nervous system (8, 11, 22). We had excluded gross fluctuations in blood pressure as a variable in these experiments and inspection of the pressure pulse showed no obvious relationship within the cardiac cycle, nor did there appear to be any relationship between the evoked potential and phases of the respiratory cycle. Lastly, there did not appear to be any correlation with intravesicular pressure.

Averaged Evoked Potentials and Prolonged Recovery Intervals. In experiments in which averaging is employed it is necessary to present many stimuli, runs of 200 not being uncommon (16). In previous experiments visual inspection had shown that there is a period of about 500 msec following an evoked potential (or the application of a stimulus) in which a second stimulus re-

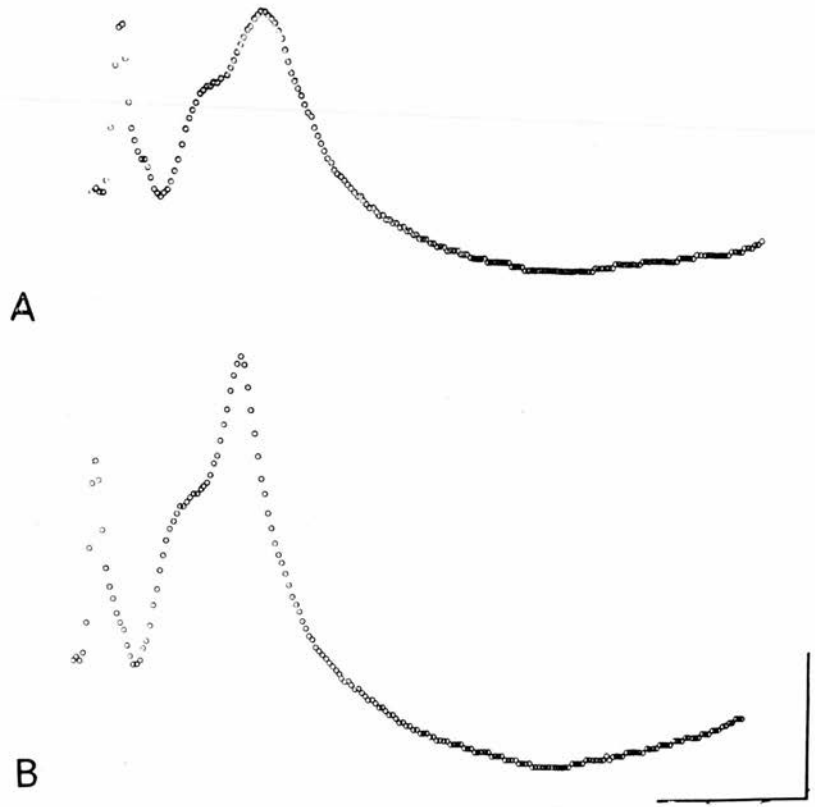

FIG. 6. Cat, chloralose 60 mg/kg. Averages of fifty evoked potentials recorded from the hypothalamus following stimulation of the contralateral forepaw at A, 4-sec intervals; B, 6-sec intervals. Calibrations: 200 μv and 100 msec.

sults in no evoked potential or in a sharply reduced evoked potential (1). To investigate the duration of this occlusive effect, averages were computed from evoked potentials following fifty stimuli delivered at regular intervals ranging from 1 to 10 sec. An occlusive effect was found to exist for intervals up to 4 sec and on occasions for as long as 6 sec (Fig. 6).

The Increase in Stimulus Interval Resulting from Use of the Discriminator Technique. Since the interval between stimuli was clearly of importance it

was necessary to know what prolongation of stimulus interval results from the use of the discriminator. In twenty-seven experiments the discriminator was set so that stimulation would be withheld until 100 msec had elapsed with no background electrical activity in excess of 10 μv positive or negative. The effect of this was to increase the mean interval between stimuli by 2.38

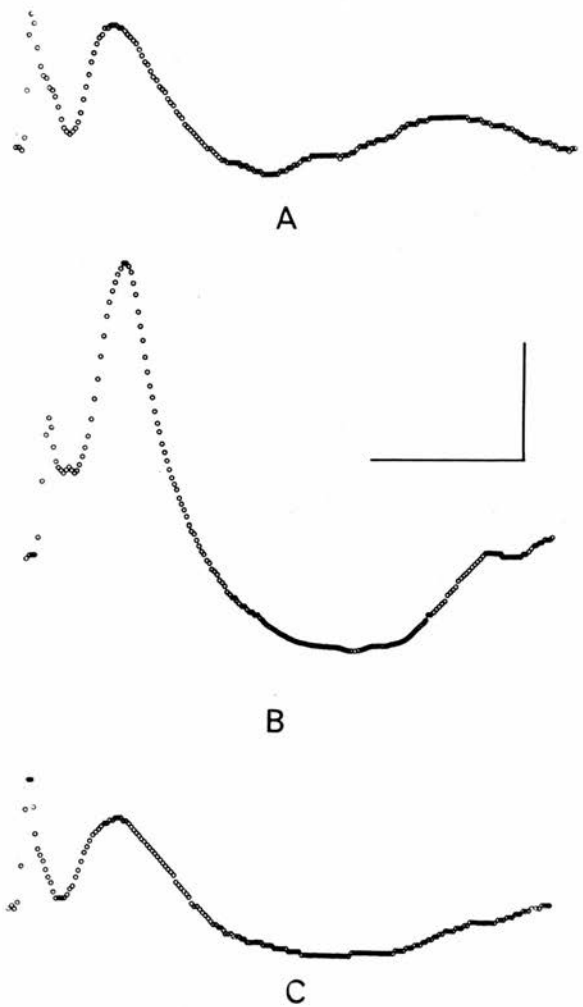

FIG. 7. Cat, chloralose 60 mg/kg. Effect of background activity on averaged evoked potentials recorded in the hypothalamus following fifty shocks delivered to the skin of a contralateral forepaw. A. Shocks at regular 6-sec intervals. B. Discriminator set, minimum interval 6 sec, period of 100 msec with no activity in excess of 10 μv negative or positive then required (mean interval now 8.17 sec). C. Shocks at regular 8.17-sec interval. Calibrations: 200 μv and 100 msec.

sec (standard deviation ± 1.62 sec; range 0.33 to 12.02 sec). Clearly, unless a minimum interval of 6 sec was specified, the use of the discrimination technique could itself increase the amplitude of a computed average potential.

Differentiation of Interval Dependent Alterations in Averaged Evoked Potentials From Those Due to Background Activity. Averaged evoked potentials were computed following fifty shocks or flashes delivered at regular 6-sec intervals. The discriminator was then employed so that following a

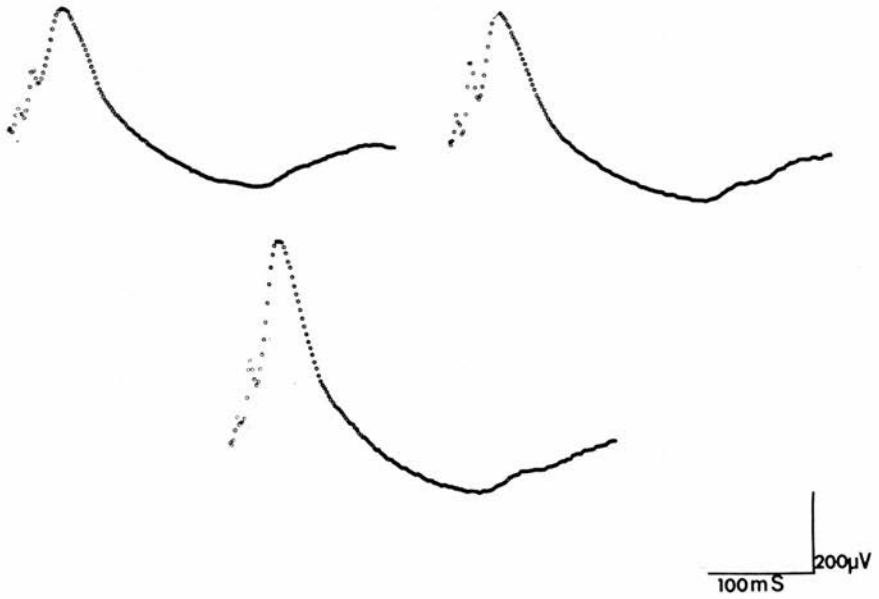

FIG. 8. Cat, chloralose 80 mg/kg. Effect of background activity on averaged evoked potentials recorded in the hypothalamus following fifty shocks delivered to the skin of a contralateral forepaw. A. Regular shocks delivered at 6-sec intervals. B. Discriminator set, minimum interval 6 sec, further period of 100 msec free of activity in excess of 10 μv negative or positive then required. C. Average computed from the stimulus sequence generated in B. Calibrations: 200 μv and 100 msec.

minimum delay of 6 sec, stimulation was further withheld until 100 msec elapsed with no background activity exceeding 10 μv negative or positive, and a further average of fifty responses computed. The mean interval was then calculated and a further fifty stimuli applied at this mean interval and the average again computed. Figure 7 is a family of curves obtained in this way and typifies all the other results obtained in these experiments, showing that the effect on an average of increasing stimulus interval alone is small compared with the effect of discriminating against background activity.

In a second type of experiment account was taken of the random nature of the time increments that the discriminator introduced. An average was again computed from fifty stimuli delivered regularly at 6-sec intervals, and the discriminator then used to generate a second average with stimuli timed in relation to background activity. The trigger pulses produced by the discriminator in this second sequence were recorded on magnetic tape. The tape was then played back to produce a third sequence of stimuli at intervals coinciding exactly with the second sequence controlled by the discriminator. As Fig. 8 shows, these experiments clearly indicate the consider-

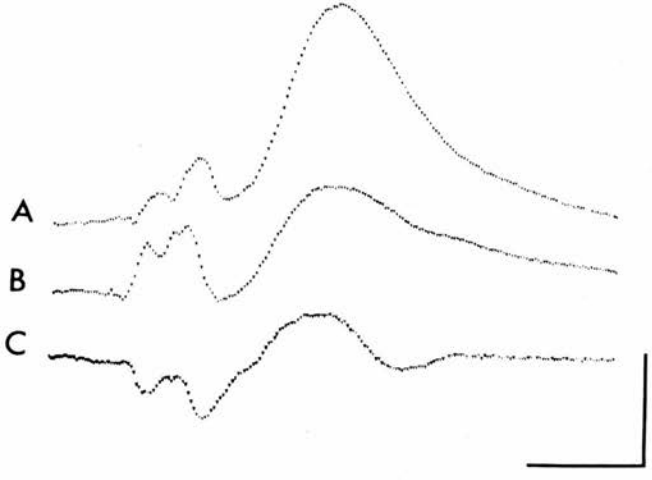

FIG. 9. Cat, chloralose 60 mg/kg. Magnitude of effect of the discriminator. A. Average of fifty evoked potentials following a shock delivered to the skin of a contralateral forepaw. Discriminator set, minimum interval 6 sec, discriminator set for further period of 100 msec with no activity in excess of 10 μv positive or negative. B. Average of potentials evoked at regular 6 sec intervals. C. Computed difference between the two averages. Calibrations: 50 μv; 50 msec.

able effect of timing stimuli in relation to background electrical activity. A quantitative measure of this effect was obtained by computing the differences between evoked potentials obtained with and without the use of the discriminator and results of such an experiment are shown in Fig. 9.

Distortion of Form in Averaged Evoked Potentials. The evoked potential recorded from the hypothalamus of the chloralose-anesthetized cat normally consists of an early low-amplitude brief negative deflection, followed by larger prolonged negative deflection. The first deflection is relatively stable and interaction usually affects the second large negative wave. The difference in stability, as might be expected, is reflected in the form of the averaged evoked potential for, in averaging without respect to background activity the

initial negative wave becomes exaggerated. When the discriminator is utilized, as Figs. 7 and 8 show, the computed wave form exhibits a large late component.

Discussion

The present experiments show that the form of an averaged evoked potential in the chloralose-anesthetized cat can be substantially affected by background electrical activity. Interactions between background activity and evoked potential are not confined to this preparation. Interactions between evoked potentials and background activity have been previously reported, although the detail of the interaction has been variously described. Bartley, and Bartley and Bishop (5, 6), recording from the visual cortex of amytal or magnesium anesthetized rabbits, ascribed the variation to a summation of the evoked potential with background electrical activity. Forbes and Morrison (17) found the secondary cortical response recorded from the somatosensory cortex of the deeply anesthetized cat, to be abolished or reduced by a preceding spontaneous wave of cortical activity. Raab and Kiang (23, 24) and Raab, Kiang and Brown (25) described a significant inverse relationship between the integrated cortical activity preceding stimulation and the amplitude of the evoked potential in the auditory cortex of the anesthetized cat. More recently, Bindman, Lippold and Redfearn (9), examined the causes of variation in potentials evoked in the cerebral cortex of the lightly anesthetized rat, following peripheral nerve stimulation, and showed these to be related to the existence of a d-c potential modified from intralaminar thalamic nuclei. Such interactions between evoked potentials and background activity are not confined to the anesthetized animal, for Bachtereva, Zontov (7), Cobb and Dawson (12) and Calloway and Layne (11) have similarly described variations in the evoked potential recorded from the skull of nonanesthetized man related to background electrical activity.

In choosing to perform our experiments on the chloralosed cat and by largely restricting recording to the hypothalamus, advantage was taken of the knowledge of the patterns of variation of evoked potentials established under the same experimental conditions. When averages are computed from electrodes placed on the scalp of the intact human, four additional problems are encountered. Firstly, there is the problem of signal-in-noise. This means that the raw data from which averaged are being computed may not be available for inspection. Secondly, in order to record without excessive interference, many recordings are made with bipolar electrodes. Under these conditions what is being recorded is the difference in potential existing between two brain regions, so that an additional set of variables is introduced into the record. Thirdly, although it is well known that background activity

does interfere with the evoked potential (7, 12) as does cardiac cycling (11) the details of the interaction are not well known. Lastly, the conscious human being is a difficult experimental animal showing reduction of response to successive batches of stimulus presentations (10), and, if visual stimuli are employed, errors due to loss of fixation (12). The chloralosed cat offers an experimental situation in which the variables are fewer and more clearly demonstrated but caution must be employed in transposing the results presented here to any other situation.

In considering how it is that stable averaged evoked potentials can be found under the recording conditions met with in human work we must assume that fluctuations are present in the background activity as well as in the evoked potentials, and that in a sufficiently large sample such fluctuations become randomized. Thus a change either in evoked potentials or background activity will lead to a change in the averaged signal. We have previously shown that the changes in evoked potentials which can be seen after the injection of small amounts of pentobarbitone sodium to a cat already anesthetized with chloralose may well be primarily due to a reduction in background activity (3, 4). It would thus seem essential that before any change in an averaged evoked potential is assigned to a given experimental maneuver or assigned to any neural function, two things must be ascertained. Firstly, it must be established that the change is not due to a change in background electrical activity and secondly, that the wave form under examination has biological reality and is not a pattern generated by the technique.

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(405) V. C. ABRAHAMS and S. FALCHETTO*

Department of Physiology, Queen's University, Kingston.

Hind leg ataxia following section of a nerve to a neck extensor muscle.

In the course of our experiments on cats concerned with the directed movements of the head and eyes we sectioned the nerve to an extensor muscle of the neck, the biventor cervicis. The postural outcome of such nerve sections was a minimal interference with head movement, but a surprising ataxia involving the hind quarters. Whilst animals were able to make adequate progress in a straight line, they showed considerable instability on rising to make a movement, or when making changes in direction. The ataxia was not permanent, and is not normally distinguishable after the fifth post-operative day. However, even some weeks after the nerve section special testing showed the existence of some deficits in jumping. Control experiments eliminated the possibility that the phenomenon originated as an after effect of anaesthesia, and the explanation of our findings may be due to integrated long spinal systems which are concerned with progression movements and head movements.

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(204) V. C. ABRAHAMS
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Cervico-lumbar spinal interactions involving a brain pathway.

In experiments reported last year we showed that section of the nerves to a neck muscle, biventer cervicis, has minor consequences for head movement, and leads to a pronounced, but transient ataxia of the hind legs. In chloralose anaesthetised cats, stimulation of biventer cervicis nerve 30 msec prior to stimulation of a hind leg nerve causes considerable facilitation of early (proprioceptive) spinal reflexes, and an abolition of late spino-bulbar-spinal reflexes. The latter may be inhibited for 500 msec. We have previously described a short latency proprioceptive pathway from biventer cervicis nerve to the superior colliculi, but this does not seem concerned with the interaction, as stimulation of the superiorcolliculi has only a slight effect. Stimulation of the central grey matter and the area of the red nucleus has marked facilitatory effects on lumbar reflexes, but since extensive lesions of these structures are without effect on the interaction, it is unlikely that the pathway involves them. Some structures critical to the interaction are supra-tentorial, for the interaction does not survive decerebration. Indeed, the pathway may be most complex, as the interaction can be reduced substantially by an auditory stimulus.

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A spinal-brain-spinal reflex and hind limb ataxia of cervical origin. V.C.Abrahams, D.Butler* and S.Falchetto*. Dept. of Physiology, Queen's Univ., Kingston, Ontario, Canada.

An ataxia of the hind limbs resembling dorsal root ataxia has been found to persist for 5 to 7 days following unilateral section of the nerves to superficial head extensor muscles. In chloralose anaesthetised cats stimulation of the central end of these nerves leads to potentiation of early components of lumbar and sacral reflexes. The pathway underlying the potentiation has a central course for spinal section at C1 abolishes the potentiation. The proprioceptive relay from head extensor muscles to the superior colliculus recently described by us is not involved in the pathway. Since the potentiation is abolished in the acutely decerebrated cat the pathway presumbly has a supra-tentorial course. (Supported by the Medical Research Council of Canada and the Wellcome Trust)

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DETERMINATION OF "SIGNIFICANT" DIFFERENCES IN UNIT DISCHARGE PATTERNS

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The individual action potential of a nerve cell or axon is readily recorded, and because of its significance as an information carrying system has been the target of a number of computer based data reduction and analysis techniques. In particular, 2 techniques, the post-stimulus time histogram (PSH), and pulse interval analysis (PIP) have been extensively used for the examination of patterns of action potential discharge following the application of stimuli. Justification for the use of these methods has been an apparent need to overcome the variability in signal that may occur. They also reduce a large amount of data to a "manageable" form.

Our experiments have been concerned with the ability of a neural system to recognize a change in an applied stimulus. This makes it necessary to be able to look at a unit discharge to see if the discharge has been altered by a change in the stimulus. Large changes are easily detected by eye, and both the PSH and the PIP can be used under some conditions where changes are less readily distinguishable. Unfortunately, these latter data processing techniques, apparently a formidable addition to the physiologists' armentarium, do have some basic weaknesses. When an animal is subjected to repeated sensory stimuli, the action potentials recorded from various brain structures as a result of such stimulation may change progressively with time. Such a change is shown in Fig. 1 where a variety of responses were obtained from a cell in the superior colliculus of the cat following a regularly repeated presentation of a flash of light. When the same data is compressed into a PSH as shown in Fig. 2, information relating to the progressive changes is lost as is the case when the same data is used to generate a PIP (Fig. 3).

Clearly, the major deficit of the PSH and the PIP is that they do not treat time as a dependent variable. In many neurophysiological experiments changes with respect to time may be critical. To overcome this handicap a technique has been developed whereby successive responses are catenated so that the entire group of responses form a pattern unique to that stimulus series. This pattern can then be compared to the pattern produced by another stimulus series to see if it is different.

This idea can be grasped by referring to Fig. 4 which is a visual representation of part of the pattern of the unit responses discussed above. The arrow represents the time when successive stimuli were applied and each spot represents a cell action potential. Only the first 350 msec. of the discharge following each stimulus are shown. Thus it can be seen that any pattern of unit activity can be reduced to a 3-dimensional array which is a complete representation of the original data. The same data can be expressed in one dimension by noting the successive intervals between unit discharges.

To treat this data so that two patterns may be compared, the pattern of action potentials is first pre-processed. This is done by scanning equal time segments within each catenated time vector. The corresponding elements of the vectors are then scanned, element by element, and a counter is incremented each time that they are different. The final value of the counter corresponds to the sum of the squares of the differences (SS) between the two patterns. The value of SS increases as the vectors become more different.

The "significance" of the magnitude of SS can be determined by comparing it to the distribution of sums obtained from vectors containing elements filled at random. This distribution is calculated from either the Binomial expansion or the Normal Distribution depending on the total number of filled elements in the two vectors.

The ideal method to determine "significance" would be to construct a histogram of the true distribution of the SS by recording and comparing a large number of patterns obtained from the preparation under control conditions. Unfortunately this procedure would leave no time for the test procedure in many experiments. As well, the average frequency of firing during the control period would have to be similar to that during the test period.

The method described here should prove useful for making fast comparisons of response patterns with a minimum of human intervention. It will be able to detect gross differences readily, but will likely be unable to detect subtle changes. However, it should not be subject to bias, fatigue and/or pregnancy.

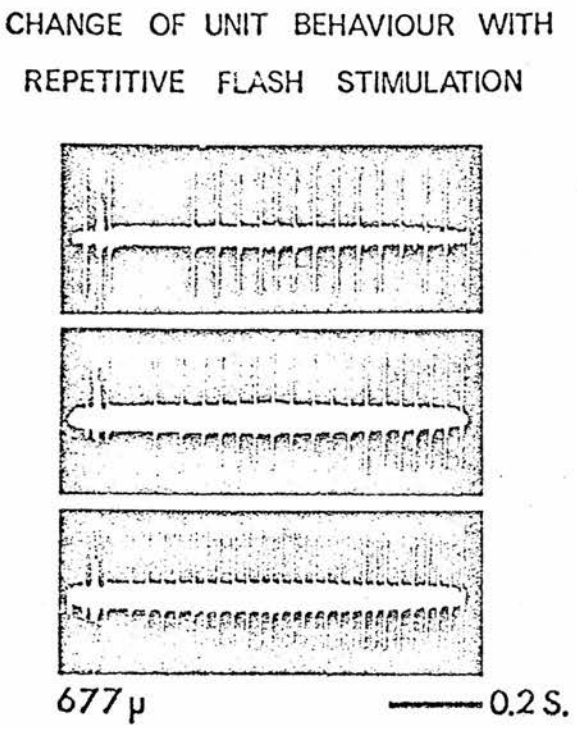

FIGURE 1

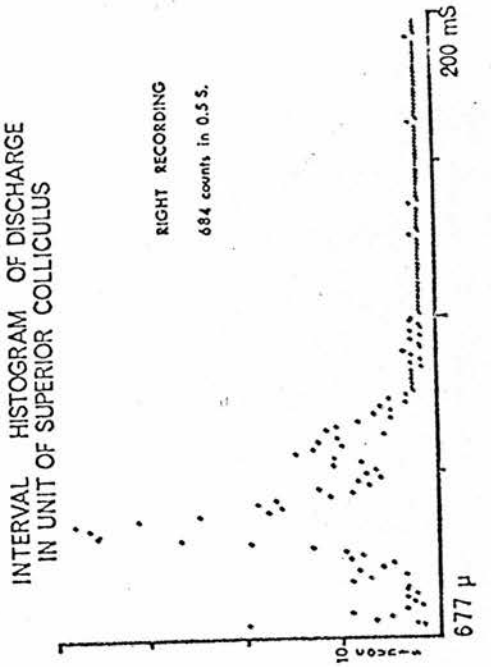

FIGURE 3

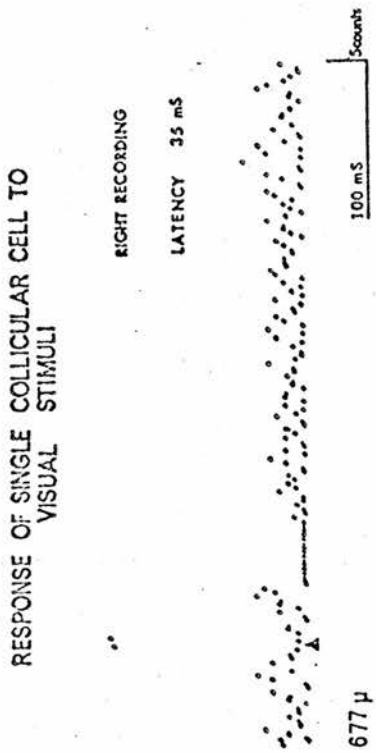

FIGURE 2

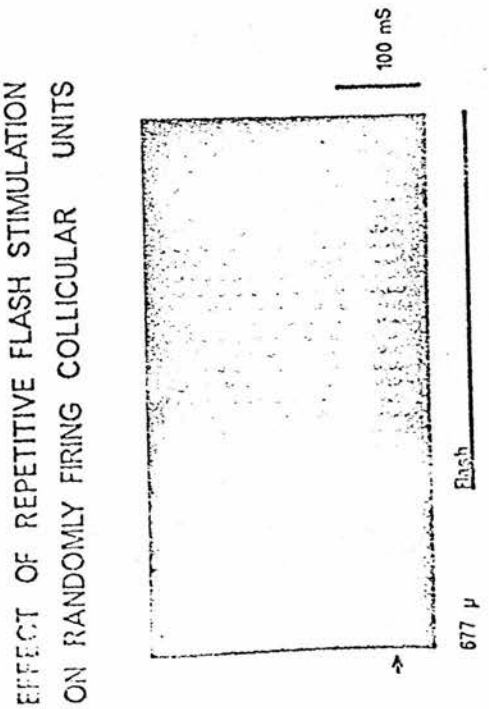

FIGURE 4

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1968

ATAXIA OF THE HIND LIMBS OF THE CAT FOLLOWING SECTION OF NERVES TO A NECK EXTENSOR MUSCLE AND A POSSIBLE REFLEX SUBSTRATE.

V. C. Abrahams & S. Falchetto. Dept. of Physiology, Queen's University at Kingston, Ontario, Canada.

In cats the section of nerves to one of the extensor muscles of the head, biventer cervicis, is followed by an ataxia of the hind legs which persists for 5 to 7 days. In attempting to find the cause of this ataxia we have found that in the cat anaesthetised with chloralose, stimulation of the central end of the nerves to biventer cervicis will grossly potentiate early spinal reflexes recorded electrically at low lumbar and sacral levels. The interaction is not due to a completely intraspinal mechanism, for it is abolished by section of the neuraxis at C1. Nor does the proprioceptive pathway from the biventer cervicis nerves to the superior colliculus previously described by us (Abrahams & Falchetto, Physiologist 9,1966) seem involved, for it has not proven possible to modulate the spinal reflexes by direct electrical stimulation of the superior colliculi. It is likely that higher centres are involved, for acute decerebration consistently abolishes the interaction between neck nerves and spinal reflexes, and the interactions do not return even after several hours. (Supported by grants from the Medical Research Council of Canada and the Wellcome trust).

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202

V.C. ABRAHAMS, D. BUTLER* and J. DAYNES*. Department of Physiology, Queen's University, Kingston. A cortical relay site in cervico-lumbar reflex interactions in cats.

In previous experiments concerned with the ataxia of the hind legs that follows section of a nerve to a neck extensor muscle, a potent cervico-lumbar reflex interaction has been described which utilises supratentorial structures. Two regions of the cerebral cortex have been found to receive proprioceptive input from the forepaws and from neck muscle, the post-cruciate dimple and the anterior pole of the suprasylvian gyrus (Oscarssen & Rosen, J. Physiol. 169, 924, 1963; Landgren & Sylfenius, Acta physiol. scand. 74, 340, 1968). Stimulation of both these cortical regions affects lumbo-sacral segmental reflexes in a manner similar to that produced by cervical input. However, ablation of the post-cruciate dimple is without effect upon cervico-lumbar interactions, but ablation of the small region of the anterior pole of the suprasylvian gyrus receiving neck proprioceptive input sharply reduces or abolishes cervico-lumbar interactions. It is concluded that this region of the suprasylvian gyrus is critical to cervico-lumbar interactions. Since this region receives input from vestibular, auditory and visual systems, as well as from proprioceptive systems, it may represent a cortical region critical in the integration of postural reflexes.

(Supported by M.R.C. grants.)

The Physiologist, 12, (1969), 154.

PROPRIOCEPTIVE RELAYS TO THE CEREBRAL CORTEX, AND THEIR SIGNIFICANCE IN INTRASPINAL ORGANIZATION. V. C. Abrahams, D. Butler* and J. Daynes*, Department of Physiology, Queen's University, Kingston, Ontario, Canada.

Previous experiments in chloralose anaesthetized cats have shown that a profound and prolonged facilitation of lumbosacral monosynaptic reflex activity follows stimulation of neck and forepaw muscle nerves. Structures critical to the interaction lie supratentorially. It has now been found that electrical stimulation of the post-cruciate dimple and the suprasylvian gyrus (both cortical regions receiving proprioceptive input) has similar effects on lumbosacral reflex excitability to neck and forepaw nerve stimulation. Ablation of the post-cruciate dimple only transiently affects cervico-lumbar interactions, whereas ablation of the anterior pole of the suprasylvian gyrus reduces or abolishes cervico-lumbar interactions for the duration of acute experiments. Thus the suprasylvian gyrus may play some role in integrating spinal reflex excitability and hence may be regarded as part of the cortical mechanisms for regulating posture. Such a view is supported by the known connections in this region which include input from many sensory systems, including the vestibular system.

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Pathways involved in potentiation of lumbo-sacral reflexes by biventer cervicis nerve stimulation. V.C.Abrahams, Dept. of Physiology, Queen's University, Kingston, Ontario.

Previous experiments have shown that an ataxia of the hind legs follows section of the nerves to biventer cervicis, a neck extensor muscle. In chloralose anaesthetised cats stimulation of the biventer cervicis nerve led to a substantial potentiation of monosynaptic reflexes, but it seemed unlikely that this mechanism underlay the interaction, for a similar monosynaptic facilitation was found to follow stimulation of fore-paw skin and muscle nerves. What now appears unique to the interactions following biventer cervicis nerve stimulation is that supratentorial structures are essential for their demonstration. In spinal cats descending interactions follow fore-paw nerve stimulation, but not stimulation of the biventer cervicis nerves. In decerebrate cats interactions readily follow fore-paw nerve stimulation, but if present from biventer cervicis nerve stimulation, they are barely discernable. Thus a unique characteristic of the interactions originating from biventer cervicis nerve stimulation is their dependence on higher brain structures. Perhaps this reflects the importance of proprioceptive input from this muscle in giving information concerning the position of the body with respect to the head, and in initiating postural reflexes related to changed head position. The input does not however seem related to body on head rotation during falling, as cats showing an ataxia following biventer cervicis nerve section still show normal righting reflexes during a fall.

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HIND LEG ATAXIA OF CERVICAL ORIGIN AND CERVICO-LUMBAR SPINAL INTERACTIONS WITH A SUPRATENTORIAL PATHWAY

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(*Received* 6 *February* 1969)

SUMMARY

1. Unilateral section of the nerves to an extensor muscle of the head, biventer cervicis, leads to an ataxia of the hind legs in cats.

2. In chloralose anaesthetized cats, shocks to the central end of the cut biventer cervicis muscle nerves leads to direct discharges over lumbo-sacral ventral roots, to a facilitation of monosynaptic reflexes in flexor and extensor muscles and to a prolonged inhibition of spino-bulbo-spinal reflexes. Such effects are not unique, but follow stimulation of skin and muscle nerves of the forepaw.

3. Supratentorial pathways are involved in descending spinal interactions in the chloralose anaesthetized cat, but are more critical for interactions taking origin in biventer cervicis nerve than in forepaw nerves.

4. In non-anaesthetized spinal cats showing interactions from forepaw nerves, no interactions could be found taking origin in biventer cervicis nerves. Descending interactions from forepaw nerves could be reduced or abolished by anaesthetic doses of chloralose.

5. The rich pathway from biventer cervicis nerve to the superior colliculus does not appear to be involved in the descending interactions reported here as neither stimulation nor ablation of the superior colliculi has any effect on descending interactions.

INTRODUCTION

In retrograde degeneration experiments to locate the motoneurones of a neck extensor muscle, biventer cervicis, the nerves to the muscle were sectioned in a series of cats. In addition to the expected deficit of head

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movement, the nerve section led to a completely unexpected, and often quite severe ataxia of the hind legs. Ataxia following interference with cervical nerves is not a completely new finding, for Cohen (1961), in experiments on monkeys found, profound disorders of gait to follow local anaesthesia of the upper three cervical dorsal roots.

We have found it possible to produce a potent cervico-lumbar interaction from stimulation of the biventer cervicis nerve, characterized by the fact that it can only be demonstrated adequately in animals with intact brains. Unlike other descending cervico-lumbar interactions (Lloyd, 1942; Lloyd & McIntyre, 1948; Gernandt & Gilman, 1960) the mechanism is not intraspinal, and unlike spino-bulbo-spinal reflexes it is not readily demonstrated in the decerebrate cat (Takagi, Matsumura, Yanai & Ogiu, 1955; Shimamura, 1961; Shimamura & Livingston, 1963; Shimamura, Mori, Matsushima & Fujimori, 1964; Shimamura & Akert, 1965; Shimamura, Mori & Yamuchi, 1967). Whether it is interference with this descending system that leads to the consequences of biventer cervicis nerve section cannot be inferred from these experiments.

METHODS

Experiments were performed on adult cats weighing 2·5 kg or more. In experiments with the nervous system intact and in which electrical recordings were made, chloralose was used as the anaesthetic, given in a single intravenous dose of 60–80 mg/kg following the induction of anaesthesia with ethyl chloride and ether. Spinal cats were prepared under ether anaesthesia. When surgical procedures were performed from which the animal was to recover, normal aseptic techniques were used and the animals were anaesthetized with a single I.P. dose of sodium pentobarbital, 30 mg/kg (Nembutal, Abbott). To abolish movement resulting from nerve stimulation in recording experiments, I.V. gallamine triethiodide (Flaxedil, Poulenc) was given in an initial dose of 10–15 mg/kg, supplemented as necessary and the animals were then maintained on positive pressure ventilation. In all experiments arterial blood pressure was continuously recorded with a Statham gauge connected to one femoral artery. Body temperature and respiration were also continuously monitored and recorded.

The extensor muscles of the cat's head consist of a deep and superficial group. Our experiments have been conducted exclusively on nerves entering a muscle of the superficial group, the biventer cervicis (Elliott, 1935). Nerves taking origin in cervical segments 1, 2 and 3 enter this muscle after some branching. In those experiments in which the consequences of nerve section were investigated, the two major muscle nerves taking origin in C 2 and C 3 were sectioned unilaterally, and on occasion the C1 nerve on the same side was also sectioned. Cine records of the animals were made before operation, and daily after operation for a period of up to 28 days. Normally records of movement and posture were made in Super 8 format at 18 frames per second. Records of cats falling were made with a 16 mm camera at 64 frames per second.

Recordings from spinal roots or peripheral nerves were made using conventional bipolar platinum wire electrodes. All nerve and nerve roots were immersed in pools containing a protective silicone oil (Medical fluid 555 or 360, Dow–Corning Silicones). No special precautions were taken to warm the pools, but the animal's rectal temperature was maintained at $37 \pm 1°$ C. Where spinal roots or peripheral nerves were stimulated, electrodes similar to the recording electrodes were used. Averages of responses were computed from twenty-five

successive signals using a Computer of Average Transients (Mnemotron Corporation). The amplified signal was fed to the averaging computer through an interface that allowed the signal voltage level to be optimized. For intracerebral stimulation a bipolar concentric electrode was introduced using conventional stereotaxic technique and the core made the cathode. The same electrode was used for making lesions using a Grass Model LMI RF lesion-making device for an appropriate period.

When investigating the effect of brain section at the midcollicular level the spinal cord and nerve dissections were first completed. The section was then performed after the carotid arteries were clipped and there was sufficient mobilization of one occipital lobe to allow a view of the colliculi. Brain anterior to the section was not removed, but by sparing a small amount of tissue at the base of the brain overlying the basilar artery and the most lateral extent of the contralateral superior colliculi, bleeding was kept to a minimum. The brains were fixed *in situ* at the termination of the experiment and examined to determine the extent of the lesion.

For histological examination the brains and cervical cords were first fixed by perfusion and then further immersed in formalin for a week and embedded in celloidin or paraffin. Sections were cut at 8–12 μ and stained with Haematoxylin-van Geisen, Luxol Fast Blue-Haematoxylin or with Nissl stain.

The following abbreviations will be used for nerves stimulated or sectioned in these experiments: biventer cervicis (BC); nerve to the lateral head of gastrocnemius (LG); nerve to medial head of gastrocnemius (MG); posterior tibial (PT); peroneal (PE); nerve to tibialis anterior (TA); the superficial radial nerve (SR); the dorsal interosseus (DI). The nomenclature is that of Elliott (1935).

RESULTS

Motor and postural consequences of unilateral section of nerves to biventer cervicis. In twenty-one cats nerves to BC muscle were sectioned unilaterally at aseptic operation. Such nerve sections led to a deficit of head movement, but not as great as expected. The animal still retained the ability to raise its head, although in the few days following operation it avoided this movement and reached objects above head level by raising its whole body. The head was not held in an abnormal position, but rotation of the head, like elevation, was avoided. The deficit was not striking, and for those who were not acquainted with the nature of the operative procedure, not readily discernible. In contrast, all animals subjected to BC nerve section suffered some ataxia of the hind legs apparently unrelated to the disturbance of head movement, and not apparently compensating for that disturbance. The degree of ataxia varied from animal to animal; approximately one third of cats had trivial defects, showing an occasional bad landing after jumping, or exhibiting occasional difficulties in executing a rapid turn. In a further one third of the animals the defect was more marked, and the animals had a distinctly odd gait with a swinging motion of the hind legs and a swaying motion of the hind quarters, particularly when executing a turn. When landing from a jump these animals usually staggered to one side (the side of the lesion) for a few paces. In the remaining one third the deficit was pronounced. The animals initially avoided movement and when cajoled into standing they would adopt a posture with the head down and

the hind legs spread wide apart. They would stand in this position without appreciable movement for many minutes and then slowly resume a lying posture. If persuaded to move they showed a staggering gait of the hind legs and would frequently collapse to one side or another when executing a turn. The phase of gross disturbance of movement was not prolonged and was generally related to the severity of the disorder. Most cats showed considerable recovery 7–10 days or so after operation. Even cats which appeared to have recovered still showed deficits when made to perform a task such as jumping. Many cats showed an unusual, exaggerated stretching behaviour after nerve section. After a period of rest most normal cats will stretch their hind legs when taking their first few steps. In cats following section of BC nerves this behaviour can become grossly exaggerated, and cats sometimes progressed with virtually every step incorporating a stretch of the hind legs.

Despite the disorders of static posture and progression, righting reflexes during a fall were still normal. Cats dropped from a height of six feet with their feet upwards righted themselves rapidly and landed with their bodies in a normal position. The landing itself was often abnormal with the animals lurching to one side.

The possibility that the ataxia of the hind legs could have been nothing more than an unusual after-effect of anaesthesia was examined in three cats in which the nerves to BC were exposed but not sectioned. Twenty-four hours following operation none of these cats exhibited ataxia of the hind legs or any other postural defects. In another six cats, when the local anaesthetic, xylocaine, was injected into the BC muscle, an unsteadiness of gait of the hind legs was seen for an hour or so.

Intraspinal descending connexions. Experiments by Lloyd (1942) and Lloyd & McIntyre (1948), and Gernandt & Gilman (1960), have shown that conditioning shocks to the brachial plexus and to forepaw skin and muscle nerves produce discharge over lumbosacral ventral roots and can affect reflex excitability in lumbo-sacral segments in spinal and decerebrate cats. Their major findings were readily confirmed in a series of ten spinal cats. Monosynaptic reflexes were found to be first inhibited by conditioning shocks to forepaw nerves and then doubled in amplitude when the interval between conditioning and test stimulus was increased. It was also found that such effects were most readily demonstrable following muscle nerve stimulation. In the same experiments the effect of shocks to BC nerves was examined. In no experiments was any direct discharge seen over lumbosacral ventral roots following BC stimulation and in eight out of ten experiments no modification of lumbosacral reflexes could be obtained as a result of prior BC stimulation. In two experiments there was a suspicion of a weak facilitation of monosynaptic reflexes but this was not

statistically supportable. In two of the cats in which no responses to BC stimulation could be obtained strychnine was given I.V. in a dose of 0·1 mg/kg. Despite the tremendous facilitation of segmental reflexes that then occurred, in one animal BC stimulation was still without effect, and in the second only some slight facilitation of 5–10 % of the monosynaptic reflex was present.

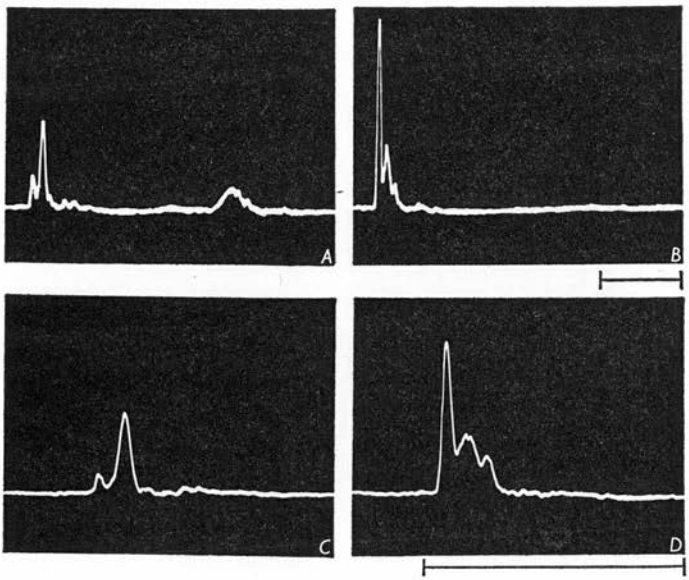

Fig. 1. Cat, chloralose 60 mg/kg. Recordings from C7 ventral root at two different sweep speeds. *A* and *C*, control reflexes following stimulation of peroneal nerve. *B* and *D*, effect on reflexes of biventer cervicis nerve stimulation 30 msec before peroneal nerve stimulation. Time marker 10 msec.

Descending spinal interactions in the chloralose anaesthetized cat. Recent experiments have shown that consistent spino-spinal effects may be exerted through pathways with a brain course in the chloralose anaesthetized animal (Shimamura *et al.* 1964; Shimamura & Yamauchi, 1967). We have looked for spino-spinal interactions in a total of seventy-four chloralose anaesthetized cats. Descending effects are readily demonstrated in such preparations, but unlike the spinal animal, not only are effects readily elicited following forepaw nerve stimulation, they are now readily elicited from BC stimulation. Conditioning shocks to BC lead to an inconstant discharge over lumbosacral ventral roots similar to that reported by Lloyd (1942) in the spinal cat, but the latency and duration of the response are both considerably longer. The latency ranged from 22 to 30 msec and the duration up to 20 msec. The amplitude of the response was variable, but never exceeded 1 mV and usually lay in the range 2–500 μV. The response

was frequency dependent, and reducing the interval between shocks below 3 sec sharply reduced the amplitude of response. In sixty-three of the seventy-four animals it was found that reflexes originating either from dorsal root or hind leg nerve stimulation were grossly affected by prior stimulation of BC, or of the forepaw nerves including SR or DI. The most obvious effect, shown in Fig. 1, was a potentiation of the monosynaptic reflex, which could be as great as 10 times control levels. Substantial

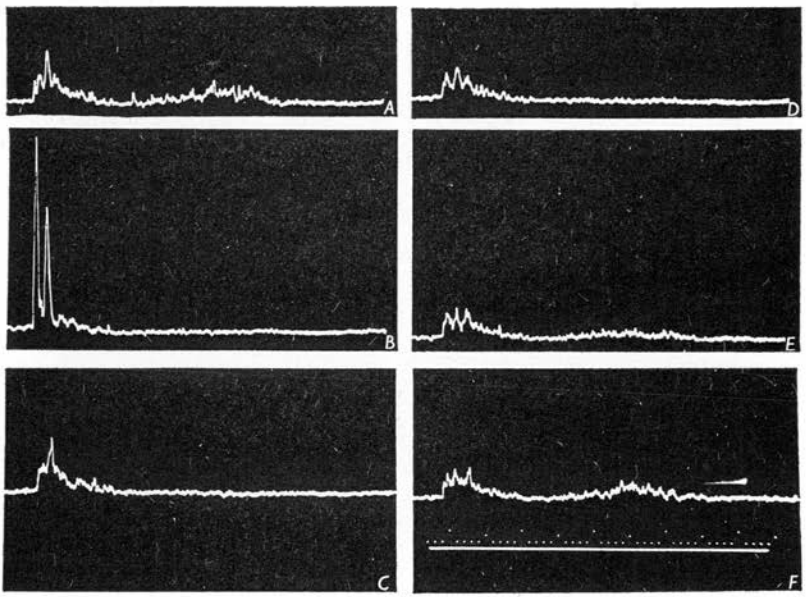

Fig. 2. Cat, chloralose 60 mg/kg. Effect of varying interval between conditioning shock to biventer cervicis on segmental and spino-bulbo-spinal reflexes recorded from L7 ventral root following posterior tibial nerve stimulation. Time marker msec; raised marks 5 msec. *A*, control, *B*, interval 30 msec, *C*, interval 100 msec, *D*, interval 130 msec, *E*, interval 150 msec, *F*, interval 170 msec.

potentiation of monosynaptic reflexes was shown by Lloyd & McIntyre (1948) to follow forepaw nerve stimulation in the spinal cat. Such facilitation was apparent when 10 msec separated conditioning and test shock, reached a peak very rapidly and the whole effect was over in about 25 msec. In our experiments the onset of the facilitation was later, rarely being present earlier than 25 msec after the conditioning shock and although a peak usually occurred about 30 msec the effects were prolonged and usually exceeded 50 msec.

The potentiation of monosynaptic reflexes was accompanied by variable effects on polysynaptic reflexes. They were depressed in twenty-five experiments, elevated in eleven, and little change could be discerned in the

remaining experiments. In a number or experiments a very early polysynaptic component was sharply facilitated and this caused the appearance of a double spike in the record (Fig. 2*B*). In contrast to the relatively variable effects on polysynaptic components the effect on the SBS reflex was a consistent and prolonged inhibition (Fig. 2). First obvious 10 msec following a conditioning shock, this inhibition was normally still evident 250 msec following the conditioning shock, and in about half the experiments was still present 400 msec after the conditioning shock.

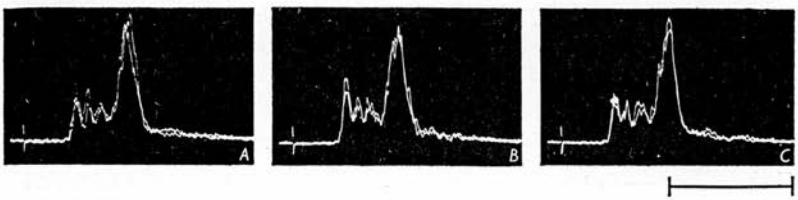

Fig. 3. Cat, chloralose 60 mg/kg. Spinal section at C1. Each panel consists of a pair of superimposed reflexes recorded from S1 ventral root following lateral gastrocnemius nerve stimulation. One trace in each pair was preceded by a conditioning shock to *A*, biventer cervicis nerve; *B*, superficial radial nerve; *C*, dorsal interosseus nerve. Time marker 10 msec.

The effects that followed BC nerve stimulation were also seen when forepaw skin and muscle nerves were stimulated. Direct responses could be recorded from lumbosacral ventral roots, although with a latency 2–5 msec less than following BC stimulation. Effects on monosynaptic and polysynaptic reflexes as well as SBS reflexes paralleled those seen following BC stimulation. In eight experiments after demonstrating the presence of interactions from BC and other forelimb nerve, the spinal cord was sectioned at C1. As was expected this led to a loss of direct responses and reflex interactions following BC stimulation. What was not expected, but was also found, was that direct responses and interactions could no longer be obtained from forepaw skin and muscle nerve stimulation (Fig. 3). These results raised the possibility that in addition to its known action in the spinal cord on lengthening and reducing the amplitude of dorsal root potentials (Eccles, Schmidt & Willis, 1963), chloralose may inhibit intraspinal interactions. To investigate this further, chloralose 60 mg/kg was given I.V. to a series of nine spinal cats. In two experiments this led to the loss of descending interactions, but in the remaining seven, interactions although reduced, were not abolished. In none of these cats was it possible to demonstrate any interaction following BC nerve stimulation.

Specific reflexes affected by descending interactions. Lloyd (1942) and Lloyd & McIntyre (1948) found that in the spinal cat descending interactions not only depended on the nerve being stimulated, but clear differences existed between effects originating from flexor and extensor muscle

nerves. Experiments were performed on fourteen chloralose anaesthetized cats in which spinal reflexes taking origin in a variety of flexor and extensor nerves were examined. The nerves stimulated included TA, MG, LG, and PE. In all experiments on chloralose anaesthetized cats, the effects of prior stimulation of a forepaw or BC nerve were as previously described (Fig. 4) and no differences were found in interactions from reflexes either of flexor or extensor origin.

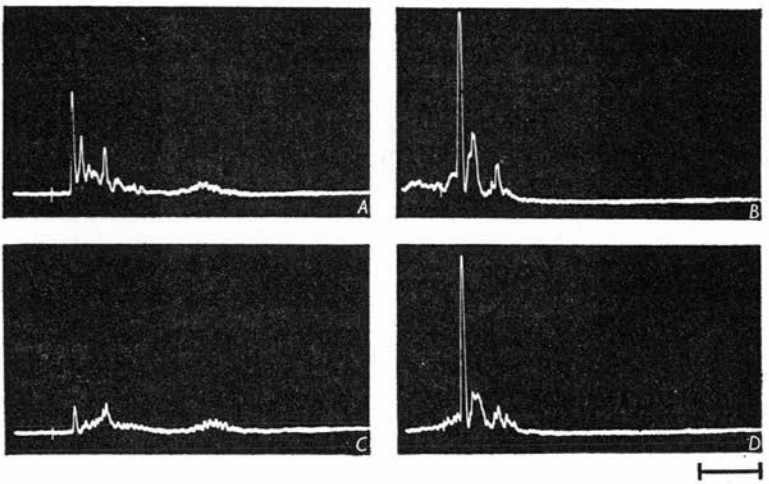

Fig. 4. Cat, chloralose 60 mg/kg. Effects of conditioning shock to biventer cervicis nerve on L6 reflexes of flexor and extensor origin. *A*, control reflex from posterior tibial nerve stimulation. *B*, effect on posterior tibial reflex of prior biventer cervicis nerve stimulation. *C*, control reflex from peroneal nerve stimulation. *D*, effect on peroneal nerve reflex of prior biventer cervicis nerve stimulation. Time marker 10 msec.

Effects of stimulation and ablation of the superior colliculus on cervico-lumbar interaction. In previous experiments, we have reported on a rich proprioceptive connexion from the BC to the superior colliculus (Abrahams & Falchetto, 1966). Since lesions of the superior colliculus lead to defects of head movements (Sprague & Meikle, 1965) the question arose as to whether this relay was of any significance in the cervico-lumbar interactions. Accordingly the superior colliculi were stimulated through stereotaxically directed electrodes both by single shocks and trains of stimuli. In nine such experiments we were unable to observe any effect on lumbosacral segmental reflexes by such stimuli, and even when the region of the superior colliculi was damaged by extensive electrolytic lesion, this was without effects on lumbosacral reflexes or interactions based on BC stimulation. When the electrode was further advanced into the brain to the area of the red nucleus, single shock stimulation led to a profound facilitation

of monosynaptic reflexes closely resembling that seen in cervico-spinal interactions. However, the red nucleus did not appear to be involved in the interactions, since lesions sufficient to eliminate the effects of stimulation were without effect on cervico-lumbar interactions.

Effects of decerebration on cervico-lumbar interactions. Since the BC initiated interactions seem to require the presence of tissue above the spinal cord, experiments were performed to see if critical areas lay above or below the tentorium. In twenty chloralose anaesthetized animals, after establishing the presence of interactions, a section of the brain was made between the superior and inferior colliculi. The immediate consequence of such a section was to abolish or sharply reduce the SBS reflexes, and to reduce the monosynaptic reflex. Within 20 min or so the monosynaptic reflex began to regain amplitude, and quite often 40 or 50 min after decerebration had regained or exceeded its original amplitude. Only in three experiments, using the averaging computer, was it possible to show an effect on spinal reflexes following BC stimulation. This effect was a facilitation of the monosynaptic reflex of 5–10%. In eleven of these experiments interactions resulting from forepaw nerve stimulation were also examined. In nine, although BC interactions were abolished, interactions were still present following forepaw nerve stimulation. Reduced far below normal levels, they were still quite strong and consisted of an increased amplitude of the monosynaptic reflex ranging from 20 to 60%. Unlike the spinal animal, cutaneous nerve stimulation was substantially more effective in facilitating the monosynaptic reflex than muscle nerve stimulation.

DISCUSSION

Proprioceptive input from the neck has previously been linked with postural reflexes concerned with orientation of the eyes with respect to the head (de Kleijn, 1918), those underlying the tonic neck reflexes of the decerebrate (de Kleijn, 1923) and body on head reflexes (Magnus, 1926). Interference with any of these systems is inadequate to explain the consequences of BC nerve section on hind leg gait found in the present experiments, and found previously to follow local anaesthetic block of the upper three cervical dorsal roots in monkey (Cohen, 1961). Electrophysiological experiments by Lloyd & McIntyre (1948) have demonstrated the presence of intraspinal pathways by which input from forepaw skin and muscle nerves can modulate excitability of lumbosacral mono- and polysynaptic reflexes. These intraspinal pathways do not appear to be available to the input from the BC nerve, but a system exists with an intracerebral course whereby lumbo-sacral reflex excitability can be modulated by proprioceptive input arising in muscles of the neck. In the chloralose anaesthe-

tized cat, these supraspinal mechanisms are apparently used in the genesis of many descending spino-spinal interactions, and intraspinal mechanisms are blocked or suppressed. Judging by the time course and nature of the descending interactions from forepaw nerves and from the BC nerves it seems likely that the same mechanism operates all these interactions, so similar are they in effect. In chloralose anaesthetized cats interactions from all these sources need the presence of supraspinal structures, but the dependence seems more marked for BC input, than for input from other nerves.

Previous experiments by Gernandt & Gilman (1959, 1961) and by Shimamura & Akert (1965), conducted principally on decerebrate cats, have demonstrated that elevation or depression of the head may modify segmental spinal reflexes, SBS reflexes and responses recorded at cervical levels as a result of vestibular stimulation. Gernandt & Gilman (1959) found that vestibular stimulation generated electrical activity at all spinal levels, but ventroflexions of the head through 30° abolished activity recorded from contralateral cervical nerves, and reduced that recorded ipsilaterally; however the effects on activity generated in the lumbosacral outflow were minimal. These effects of head movement were probably associated with input from the dorsal muscles of the neck, for effects similar to those produced by head flexion could be obtained from pinching these muscles, but not from pinching other muscles or the skin (Gernandt & Gilman, 1959). Since the experimental animals were decerebrated the effects from neck muscles must have been due to the operation of infratentorial systems. Not only do these experiments show that neck muscle proprioceptive input can override the output from vestibular mechanisms in the decerebrate preparation, but also, in this preparation, that effects from neck muscle proprioceptors on the lumbo-sacral outflow were weak, supporting our present findings. Further evidence for a need for supraspinal structures in some spino-spinal interactions comes from further experiments of Gernandt & Gilman (1961), in which they showed that although cervical segmental reflexes were sharply reduced by neck ventroflexion both before and after a C1 spinal section, in these same experiments (in which cats were decerebrated initially) lumbosacral responses were unaffected by head position. Gernandt & Gilman (1961) also showed that spinal input could override vestibular effects. Shocks were applied to the vestibular nerve and preceded by movements of the neck or by shocks to cervical dorsal roots. Flexion of 5° was sufficient to reduce the vestibular response recorded from the radial nerve, and stimulation of the C7 dorsal root abolished the response.

In decerebrate cats with the 8th nerve sectioned, head depression leads to an augmentation of the SBS reflex, and elevation leads to a depression of the SBS reflex (Shimamura & Akert, 1965). Since these responses were

recorded from lumbo-sacral roots, there is ample evidence that input from neck proprioceptors can operate to affect non-segmental lumbo-sacral reflexes in the decerebrate. However, in these experiments, unlike those of Gernandt & Gilman (1959, 1961), there is no reason to assign the effects of head movement specifically to muscle proprioceptors, and receptors from intravertebral structures such as those that underly the tonic neck reflexes of the decerebrate (McCouch, Deering & Ling, 1951), may be involved. Since muscle pinching was as effective a stimulus in the experiments of Gernandt & Gilman (1961) as head movement, the possibility of vertebral receptors playing a role in the modulation of vestibular responses was reduced.

The phenomenon of ataxia following interference with neck muscles has occasioned little attention, although there are clinical reports that suggest a similar phenomenon may exist in man (Cope & Ryan, 1959; Gray, 1956; Weeks & Travell, 1955). There is evidence that it may have been observed in the nineteenth century, for Claude Bernard (1865), in his book, *An Introduction to the Study of Experimental Medicine*, refers to experiments by Magendie in which (in the words of one of his translators) he found that removal of the cerebrospinal fluid led to a 'kind of titubation in animals and a characteristic disturbance in their motions'. Subsequently Magendie informed Bernard that another experimenter was interrupted in his experiments after having cut the neck muscles, but before incising the atlanto-occipital membrane. This preliminary dissection itself was then found to lead to the titubation, and it is this unknown experimenter who first seems to have observed the unexpected postural consequences of interference with neck muscles.

The consequences of the section of the BC nerve, taken together with these experiments of Gernandt & Gilman (1959, 1961), form a powerful argument for suggesting that the input from neck muscles is a critical factor in the reflex regulation of posture. It would seem that this input can modulate, or interact with, output generated on the basis of vestibular sensory information to control a great deal of the reflex excitability of the spinal cord. The effects probably add to a pool of proprioceptive information, for as we have seen, not only are the consequences of BC nerve section variable, but the animal can compensate for this lesion in a relatively short space of time.

One last point concerns not the special function of input arising from the BC muscle nerve, but the general function of reflex interactions in the spinal cord involving supratentorial pathways. Recently, Mori & Brookhart (1968) have investigated the response of muscle groups in the dog's hind leg when animals were subjected to a headward displacement. Muscles were found to respond with a variable latency, with one group

active 25 msec after displacement and a second group active 55 msec after displacement. In seeking an explanation of such unexpectedly long latencies, Mori & Brookhardt (1968) suggested that long spinal or SBS reflexes might be involved. It is equally likely that the mechanisms described here may underly the response either through a direct effect on motoneurones, or by an indirect effect on the excitability of the myotatic reflex. The participation of the supratentorial structures is presumably necessary for the total integration of muscle response operating either directly on motoneurones, or indirectly by manipulation of excitability in segmental spinal reflexes.

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Proprioceptive projection areas of the cerebral cortex and their relation to cervico-lumbar spinal interactions

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It is an old observation that interference with structures of the neck can lead to instability of gait (Bernard, 1865). This early observation was confirmed and extended by Cohen (1961) who showed that local anaesthesia of the upper three cervical dorsal roots in monkeys led to a transient gross ataxia. Similar transient disorders of gait were found in the cat (Abrahams & Falchetto, 1969) following section of nerves to a neck extensor muscle, biventer cervicis (BC). In seeking an explanation of this ataxia, it was found in the chloralose anaesthetized cat, that electrical stimulation of the central cut end of the BC nerve or forepaw skin and muscle nerves substantially potentiate lumbosacral monosynaptic reflexes. Unlike the cervico-lumbar interactions described in the spinal cat by Lloyd (1942) and Lloyd & McIntyre (1948), interactions in the chloralose anaesthetized cat have long latencies, exceeding 25 msec, and a total duration exceeding 30 msec. These interactions are dependent on structures above the spinal cord (Abrahams, Butler & Falchetto, 1968) and in the decerebrate preparation only weak cervico-lumbar interactions can be found. The present experiments show the interactions to be dependent upon the integrity of regions of the cerebral cortex.

Two cortical areas receive proprioceptive input from forepaws and neck, the post-cruciate dimple (Oscarsson & Rosén, 1963) and the anterior pole of the suprasylvian gyrus (Landgren & Silfvenius, 1968). Single shock stimulation to both these cortical areas produces effects on lumbo-sacral reflexes resembling cervico-lumbar interactions. The most noticeable effect is a potentiation of monosynaptic reflexes after a latency of some 15 msec with a duration of 30–40 msec. The post-cruciate dimple is not critical to cervico-lumbar spinal interactions, for its bilateral excision produces only a transient reduction of cervico-lumbar interactions. On the other hand, excision of the contralateral anterior pole of the suprasylvian gyrus virtually abolishes cervico-lumbar interactions from the BC nerve and sharply reduces interactions from forepaw skin and muscle nerve. This area of the suprasylvian gyrus has input from the vestibular apparatus and other major sensory systems (Landgren, Silfvenius & Wolsk, 1967). The present findings are consistent with the view that it may be concerned with the integration of postural reflexes, in part exerting its effects by modulating spinal reflex excitability.

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explained by the fact that before the pentobarbitone sodium injection a great number of stimuli have fallen during periods of pronounced background activity, during which the evoked responses have been absent or small.

When, however, the circuit described by McDonald (1963) is used, so that stimulation is restricted to periods when background activity is minimal, both before and after the injection of pentobarbitone sodium, then the injection is seen to reduce the averaged evoked potentials. It is therefore essential, when comparisons are made by computer averaging techniques with evoked potentials, to ensure that stimuli are always applied at comparable times in relation to background electrical activity.

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Peripheral pathways and properties of uterine afferents in the cat

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ABRAHAMS, V. C., and TEARE, J. L. 1969. Peripheral pathways and properties of uterine afferents in the cat. Can. J. Physiol. Pharmacol. **47**, 576–577.

Recordings were made from pelvic, hypogastric, and uterine nerves of the nonpregnant cat during electrical stimulation, manual compression, and inflation of the uterus and vagina. Unit recording showed that most but not all afferents from the uterus and vagina travel in the hypogastric, where they may constitute about 5% of the afferent fibers. Receptors were both restricted field and wide field, and although readily excited by manual compression they were less readily excited by increased intrauterine pressure.

Introduction

Little is known of uterine afferents in the cat. The rabbit uterus is known to have mechanoreceptors (Bower 1959; 1966*a*, 1966*b*), and afferent pathways from the uterus and cervix relaying to the hypothalamus and cortex have been demonstrated in the cat (Abrahams *et al.* 1964; Béfort and Albe-Fessard 1966). In the present experiments electrophysiological techniques have been used to define the peripheral afferent pathways from the uterus and vagina in the cat and to examine the distribution and some of the properties of the receptors.

Methods

Experiments were performed on 38 female cats each anesthetized with a single intravenous dose of chloralose (60 mg/kg) after the induction of anesthesia with ethyl chloride and ether. The pelvic area was exposed with a long midline incision, and the uterus brought into view by displacement of the intestines and bladder. Nerves were protected with Dow-Corning Medical Fluid 360. In experiments in which compound action potentials were very small, averages were constructed by using a computer of average transients to improve the signal-to-noise ratio. Records of unit data were made on magnetic tape and were photographed. Receptors of the uterus, and other hollow viscera, were activated by direct electrical stimulation, or by light compression with insulated forceps. In some experiments intrauterine pressure was increased by inflation with air. Recordings were made from the whole hypogastric or pelvic nerves, from filaments dissected from the nerves, or from fine nerve filaments leaving the uterus (uterine nerves).

Results

Langley and Anderson (1894, 1895 *a*, *b*, and *c*; 1896 *a* and *b*) showed that the uterus and vagina are innervated by the pelvic and hypogastric nerves with no contribution from the sacral nerves. Recordings made from the pelvic nerve show that although it carries many afferents from bladder and intestine, only rarely does it carry afferents from the vagina, and never, in our experiments, from the uterus. Of the pelvic nerves examined in 25 cats, activity was found in only two following electrical stimulation of the vagina. Electrical stimulation or manual compression of the uterine horns never led to detectable electrical activity either in the whole pelvic nerves or in filaments dissected from whole nerves. In contrast, electrical stimulation of the vagina and uterus commonly led to the appearance of compound action potentials in the peripheral stump of the cut hypogastric nerve, and manual compression of the uterus led to the discharge of individual units. The peak amplitude of compound action potentials recorded from the hypogastric nerve was only 2.5% of the amplitude of a compound action potential resulting from direct supramaximal electrical stimulation of the whole nerve, suggesting that vaginal and uterine afferents constitute only a small fraction of the afferent fiber. Most afferents arise from other hollow viscera, and unit discharge was readily caused by manual compression of the bladder and intestine. Measurements of conduction velocity showed uterine afferents to conduct at velocities ranging from 0.84 to 2 m/s.

In 19 cats recordings were made directly from the uterine nerves. All nerves showed the presence of unit activity following compression of an ipsilateral uterine horn. On 22 occasions, during recording from a uterine nerve which

responded to manual compression of the uterus, a pressure of up to 100 mm Hg was applied to the lumen of the uterus. On five occasions only did this maneuver lead to recognizable unit activity in the nerve. In a number of experiments the uterus was lightly compressed with forceps over its entire surface during recording from a single unit in the uterine nerve. In this way 18 units were found serving almost the whole of one horn and 12 were found to have receptive fields that were restricted to an area occupying about 1 cm^2 on the uterine horn.

Discussion

Most afferents from the uterus travel in the hypogastric nerve and appear readily excited by manual compression of the uterus and less readily excited by distention of the uterus. Since these experiments were performed on nonpregnant cats, these findings may simply relate to the lack of distensibility of the nonpregnant uterus.

The proportion of sensory fibers in the hypogastric nerve that originate in the uterus or the vagina is not large, but if the amplitude of the compound action potential is used as an indication, then about 5% of afferent nerves in the hypogastric originate in the generative organs. This figure is arrived at by assuming the hypogastric nerve to be evenly divided between sensory and motor fibers and assuming the amplitude of the compound action potential to be related to the numbers of individual fibers conducting. Receptors in the generative tract might be associated with the vascular supply or they may play a more direct role in the physiology of the uterus, perhaps in the regulation of neurohypophyseal hormone release, since it is known that pathways from the uterus relay to hypothalamic regions concerned with the release of neurohypophyseal hormones (Abrahams *et al.* 1964; Béfort and Albe-Fessard 1966; Bisset *et al.* 1966).

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A NEW APPROACH TO BLADDER EVACUATION: STUDIES IN DOGS

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ABSTRACT

A mechanical device to evacuate the urinary bladder of the intact dog has been studied. Both acute and chronic studies to determine the effectiveness of this form of evacuation have been carried out. It was clear that excellent voiding could be produced on acute experiments. However, with chronic use all animals developed serious complications, such as peritoneal adhesions, peritonitis, and severe contracture of the bladder, which prevented further testing of the device. This is felt to be due to the vigorous contractions of the normal bladder against the relatively rigid device. It is felt that further work is needed and that the device should be tested on animals with denervated atonic bladders.

Despite significant improvement in the management of patients with neurogenic vesical dysfunction, the treatment of the hypotonic bladder, frequently seen among diabetics and others, remains unsatisfactory (1).

Currently, when simple measures such as multiple voiding and administration of drugs (e.g., Urecholine) fail to reduce the residual urine to acceptable levels, transurethral resection of the bladder neck or Y-V-plasty is done to reduce the outflow resistance and restore the "detrusor balance" (2). Because of the development of bladder neck contracture and other complications following transurethral resection, a reported 20 to 40 per cent of these patients continue to build up residual urine, resulting in progressive deterioration of the upper urinary tract and urinary diversion in one form or another (3).

Urinary diversion, except when done as a life-saving procedure, has been found to be unsuitable because of the high incidence of postoperative complications and its failure to provide social acceptability. There is therefore a great need for investigation of new methods, based on physiologic principles, for improving vesical emptying in these patients.

Bradley et al. and others (4–9) have reported encouraging results with the use of electrical stimulation to produce micturition in dogs. However, serious problems such as pain, current spread, and outflow resistance have been encountered with its use in humans (10).

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This study was supported by Grant MA 2270 from the Medical Research Council of Canada.

With a view to improving the intravesical pressure without any increased outlet resistance, we have designed a mechanical encapsulating device in our laboratory and here report the results of our initial studies in dogs.

MATERIALS AND METHODS

Our method is based on the proposal to surround the urinary bladder with a double-walled casing of a flexible but relatively inextensible material. Upon inflation of the interwall cavity with a suitable fluid under pressure, the inner wall of the casing will collapse onto the bladder and increase the intravesical pressure sufficiently to cause micturition. Our initial efforts have been directed to the design of a suitable bladder casing and this report is limited to the description and evaluation of this casing.

The device consists of a pear-shaped casing of 0.020-inch medical grade Dacron-reinforced silicone rubber (Fig. 1) which has an inner wall of the same material covering one-half of the dome. It is designed to fit the moderately distended dog bladder. To assist fixation of this device to the bladder neck, a Dacron mesh skirt is fixed to the free edge. The device has been constructed in three different sizes (120, 150, and 200 ml) to accomplish proper fitting of the bladder in each animal at the time of surgery. A silicone rubber tube 20 cm long and of 4-mm outside diameter is attached to the apex

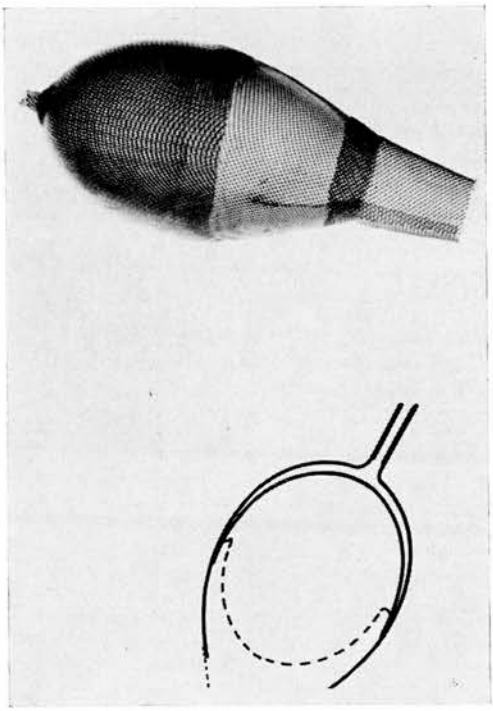

FIG. 1. Photograph of a silicone rubber casing with diagram of cross-section to show construction. Bold dashed line shows position of inner wall when interwall cavity inflated. Fine dashed line shows Dacron mesh skirt.

of the dome to give access to the interwall cavity. When fluid pressure is applied to this tube, the inner wall of the casing inverts to sweep the full volume of the casing. The end of the tube is sealed with silicone rubber.

Ten dogs of either sex and weighing from 25 to 30 kg were used in this study. In the first five dogs (group A) the following technique was used.

Under i.v. Nembutal anesthesia the bladder was exposed through a lower midline abdominal incision. It was filled through a urethral catheter with saline under gravity. With two pairs of hemostats holding the Dacron mesh skirt, the device was slipped over the dome of the bladder and was fixed by suturing the skirt to the bladder neck with 0000 chromic interrupted sutures. Care was taken to avoid the mucosa. To prevent kinking and obstruction of the ureters, windows were cut in the skirt for the ureters to enter the bladder. The rectus muscles and fascia were reapproximated and the tube connecting the interwall cavity was brought out through the incision. The skin incision was closed with 00 silk.

At the end of surgery, an appropriate volume of saline was injected into the cutaneous end of the tube by means of a 21 gauge hypodermic needle. A good flow of urine per urethra was obtained in each case, and good bladder emptying was achieved, with virtually no residual urine. Following emptying, the driving fluid was withdrawn. The tests for voiding were repeated postoperatively at weekly intervals.

The remaining five dogs (group B) were employed as a control group. In these animals, the device consisted of a single-walled casing without the tube. The implantation technique was the same except that a test for voiding was not carried out. All animals in groups A and B received antibiotics during the immediate postoperative period.

RESULTS

Group A. In an acute experiment with the bladder held to avoid errors due to pulling on the neck we compared the threshold voiding pressure with manual expression to that with electrical stimulation. Although a manually induced pressure of approximately 30 mm Hg would produce a steady stream, electrically induced pressures of up to 40 or 50 mm Hg were required to produce a similar stream. Pressure measurements were made during the implantation of two bladder casings by means of the urethral catheter. These showed that with a filled bladder having a resting pressure of approximately 15 mm Hg the intravesical pressure was 5 mm Hg higher than the fluid pressure applied to the casing over a range of 20 to 60 mm Hg applied pressure.

All five animals in group A survived the operation and were sacrificed at intervals of 1, 2, 3, 4 and 6 weeks after surgery. On the day of surgery, good emptying of the bladder was noticed following the injection of saline into the inlet tube in all the animals. However, all subsequent testing of the device produced only fair to poor emptying. In one animal, injected saline was seen coming out of the urethra because of the development of a fistulous communication between the bladder and the device.

In two other animals, the inlet tube retracted inside the abdomen with

TABLE 1.

Dog No.	Survival Period	Postoperative Condition	Postoperative Functional Status	Autopsy Findings
	wks			
18	1	Abdominal distension with lethargy. Silicone tube retracted inside the abdomen.	Good at the time of surgery.	Large thick brownish fluid in the abdomen with signs of peritonitis. Bladder remained contracted and thickened.
19	2	Abdominal distension with lethargy. Silicone tube retracted inside the abdomen.	Good at the time of surgery.	Large thick brownish fluid in the abdomen with signs of peritonitis. Bladder remained contracted and thickened.
7	3	Healthy but incontinent.	Good at the time of surgery, but fluid could not be recovered from the casing.	Signs of peritonitis with fistulous communication between the bladder and the casing. Bladder remained small and contracted.
9	4	Healthy but incontinent.	Good at the time of surgery, but fluid could not be recovered from the casing.	Signs of peritonitis with fistulous communication between the bladder and the casing. Bladder remained small and contracted.
12	6	Healthy but incontinent.	Good at the time of surgery.	Massive adhesions with signs of peritonitis. Bladder wall thickened and contracted; also fluid accumulation as in dog 18.

marked abdominal distention. Autopsy revealed signs of severe peritonitis. Constant dribbling of urine, due to the development of a small capacity contracted bladder, was noticed in the remaining two animals. The autopsy findings are given in Table 1.

Voiding pressure studies by means of suprapubic catheters were not possible because of the presence of the casing. Detailed followup pressure studies by means of urethral catheters were not possible because of the postoperative complications described.

Group B. Owing to the high incidence of infection noticed in group A, extra measures were taken to eliminate infection in the animals in group B. No tube was left outside the abdominal wall.

All five animals survived the operation and were active and healthy postoperatively. The incisional wound healed without infection in all of them. Intravenous pyelograms done 2 weeks after surgery showed normal upper urinary tracts in all the dogs. Animals were sacrificed at 2, 3, 4, 11 and 15 weeks after surgery to study changes on the bladder wall. In three animals, the silastic casing was found to have separated from the Dacron mesh skirt and was lying free in the peritoneal cavity. The bladder itself appeared to be completely normal. The casing remained intact in the remaining two animals and was seen covered by omentum. On sectioning through the casing, a cavity was seen between the casing and the bladder wall, containing about 8 to 10 ml of colorless, slightly viscous fluid. The area of the bladder under the casing remained contracted and was coated with a yellowish brown

FIG. 2. Contracted bladder under casing

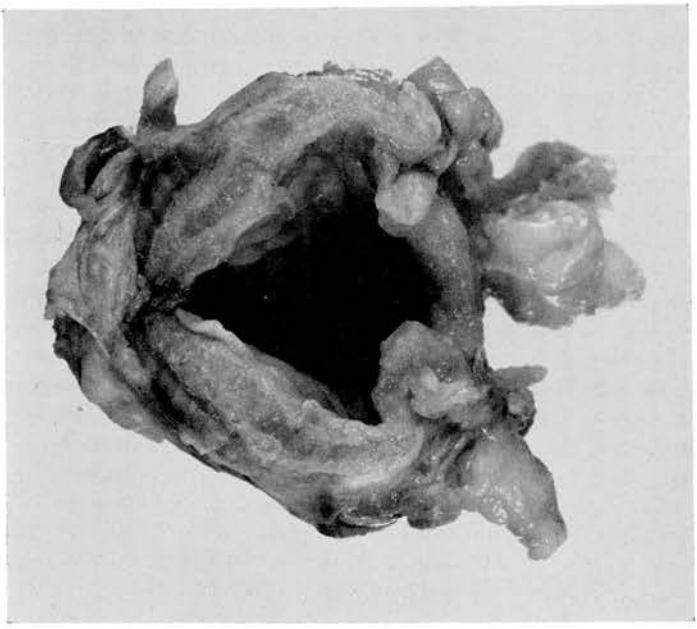

FIG. 3. Bladder opened to show the considerable thickening of the wall

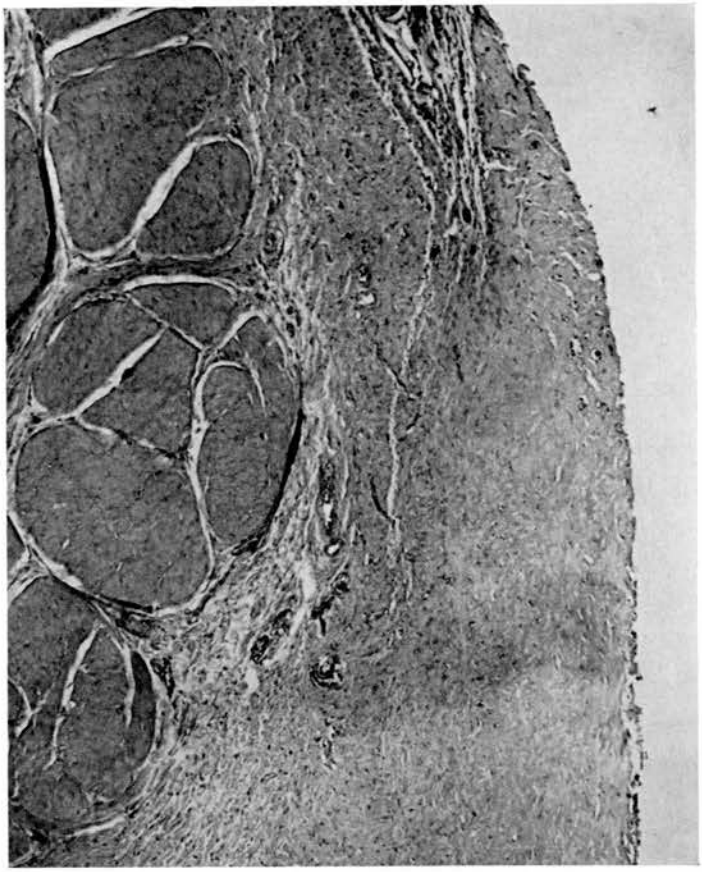

FIG. 4. Microphotograph showing severe fibrous tissue infiltration of the bladder wall

TABLE 2.

Dog No.	Period of Service	Postoperative Condition	Postoperative IVP	Autopsy Findings
	wks			
90	3	Active and healthy; no complications.	Normal BUN; IVP normal except for deformity over bladder.	Fluid (5-6 ml) between casing and bladder; marked thickening of bladder wall with contraction of area under casing.
91	2	Active and healthy; no complications.	Normal BUN; IVP normal.	Bladder casing found separated from Dacron skirt and lying free in peritoneal cavity. Normal bladder.
92	11	Active and healthy; no complications.	Normal BUN; IVP normal except for deformity over bladder.	Bladder casing found separated from Dacron skirt and lying free in peritoneal cavity. Normal bladder.
93	15	Active and healthy; no complications.	Normal BUN; IVP normal except for deformity over bladder.	Bladder casing found separated from Dacron skirt and lying free in the peritoneal cavity. Normal bladder.
94	4	Active and healthy; no complications.	Normal BUN; IVP normal except for deformity over bladder.	Fluid (5-6 ml) between casing and bladder; marked thickening of bladder wall with contraction of area under casing.

exudate (Fig. 2). Considerable thickening of the bladder wall was also noticed (Fig. 3). Histologic examination revealed severe fibrous tissue infiltration of the bladder wall (Fig. 4). Bacteriologic studies carried out on all the animals during autopsy showed no evidence of infection. The postoperative findings are summarized in Table 2.

DISCUSSION AND CONCLUSIONS

From the results of our study in group A, it becomes clear that excellent voiding can be produced in every animal on the day of surgery. However, with chronic postoperative use all animals developed serious complications, such as peritoneal adhesions, peritonitis, and severe contracture of the bladder, which prevented further testing of the device. To find out whether these complications were due to tissue reaction to the material, a series of experiments was conducted with patches of silicone rubber applied to dog bladder. Only minimal tissue reaction resulted (see APPENDIX). However, when silicone rubber was placed around the bladder as an encapsulating device, as was done in group B, fairly serious reaction was noted in the form of fibrous tissue infiltration and thickening of the bladder muscle. Also, the area of the bladder under the device became contracted in the two animals in which the casing remained in position. Such a serious reaction might have been due to the frequent vigorous contractions of the normal bladder enclosed by the relatively rigid casing, which produced a shearing stress. The same stress was probably responsible for the separation of the casing noted in three animals of group B. Such an effect may not be produced were the device to surround an atonic bladder and voiding produced only at the time fluid enters the space between the walls of the casing. It is felt that further work is needed in developing a self-adjusting emptying mechanism to achieve controlled micturition, and we recommend that the device be tested on animals with denervated atonic bladders.

APPENDIX

The Effect of Silicone Rubber Patches on the Urinary Bladder in Dogs

A study of the tissue reaction of the serosal surface of dog urinary bladder to silicone rubber was investigated in a series of patch tests.

Twenty-four adult male and female dogs weighing 12 to 20 kg were used in the study. They were divided into two groups. Group 1 consisted of 12 dogs in which a 2- by 2-cm square patch of silicone rubber was sutured to the serosal surface of the dome of the bladder. Group 2 consisted of the same number of animals in which similar patches of silicone rubber reinforced with Dacron mesh were used.

The animals were sacrificed at fixed intervals varying from 1 to 32 days, and at autopsy bacteriologic and histologic studies of the bladder were carried out. In each dog the omentum covered the patch but was easily freed without causing bleeding. There was a definite cavity between the patch and the bladder, containing 2 to 3 ml of clear viscous fluid with hyperemia of the

serosal surface and some thickening of the bladder musculature. No abnormality was seen in the mucosa, nor were there any gross changes in the other abdominal viscera. Only four of the animals showed any significant growth of Staphylococci from the peritoneal cavity or the fluid under the patch. The histologic findings in both groups were identical and consisted of a thin layer of connective tissue coating the serosal surface, associated with focal hemorrhages with a few polymorphs and lymphocytes. No plasma cells or giant cells were seen. In a few dogs, the muscle underlying the serosa showed fibrous tissue infiltration.

In this study only minimal histologic evidence of tissue reaction to silicone rubber was noted and the presence of Dacron mesh did not seem to affect the tissue tolerance. This was not the finding with the whole-bladder emptying device. We can only conclude that bladder movement is the predominant factor producing the extensive reaction observed.

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Can. Physiol., 1, 1969, 37.

Intraspinal mechanisms in cortically mediated descending cervico-lumbar reflex interactions. V. C. ABRAHAMS, Dept. of Physiology, Queen's University, Kingston, Ontario.

In previous experiments we have shown that a prominent characteristic of cortically mediated descending cervico-lumbar reflex interactions is a prolonged and selective facilitation of lumbosacral monosynaptic reflex excitability. Current experiments show that this facilitation is not likely to be due to increased postsynaptic excitability, since then both monosynaptic and polysynaptic reflexes are enhanced. No evidence has been found in favour of the monosynaptic facilitation being due to presynaptic mechanisms. Excitability of Gp. 1 terminals in the ventral horn is unaltered early in the interaction, when the facilitation is maximal. Later in the interaction, there is an increase in the excitability of the Gp. 1 axons, but on the basis of current theory, this should lead to a fall in reflex excitability, not the observed increase. Additional evidence against a presynaptic mechanism comes from examination of the dorsal root potentials (which should reflect primary afferent depolarization). No firm relationship can be observed between dorsal root potentials and monosynaptic reflex facilitation. It is concluded that the mechanism underlying the prolonged monosynaptic facilitation is either remote (dendritic) in origin, or is dependent on some hitherto unexplained neuronal property.

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IEEE, Student J., 8, (1970), 35-38.

Computer diagnosis

The beat of your heart can now be analyzed by computer. Here, a Canadian physiologist describes the automated electrocardiogram.

Vivian C. Abrahams

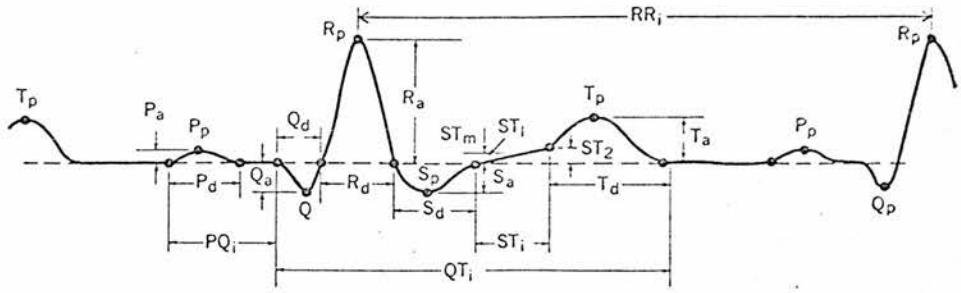
THE CONCEPT that disease can be diagnosed and correct treatment determined with the aid of a machine is not new, but until recently the technique had mainly been employed by those more concerned with parting fools from their money than with serious machine diagnosis of disease. In the last eight or nine years, serious attempts have been made to create machines that will fulfill all or some part of the task of diagnosis. The techniques employ either conventional computers, handling coded information, or special purpose machines to handle one or a few specific tasks.

In medical administration the computer has been adopted to create files, to ease the problem of handling medical information, to regulate the automated devices that perform laboratory tests, and even to compile the data output to aid diagnosis. As yet, the computer has not replaced the doctor in assembling all the information obtained from a patient, and in presenting a diagnosis in a reliable and acceptable fashion.

The process of medical diagnosis is based on an ordered sequence of examinations. The patient is questioned to obtain his history. He is then subjected to a physical examination in which he should be completely examined, not only to explore further the symptoms that first brought him to the doctor, but also to detect obvious signs of other diseases. Next, laboratory tests are performed. These may be X-rays, they may include biochemical analysis of body fluids, or they may involve highly specialized techniques based on the distribution of isotopes. If possible, tests are performed that enable the doctor to verify what he thinks the condition may be.

Often the task is complicated because of the presence of multiple disorders, the effects of previously administered medication, or because the patient has lapses of memory and either fails to supply vital data or supplies incorrect data. The successful doctor often needs to use intuition and subtlety in securing the information he needs to aid his diagnosis. Nonetheless, since diagnosis involves an ordered sequence of logical questions and answers, a computer should be able to perform the task. The job is enormous, the cost huge. At the moment the main benefit in performing such a task is to explore its feasibility and to learn more concerning the nature of the problems that must be overcome.

Partial diagnostic systems and aids have been created and are in use in the United States and Sweden. In such



EKG parameters measured		
Amplitudes	Durations	Intervals
P_a (type)	P_d	PQ_i
Q_a	Q_d	ST_i
R_a	R_d	QT_i
S_a	S_d	RR_i
T_a	T_d	
ST_I		
ST_m		
ST_2		

Fig. 1. A single cycle of the EKG shows the measurements of amplitudes, durations, and intervals that are utilized in analysis.

systems the computer may be used to perform preliminary patient screening. Usually the patient answers a questionnaire using mark-sense cards. The gathered data is compiled and on the basis of the compilation, the patient may be directed to an appropriate service or laboratory. There is an obvious benefit here in saving the doctor's precious time.

Not everyone is happy with this type of system because of the unreliability of data provided by patients. Studies have been made to check the validity of data obtained from patients, and also the reliability of laboratory tests. There is a surprising amount of error present in both types of data.

One of the assets of computerized medical laboratories is that the examination of laboratory generated data acts as a good form of quality control. For this reason, and also because of the sheer enormity of the task of total diagnosis, the greatest progress has been made in more limited diagnostic tasks. Signals generated by the body, are readily available in electrical form, and when properly analyzed, can be used as a basis of a diagnostic routine. Such signals include the electrical activity associated with each beat of the heart, the electrocardiogram (EKG), the electrical activity generated by the brain that can be recorded from the scalp, the electroencephalogram (EEG), the specific change in brain electrical activity that follows a sudden sensory stimulus, the evoked potential, and the electrical signal that can be generated by a transducer recording the flow of gases into and out of the lungs, or pressure or blood flow in the circulatory system.

For the last few years here at Queen's University in Ontario, we have been concerned with the development of an operational system for the computer diagnosis of EKG's to determine whether a service can be provided at a realistic cost to give doctors in a wide and lightly populated area access to EKG analysis.

Our work was based on that of Dr. Cesar Caceres and his colleagues, then in the Public Health Service in Washington, D.C. The EKG is conventionally recorded as a potential change between two points in the body surface and consists of a regularly repeated series of complex waveforms (Fig. 1). By using a computer to measure the time between various waves and the amplitude of the waves, Dr. Caceres and his colleagues developed a program that was able to create an EKG diagnosis (Fig. 2). Our aim was to make such a system generally available. First, the Ca-

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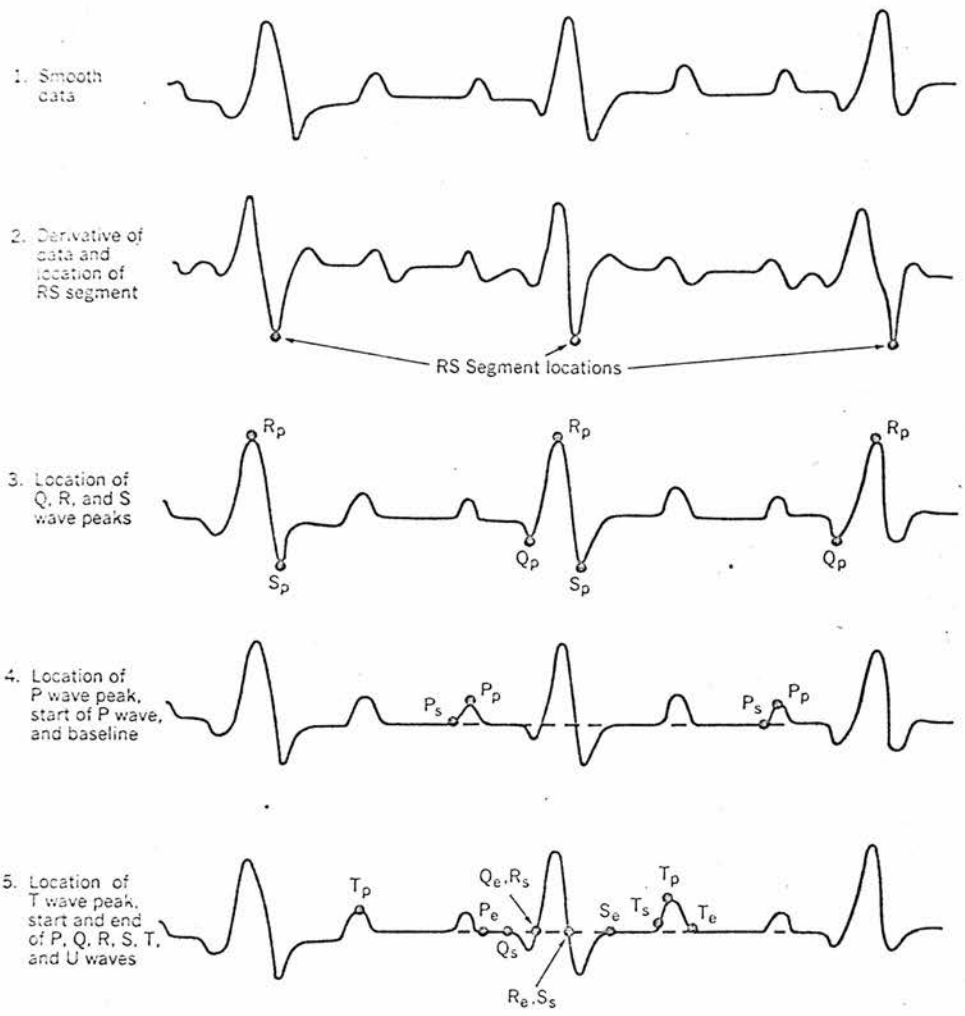

Fig. 2. With the Caceres Program the sequence of signal treatment underlies computer analysis of EKG's.

ceres program was translated from the machine language in which it was originally compiled to FORTRAN. Although such program translation is not justifiable on economic grounds, it is essential if the program is to run on available computers. At this stage we found that even with the large 360/50 available to us, the central processing unit (CPU) time usage raised the cost of computer analysis above the cost of conventional techniques. It is possible, however, to reduce this cost by recording the electrocardiogram in a different fashion.

Conventional EKG's are called 12-lead EKG's, as the potentials are recorded serially from twelve different arrangements of electrodes on the body surface. The same amount of information may be obtained by recording simultaneously from leads disposed around the body and resolving the recorded potential changes to give three orthogonal vector components. This technique is called vectorcardiography (VCG), but it is not as widely employed as the 12-lead system. From the point of view of computer programming, VCG, is far preferable and programs for VCG analysis have been written in a number of laboratories.

The problem that we faced was the difficulty in gaining acceptance for a computer based system for general use; one for our particular needs, utilizing VCG, was likely to die at birth. We therefore decided to stay with the 12-lead system whose printout is full of familiar detail for the doctor, and hoped that once the use of the computer was accepted, we could try to wean out doctors to the VCG and then show real economies.

The next problem that arose concerned capture of the signal. Ideally the information should come straight from the patient to the computer and some display of the diagnosis should be instantaneously returned to the bedside. Such a system while feasible is not economically viable. The data transmission system must incorporate some form of coding that allows patient identification and transmission of data other than the EKG vital to the diagnosis. Originally, the data capture system was based on an FM tape recorder that was wheeled from bed to bed. Such carts proved too costly for wide distribution, and now one cart is centrally located and connected to a dial-up telephone system. The new bedside unit is a relatively low-cost

Fig. 3. Bedside equipment for EKG capture of computer analysis includes the strip chart recorder that produces a conventional record. The acoustic coupler transmits this information together with appropriate codes to a central capture site.

acoustic coupler with a built-in coder (Fig. 3). The 360/50 available to us is used extensively in the time sharing mode and offers Watfor[1] terminals, CRO displays, and keyboard terminals with a variety of languages. In addition, there is access to the 360/50 from a number of small remote computers. The taped data is fed to the small computer control system through the analog-to-digital converter of a Linc-8. The control system operated by a PDP-9 allows the information to be stored in the 360/50 as digital tape and when needed the program is run and the conventional printout is obtained.

The system is clumsy; however, this does not detract from its major purpose, which is to allow doctors to use a computer-based system to test it for convenience, reliability, and general acceptability, and to identify the ways in which costs may be reduced to bring the system to a financially acceptable level. If these criteria are met, and the system meets the need of the rural hospital or isolated practioner, then we must improve the engineering to reduce costs and improve convenience. The first criterion of any computer-based diagnostic system is that it must be completely acceptable to the profession that is to use it. By starting small it is possible that such systems will be developed. From there it may be a large jump to the total diagnostic machine, but a necessary jump to make. In our own experience, the cost of data transmission and peripheral equipment is a major hurdle. Perhaps if systems do become accepted on a wider basis we may realize the economies of scale and produce low cost peripheral equipment that will make a system widely available at a reasonable cost. After a decade of vigorous growth we soon will approach infancy.

[1] The term "Watfor" is a commonly used term for a compiler for Fortran use on the IBM 360 series. The compiler has the great virtue of being a "load and go" system and therefore is extremely quick. It is, however, not suitable for long jobs. We use Watfor terminals for student use and can get up to 1000 or more jobs a day.

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CERVICO-LUMBAR REFLEX INTERACTIONS INVOLVING A PROPRIOCEPTIVE RECEIVING AREA OF THE CEREBRAL CORTEX

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SUMMARY

1. In the decerebrate cat descending cervico-lumbar reflex interactions from neck muscle nerves are absent, and those from forepaw nerves are reduced or absent.

2. Lesions restricted to the anterior pole of the suprasylvian gyrus regularly abolish descending cervico-lumbar reflex interactions from neck muscle nerves. The same lesions may reduce or abolish descending cervico-lumbar reflex interactions from forepaw nerves.

3. The ascending cortical looping pathway utilized in descending cervico-lumbar reflex interactions is crossed, and the descending pathway from the cerebral cortex is bilateral.

INTRODUCTION

Apart from the operation of hopping and placing reflexes, the role of the cerebral cortex of the cat in posture has not been well defined. The region of cortex involved in hopping and placing reflexes is restricted largely to the pericruciate area (Bard, 1931, 1933). As might be expected, the pericruciate area receives connexion from proprioceptive afferent systems (Mountcastle, Covian & Harrison, 1952; Gardner & Haddad, 1953; McIntyre, 1953; Amassian & Berlin, 1958; Oscarsson & Rosen, 1963), but this region is not unique in this respect, and such input goes to the second somatosensory area (Mountcastle *et al.* 1952; Gardner & Haddad, 1953), the region of the postcruciate dimple (Oscarsson, Rosen & Sulg, 1966) and the anterior pole of the suprasylvian gyrus (Landgren, Silfvenius & Wolsk, 1967*a*; Landgren & Silfvenius, 1968, 1969). The existence of these widespread cortical proprioceptive connexions has not been adequately explained and may be related to hitherto unexamined cortical function in posture. The experiments described here show that cortical proprioceptive

receiving areas are involved in spinospinal descending reflex interactions. One region in particular, the anterior pole of the suprasylvian gyrus (ASSG) is critically involved in such interactions, and is essential for neck muscle proprioceptors to exert effects at lumbosacral levels of the cord. The experiments were prompted by our previous finding (Abrahams & Falchetto, 1969) that intraspinal systems for cervico-lumbar interactions (Lloyd, 1942; Lloyd & McIntyre, 1948) are suppressed in the chloralose anaesthetized cat in favour of systems utilizing supratentorial structures.

METHODS

Experiments were performed on cats anaesthetized with a single intravenous dose of chloralose (60 mg/kg) given after the induction of anaesthesia with ethyl chloride and ether, or on non-anaesthetized cats following a mid-collicular decerebration under methoxyfluorane anaesthesia (Metofane, Pitman-Moore). Nerves prepared for stimulation included the posterior-tibial, peroneal, superficial radial, dorsal interosseus and nerves leaving the biventer cervicis muscle. All nerves were bathed in protective pools of silicone oil (Medical Fluid 360, Dow-Corning Silicones Ltd.). The lumbosacral cord was exposed from L5 to S2, appropriate ventral roots prepared for electrical recording, and the exposed cord was also bathed in a pool of silicone oil. Animals were routinely injected with gallamine triethiodide (Flaxedil, Poulenc) to abolish reflex movements and were artificially ventilated. Blood pressure, respiration and body temperature were continuously monitored during experiments. Body temperature was maintained within normal limits by a heated table. Records were normally made photographically, but where differences in electrically recorded spinal reflexes were small, averages were constructed from twenty-five successive reflexes using a Computer of Average Transients, Model 400B (Mnemotron Corporation).

When the cerebral cortex was to be stimulated or ablated, it was first exposed by wide removal of bone and kept moist by the repeated application of silicone oil. The cerebral cortex was stimulated with 2 msec square-wave pulses using a bipolar platinum electrode with ball-shaped tips 0·5 mm in diameter, separated by 1 mm. Plotting of responsive cortical areas was based on stimulation at or close to threshold. Ablation was restricted as far as possible to removal of the superficial grey matter using sub-pial suction. Bleeding after ablation was controlled by the application of gelatine sponge. Brains were perfused with formalin at the termination of the experiment and the extent of the lesion determined from brain sections.

RESULTS

Cervico-lumbar interactions in the non-anaesthetized decerebrate cat and the actions of chloralose. Decerebration of the chloralose anaesthetized cat was previously found to abolish or reduce pre-existing cervico-lumbar interactions, the effects being most pronounced on the interactions that follow stimulation of the biventer cervicis nerve (Abrahams & Falchetto, 1969). To ensure that the loss of biventer cervicis interaction in these experiments was unrelated to the presence of anaesthetic doses of chloralose,

eight surgically decerebrate cats were prepared under Metofane anaesthesia. Two hours were allowed for the anaesthetic to wear off and the effects of prior stimulation of superficial radial, dorsal interosseus and biventer cervicis nerves on lumbosacral reflexes then investigated. Even with the use of the averaging computer, no interactions were found in four cats. Superficial radial and dorsal interosseus nerve stimulation in the remaining four cats led to a 10–50 % facilitation of the monosynaptic reflex (Fig. 1). Only in one of these experiments was an interaction found to follow biventer cervicis nerve stimulation and that was very weak.

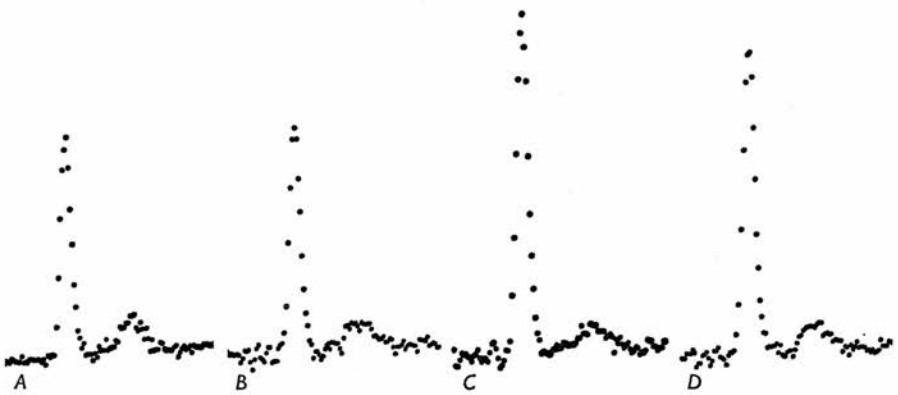

Fig. 1. Decerebrate cat. Reflexes recorded from L7 ventral root following stimulation of the posterior-tibial nerve. Each record average of twenty-five consecutive reflexes. *A*, control reflex; *B*, reflex preceded by biventer cervicis nerve stimulation; *C*, reflex preceded by dorsal interosseus nerve stimulation; *D*, reflex preceded by superficial radial stimulation. Conditioning interval, 30 msec. Time calibration: 28 mm = 10 msec.

Chloralose was then administered in the normal anaesthetic dose of 60 mg/kg to the four cats showing descending cervico-lumbar interactions. The effect of chloralose in these experiments was not to increase the amplitude of monosynaptic reflexes, as in the spinal cat (Shimamura, Yamauchi & Aoki, 1968) but to reduce the amplitude of monosynaptic reflexes without altering patterns of interactions.

Effect of stimulation and ablation of the cerebral cortex on cervico-lumbar interactions. The regions of the cerebral cortex that receive proprioceptive input are located in the frontal pole (Mountcastle *et al.* 1952; Gardner & Haddad, 1953; Amassian & Berlin, 1958; Oscarsson & Rosen, 1963; Landgren *et al.* 1967*a*; Landgren & Silfvenius, 1968, 1969). In sixteen cats the exposed surface of the frontal pole was systematically explored, stimulating with single pulses 10–50 msec before the elicitation of lumbosacral reflexes. Not only were effects on the lumbosacral cord elicited from

the pericruciate area, as was to be expected (Bernhard, Bohm & Taverner, 1954; Stewart, Preston & Whitlock, 1968), but stimuli localized to the postcruciate dimple (PCD) and the ASSG also had effects on lumbosacral reflex excitability. As Fig. 2 shows, these effects closely resembled those following biventer cervicis, dorsal interosseus or superficial radial nerve stimulation. There was an early inconstant discharge over the ventral roots preceding a period of monosynaptic reflex enhancement. There was also a prolonged inhibition of the SBS reflex (Shimamura, 1961; Shimamura & Livingstone, 1963). These effects appeared about 10 msec earlier than the consequences of biventer cervicis, dorsal interosseus or superficial radial nerve stimulation in the same preparation.

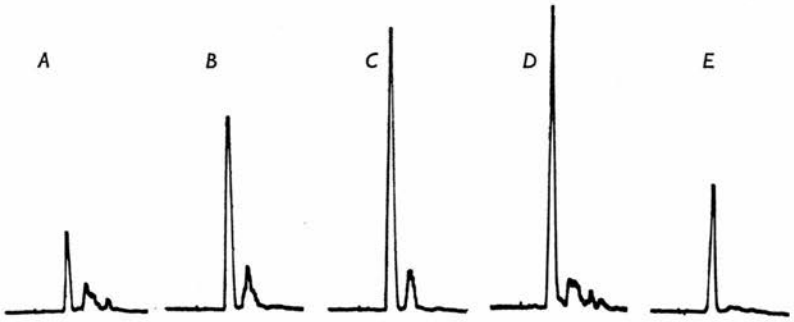

Fig. 2. Cat, chloralose 60 mg/kg. Effect of single shock stimulation of the PCD on lumbosacral reflexes. *A*, control reflex; *B*, cortical stimulation 10 msec; *C*, 20 msec; *D*, 30 msec; *E*, 40 msec before eliciting reflex. Time calibration: 28 mm = 20 msec.

The effect of cortical ablation on cervico-lumbar interactions was tested in a number of ways. In three cats the frontal pole was progressively extirpated starting anteriorly at the orbital sulcus. Transient small depressions followed ablations in the pericruciate area, but recovery was complete within 10–15 min. Only when the lesion encroached on the ASSG were interactions sharply reduced or abolished, and then there was no recovery within the remaining 3 hr or so of the experiment. In nine of the experiments in which the cortex was stimulated, the PCD was subsequently excised bilaterally. This led to some reduction of interactions from dorsal interosseus superficial radial nerve stimulation, but the consequences of biventer cervicis nerve stimulation were largely unaffected (Fig. 3). In these same experiments when the lesion was then extended to include the area of the ASSG, interactions from the biventer cervicis nerve were promptly lost and did not return for the duration of the experiment. Including these experiments and others in which the lesion was confined to the ASSG a total of eighteen small unilateral lesions of the type illu-

strated in Fig. 4 were made. In five experiments this led to the total loss of interactions from forepaw and neck muscle nerves (Fig. 4). In a further five experiments the effects of biventer cervicis stimulation were abolished, but some small interactions could still be obtained from superficial radial

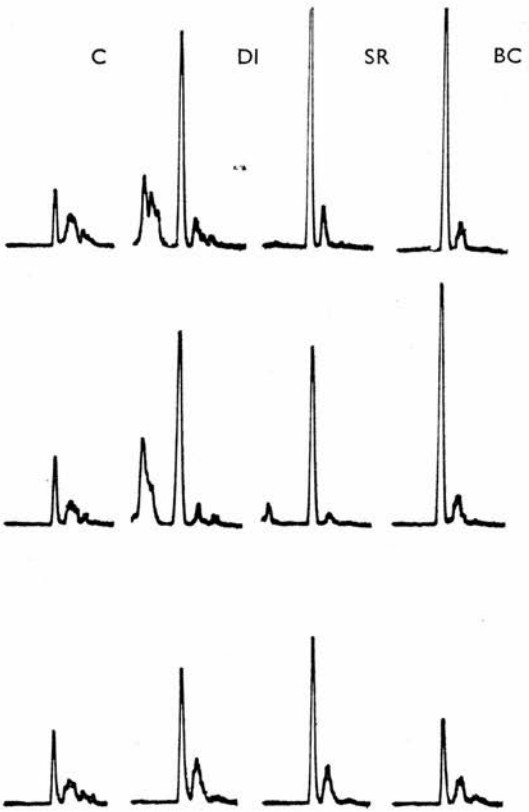

Fig. 3. Cat, chloralose 60 mg/kg. Effect of progressive cortical ablations on cervico-lumbar reflex interactions. Top row, control reflex, dorsal interosseus, superficial radial and biventer cervicis interactions before ablations. Middle row, control, same interactions as top row, 20 min after ablation of pericruciate region and PCD. Bottom row, control, same interactions as top row 2 hr after extension of lesion to include the anterior pole of the suprasylvian gyrus. Time calibration: 28 mm = 20 msec.

and dorsal interosseus nerve stimulation. In three experiments both biventer cervicis and dorsal interosseus interactions were lost but the effects of superficial radial nerve stimulation were undiminished. In three experiments only interactions from biventer cervicis nerve were lost. In the remaining two experiments the lesions did not affect the interactions.

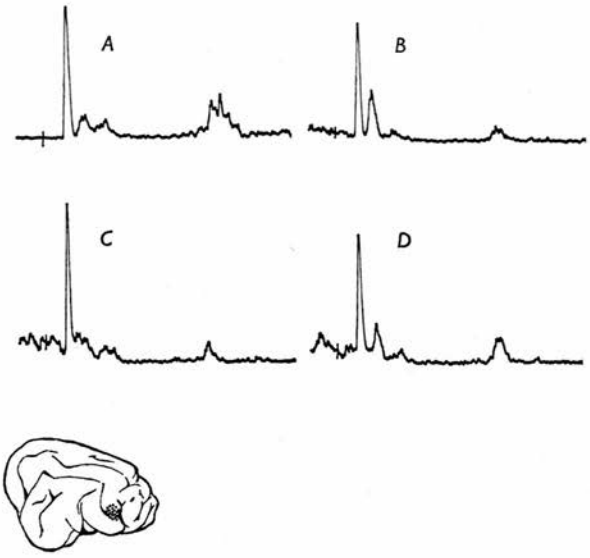

Fig. 4. Cat, chloralose, 60 mg/kg. Loss of interactions after restricted lesion of suprasylvian gyrus. *A*, control reflex; *B*, prior biventer cervicis stimulation; *C*, prior superficial radial stimulation; *D*, prior dorsal interosseus stimulation. Extent of lesion shown on inset diagram. Time calibration: 13·5 mm = 20 msec.

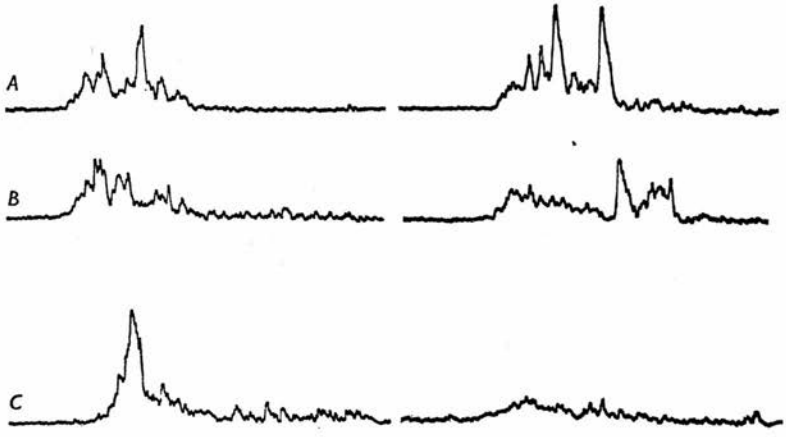

Fig. 5. Cat, chloralose 60 mg/kg. Selective loss of dorsal interosseus direct response following bilateral ablation of the PCD. Activity in L7 ventral root, left, before PCD ablation; right, after PCD ablation. *A*, superficial radial stimulation; *B*, biventer cervicis stimulation; *C*, dorsal interosseus stimulation. Time calibration: 20 mm = 20 msec.

The pathway from neck muscle and forepaw nerves to the cortex is crossed. After a lesion of the ASSG had abolished interactions from contralateral nerve stimulation interactions were still readily obtained following ipsilateral forepaw or neck stimulation. The descending pathway is bilateral, for the interactions from the ipsilateral forepaw or neck nerve were exerted equally on reflexes obtained from either side of the lumbosacral spinal cord.

Effects of cortical ablation on ventral root discharge. Stimulation of nerves entering the cord at cervical levels not only led to a potentiation of lumbosacral monosynaptic reflexes but also to an inconstant discharge over the lumbosacral ventral roots (Abrahams & Falchetto, 1969). This direct response was also affected by lesions of the frontal pole. Frequently the reduction or loss of an interaction was accompanied by the loss of a direct response. Examples of this can be seen in Fig. 3. On many occasions there was a selective loss of direct responses arising from stimulation of one nerve. Lesions of the ASSG usually abolished direct responses from the biventer cervicis nerve, even when the interaction was only reduced. Fig. 5 illustrates another instance of selective loss of one direct response; in this case the dorsal interosseus response was abolished by bilateral ablation of the PCD.

DISCUSSION

The experiments reported here reinforce our previous observations (Abrahams & Falchetto, 1969) that descending cervico-lumbar reflex interactions are more consistently observed and are greatly enhanced when supratentorial structures are present. Additionally, evidence is now presented to implicate the cerebral cortex in descending cervico-lumbar reflex interactions. A small region of the ASSG which approximates to the limited area receiving proprioceptive input from neck muscles (Landgren & Silfvenius, 1969) seems critical for descending reflex interactions from neck muscle nerves. The relationships between the cerebral cortex and descending reflex interactions from forepaw nerves is rather different. As these and our previous experiments have shown (Abrahams & Falchetto, 1969) such descending interactions from forepaw nerves can be obtained in the decerebrate cat. However, the presence of an intact cortex gives rise to interactions of greatly enhanced magnitude which are more regularly obtained. Yet we have found that ablation of quite restricted regions of the cerebral cortex will interfere with even these interactions. Regardless of the exact functional relationship of the cerebral cortex in descending cervico-lumbar reflex interactions, the fact that it plays some role is evidence that the cortex is involved in the co-ordination of intraspinal mechanisms concerned with posture.

The cerebral cortex of the cat has usually been regarded as of minor importance in the reflex regulation of posture. This view largely arose from the wide range of postural reflex capability of subcortical structures (Magnus, 1926) and from the study of chronically decorticate cats, which are well able to stand and walk (Dusser de Barenne, 1919; Schaltenbrand & Cobb, 1930; Bard, 1931, 1932, 1933; Bard & Rioch, 1937; Bard & Mountcastle, 1948). Bard (1931, 1932) makes it clear that normality of locomotion in decorticate cats is relative and he refers (Bard, 1931) to 'approximately normal locomotion' and (Bard, 1932) to 'relative normality of locomotion'. Schaltenbrand & Cobb (1930) describe their long surviving decorticate cat as having an instability of gait and walking on a wide base. Other defects of locomotion in decorticate cats were described by Bard & Rioch (1937) and Bard & Mountcastle (1948) and included unusual head position and an odd gait with a retardation of the initiation of stepping. There is some similarity between these defects and those that follow section of neck muscle nerves and which prompted the present enquiry (Abrahams & Falchetto, 1969).

As well as having locomotor deficits the decorticate cat has deficits of static posture. There is a gradual and uncorrected sliding out of position so that ultimately the animal may sustain a grotesque position (Dusser de Barenne, 1919; Rademaker, 1930; Schaltenbrand & Cobb, 1930; Bard, 1933). This deficit was thought by Rademaker (1930) to be due to the loss of placing and hopping reflexes. These reflexes Rademaker (1930) viewed as dependent on cortical integrity. Bard (1933) showed that indeed in the cat that placing and hopping reflexes depended on the integrity of a small pericruciate cortical region. However, cats with this cortical region ablated, and with no placing and hopping reflexes, still exhibited oddities of static posture (Bard, 1933). Thus, neither static posture nor locomotion in the decorticate can be regarded as normal, and the deficits are substantial. The corollary of this is that the cortex of the cat does have a major role to play in the reflex regulation of posture. In the present experiments we have largely confined ourselves to an examination of monosynaptic reflex excitability in the lumbosacral cord. The ability of the cortex to manipulate lumbosacral monosynaptic reflex excitability is well known from previous work (Bernhard *et al.* 1954; Stewart *et al.* 1968). What has now been demonstrated is that the cortex is involved in generating this output at the lumbosacral level from information entering at the cervical level. Such a system, operating through monosynaptic reflex excitability, is well adapted to stabilize posture in the presence of varying loads. This the hind legs of the decorticate dog cannot do and they bend when slight pressure is placed on the pelvis (Rademaker & Winkler, 1928). Whether or not this is a function of the ASSG cannot at present be said. Little is

known concerning the functional significance of the ASSG, but it does receive extensive connexions from major sensory systems. In addition to the proprioceptive connexions previously mentioned, the ASSG has input from the vestibular system (Walzl & Mountcastle, 1949; Kempinsky, 1951; Mickle & Ades, 1952; Andersson & Gernandt, 1954; Landgren, Silfvenius & Wolsk, 1967*b*), from skin (Landgren *et al.* 1967*a*; Landgren *et al.* 1968) and from visual and auditory systems (Landgren *et al.* 1967*b*; Landgren & Silfvenius, 1968).

It may be of some significance that it is descending cervico-lumbar reflex interactions from neck muscle receptors that are particularly dependent on the integrity of the ASSG. In a consideration of reflexes originating from neck structures it has generally been suggested that the receptors are not in muscle, but lie in deeper structures. Tonic neck reflexes are dependent on vertebral structures (McCouch, Deering & Ling, 1951). Cervical nystagmus that follows the injection of local anaesthetic into neck muscles is attributed to interference with deep receptors rather than interference with muscle receptors (Biemond & de Jong, 1969). (However, in this work reference is made to experiments of Longet dating from 1845 in which he described disorders of gait that resulted from interference with the cervical musculature in dogs, horses, sheep, cats, rabbits and copybaras.) Nonetheless, in man, neck muscles have a high density of muscle spindles with levels approaching those of the intercostals (Voss, 1958). Since the intercostals have a unique system of reflex control through the fusimotor system (Sears, 1964) it might be argued that a similar control system exists from neck muscles and this explains the high density of spindles. Such a hypothesis is not borne out by the consequences of section of neck muscle nerves, for this leads to minimal interference with head movement but to an interference with hind leg gait (Cohen, 1961; Abrahams & Falchetto, 1969). It seems likely therefore that the richness of the spindles in neck muscle relates more to a sensory function with respect to head position and loading. In the cat, the head represents a substantial proportion of the total body mass, perched at one extremity well away from the centre of gravity. Head movements inevitably will alter loading on the muscles supporting the body. It should be no surprise that input related to head position should control a system underlying the type of reflex interactions that has been described here.

Some additional mention should be made of other events in the lumbosacral cord that follow stimulation of forequarter nerves. Characteristically, the chloralose anaesthetized cat will show a vigorous jerk after somatic or auditory stimulation. This 'chloralose jerk' was shown by Adrian & Moruzzi (1939) to involve the cerebral cortex, since it was accompanied by discharge in the pyramidal tracts. Defined electro-

physiologically, chloralose jerks are 'a reflex discharge which can be recorded from somatic motor axons, lasting some 10–25 msec and with a latency varying between 10 and 80 msec depending on the stimulation and recording site' (Devananden, Eccles, Lewis & Stenhouse, 1969). What has been called in this and previous work the 'direct response' (Abrahams & Falchetto, 1969) should be considered synonymous with the chloralose jerk. Intracellular records by Devananden *et al.* (1969) show that the direct discharge results from EPSPs generated in the motoneurones. These EPSPs are followed by a prolonged (1–200 msec) hyperpolarization which does not have IPSP characteristics. Descending reflex interactions are characterized by a prolonged monosynaptic facilitation which occurs during this period of motoneurone hyperpolarization. It would seem likely therefore that they utilize a system, either presynaptic or remote, that has the ability to selectively enhance the excitability effects of group 1A afferent terminations. The existence of such a cortically operated system must, of necessity, presume a substantial cortical role in the reflex regulation of posture.

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INTRASPINAL EXCITABILITY CHANGES DURING CORTICALLY MEDIATED DESCENDING CERVICO-LUMBAR REFLEX INTERACTIONS. Vivian C. Abrahams, Dept. of Physiol., Queen's Univ., Kingston, Ontario

Previous experiments have shown that a prominent characteristic of cortically mediated descending cervico-lumbar reflex interactions is a prolonged and selective facilitation of lumbosacral monosynaptic reflex excitability. Because of the selective effects on the monosynaptic reflex, it is unlikely that a postsynaptic mechanism is responsible. It has not proven possible to find evidence for a presynaptic mechanism. Tests of terminal excitability in gastrocnemius nerves have shown that there are changes after stimulation of nerves entering the cervical cord, but these occur too late to explain the facilitation, and further, the changes should lead to inhibition of the monosynaptic reflex not to a facilitation. Examination of dorsal root potentials associated with the descending interactions has also failed to provide evidence for a presynaptic mechanism. It is concluded that the selective facilitation of the monosynaptic reflex either involves a remote (dendritic) or some other hitherto unexplained system. (Supported by the Medical Research Council of Canada.)

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Individual mechanisms activated in descending spino-spinal activity in the chloralose anaesthetized cat

By V. C. Abrahams. *Department of Physiology, Queen's University at Kingston, Ontario, Canada*

Spino-spinal activity in the chloralose anaesthetized cat is mediated via supraspinal structures (Alvord & Fuortes, 1954; Shimamura & Yamauchi, 1967; Devanandan, Eccles, Lewis & Stenhouse, 1969; Abrahams & Falchetto, 1969) utilizing the cerebral cortex (Adrian & Moruzzi, 1939; Ascher, Jassik-Gerschenfeld & Buser, 1963; Abrahams, 1970). When a forepaw or neck nerve is stimulated a discharge is recorded in the lumbosacral ventral roots with a latency of 15–25 msec lasting 10 or 15 msec (Alvord & Fuortes, 1954). If lumbosacral reflex excitability is tested during the discharge both monosynaptic and polysynaptic reflexes are found to be greatly potentiated. The spino-bulbo-spinal reflex (Shimamura & Livingstone, 1963) is abolished prior to the appearance of the ventral root discharge, and remains abolished for about 400 msec. Following the ventral root discharge, the monosynaptic reflex remains enhanced for about a further 60 msec, but polysynaptic reflexes are now reduced or abolished. The differential effect on monosynaptic and polysynaptic reflexes makes it unlikely that at this time there is a generalized increase in motoneurone excitability of the type present during the ventral root discharge.

The selective potentiation of monosynaptic reflex excitability might be due to a pre-synaptic mechanism. Experimental testing of this hypothesis proves it not to be correct. Rather than the period of pre-synaptic excitability that might be expected to occur, a period of pre-synaptic inhibition of Gp 1 A fibres was found. The existence of presynaptic inhibition with a peak 40–60 msec after stimulation was confirmed by the finding that dorsal-root potentials are regularly recorded in lumbosacral dorsal rootlets following cervical nerve stimulation.

Thus, at least four distinct events occur in the lumbosacral cord in descending spino-spinal activity: (1) a period of enhanced motoneurone excitability, presumably due to EPSP activity (Devanandan *et al.* 1969) and coinciding with the ventral root discharge, (2) a period of presynaptic inhibition of Gp 1 A fibres, (3) a prolonged inhibition of spino-bulbo-spinal activity by an unknown mechanism, and (4) a late and prolonged monosynaptic reflex facilitation, also by an unknown mechanism. Each of these events may depend on the existence of separate systems, each with a separate intracerebral course since discrete cortical lesions lead to loss of one or another of the four events with little or no effect upon the others. Further, cortical lesions at some sites affect descending effects from one particular nerve without having effect on the response to stimulation of another nerve.

These experiments suggest that there are a number of parallel pathways open in the chloralose anaesthetized cat whereby effects can be exerted on the spinal cord. These mechanisms may represent some or all of the ways by which sensory systems can affect posture.

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SPINO-SPINAL MECHANISMS IN THE CHLORALOSE ANAESTHETIZED CAT

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SUMMARY

1. In chloralose anaesthetized cats the events in the lumbosacral cord that follow stimulation of a forepaw or neck nerve have been examined.

2. Four events take place in the lumbosacral cord after forepaw or neck muscle nerve stimulation. These are: (1) a brief period of generalized enhanced motoneurone excitability, (2) a prolonged and as yet unexplained enhancement of monosynaptic reflexes, (3) a period of presynaptic inhibition in muscle and skin afferent fibres, and (4) a prolonged inhibition of spino-bulbo-spinal reflexes.

3. A variable and sometimes selective reduction of one or another descending effects follows the placing of small lesions in the anterior portion of the suprasylvian gyrus.

INTRODUCTION

The chloralose-anaesthetized cat responds reflexly to almost any form of sensory stimulus by a characteristic jerk. Sometimes studied from a pharmacological standpoint (Hanriot & Richet, 1893; Alvord & Fuortes, 1954; Devanandan, Eccles, Lewis & Stenhouse, 1969*a*, *b*), sometimes mentioned as a curiosity in the course of other investigations (Adrian & Moruzzi, 1939), the neurophysiological events underlying the phenomenon have some unique characteristics. In particular, spino-spinal effects are mediated by the well-known intraspinal pathways (Sherrington & Laslett, 1903; Lloyd, 1942; Lloyd & McIntyre, 1948) but utilize pathways which loop supraspinally and in which cerebral cortex has an important role (Adrian & Moruzzi, 1939; Ascher, Jassik-Gershenfeld & Buser, 1963; Devanandan *et al.* 1969*a*; Abrahams & Falchetto, 1969; Abrahams, 1970*a*). In the present work some of the events that take place in the lumbosacral cord as a consequence of activity in these looping pathways have been examined.

METHODS

All experiments were performed on cats anaesthetized with a single intravenous injection of chloralose (60 mg/kg) following the induction of anaesthesia with ethyl chloride and ether. Body temperature of the cats was controlled at or close to 38° C by a heated table. Blood pressure and respiration were continuously monitored and recorded. Appropriate dorsal and ventral roots of the lumbosacral area were exposed and placed on bipolar platinum electrodes for electrical recording. Nerves of the neck, fore and hind paws were also dissected free and placed on bipolar platinum electrodes which were used for electrical recording and stimulation. All exposed nervous tissue was bathed in a silicone oil (Medical Fluid 360, Dow Corning Silicone) in skin flap baths in the usual manner. Amplification was by a.c. amplifiers including that for the recording of dorsal root potentials (DRPs) when an over-all time constant of 1 sec was used. Records of oscilloscope traces were made photographically.

The technique for testing the excitability of the termination of axons within the spinal cord followed that of Wall (1958). A stainless steel or tungsten micro-electrode (Transidyne type 415–20 or 405–20) was introduced into the spinal cord with a micromanipulator at an angle of about 20° so that the electrode tip would ultimately rest in the ventral horn. This was done either after section of all ventral roots on one side of the cord from L 4 to S 2, or with all ventral roots except L 7 and S 1 sectioned. In the latter experiments the L 7 and S 1 roots were dissected free and a ligature placed round each root so that the roots might be cut during the course of the experiment.

Conduction velocities of fibres in the superficial radial and dorsal interosseus nerves were measured by placing bipolar recording electrodes on the appropriate branch of the brachial plexus proximal to the stimulus site. Because of the short length and rapid conduction velocity of fibres in the biventer cervicis nerve, it was not possible to measure conduction velocity in this way. Records were made from a dorsal rootlet of one of the first three cervical roots at the point of cord entry either by a fine pair of bipolar electrodes placed under the rootlet or by a stainless steel micro-electrode just entered into the rootlet at the point of cord entry.

All brain lesions were placed using subpial suction. Positioning of the lesion was verified by examination of sections cut from the formalin-fixed brain.

RESULTS

L 7 direct discharges and segmental reflex excitability changes

As is well known (Alvord & Fuortes, 1954; Abrahams & Falchetto, 1969; Abrahams, 1970*a*), stimulation of a forepaw or neck muscle nerve in the chloralose-anaesthetized cat leads to a discharge in lumbosacral ventral roots after a latency of 12–15 msec. The duration of this discharge is between 8–20 msec and when segmental reflexes are tested during the discharge both monosynaptic and polysynaptic reflexes are found to be greatly enhanced. The monosynaptic reflex facilitation outlasts the duration of direct discharge and may persist for a further 40–60 msec. After the direct discharge polysynaptic reflexes are usually reduced below control levels and may even be abolished (Fig. 1). In a small proportion of animals the direct discharge is weak and the course of interaction in such

experiments is somewhat different. The facilitation of polysynaptic reflexes is minimal, although the monosynaptic reflex still shows a prolonged period of facilitation which commences 25–30 msec after forepaw or neck muscle nerve stimulation.

The effects on polysynaptic reflexes may be examined in reflexes elicited by sural nerve stimulation, or by reflexes recorded from a ventral root

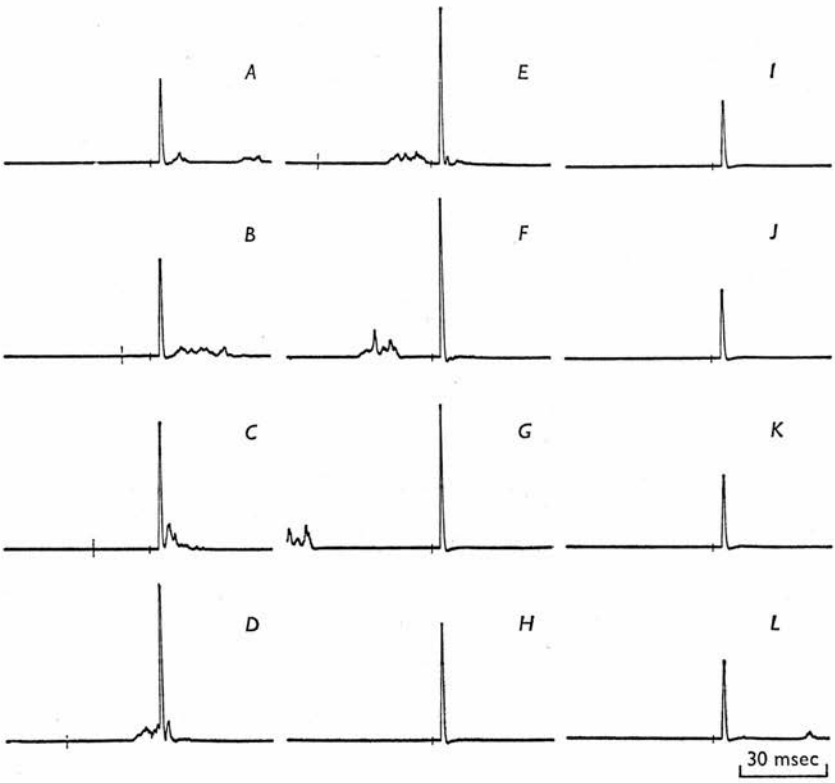

Fig. 1. Cat, chloralose 60 mg/kg. Effect of neck muscle nerve stimulation on lumbosacral reflexes. *A*, control reflex. Effect on reflex of stimulation of biventer cervicis nerve at *B*, 10 msec; *C*, 20 msec; *D*, 30 msec; *E*, 40 msec; *F*, 50 msec; *G*, 80 msec; *H*, 100 msec; *I*, 200 msec; *J*, 300 msec; *K*, 400 msec; *L*, 500 msec before the reflex. Recordings from L7 ventral root. Reflex elicited by posterior tibial nerve stimulation.

one or more segments removed from that showing the presence of monosynaptic reflexes. Typical results of such an experiment are shown in Fig. 2. The polysynaptic reflex vanishes when the interval between conditioning and test stimulus is as brief as 10 msec. When the direct response and the polysynaptic reflex coincide, then the response is enhanced. When

the test reflex occurs late in the direct response it is weakened, and it remains depressed for intervals up to and exceeding 70 msec.

In chloralose anaesthetized cats a third reflex is seen about 10 msec after the end of the main polysynaptic elevation. This is the spino-bulbo-spinal (SBS) reflex (Shimamura & Livingstone, 1963). As previously reported (Abrahams & Falchetto, 1969), this reflex is inhibited within 10 msec of forepaw or neck muscle nerve stimulation and remains inhibited for periods up to or exceeding 400 msec.

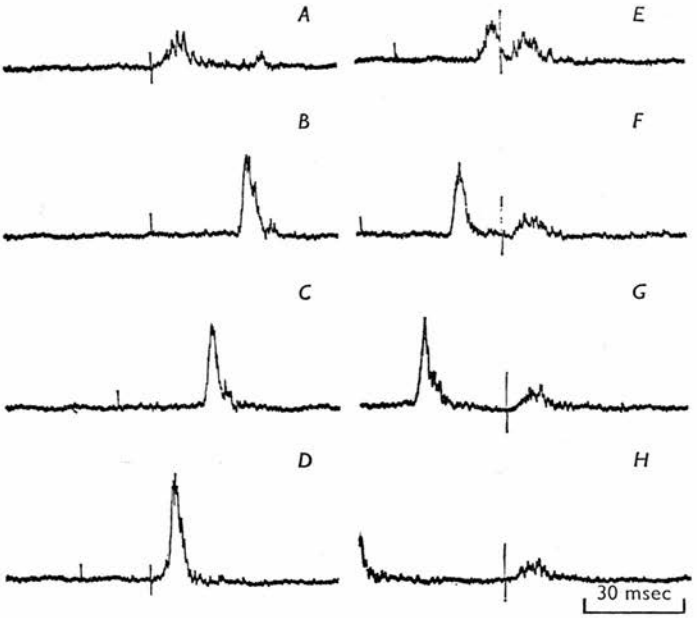

Fig. 2. Cat, chloralose 60 mg/kg. Effect of forepaw muscle nerve stimulation on lumbosacral polysynaptic reflex. *A*, response to stimulation of sural nerve; *B*, response to stimulation of dorsal interosseus nerve. Effect on reflex of stimulation of dorsal interosseus nerve at *C*, 10 msec; *D*, 20 msec; *E*, 30 msec; *F*, 40 msec; *G*, 50 msec; *H*, 70 msec before the reflex. Note inhibition of polysynaptic reflex at short conditioning test intervals, and its reduction at longer intervals. Records from L7 ventral root.

Terminal excitability in the lumbosacral cord in descending interactions

The obvious explanation of the prolonged and selective facilitation of lumbosacral monosynaptic reflexes would be a descending presynaptic action on group Ia fibres. This hypothesis was tested in twenty cats with the method of Wall (1958). No evidence could be found for a presynaptic effect that could be responsible for the prolonged and selective mono-

synaptic facilitation. On the contrary (Fig. 3), evidence has been obtained for a period of depolarization with its peak 40–60 msec after forepaw or neck muscle nerve stimulation. It is possible, with slight modification of the Wall (1958) technique, to simultaneously test terminal excitability and excitability in the orthodromic pathway. With the L 7 and S 1 ventral roots intact, the electrode was placed 1–2 mm into the cord at the level of

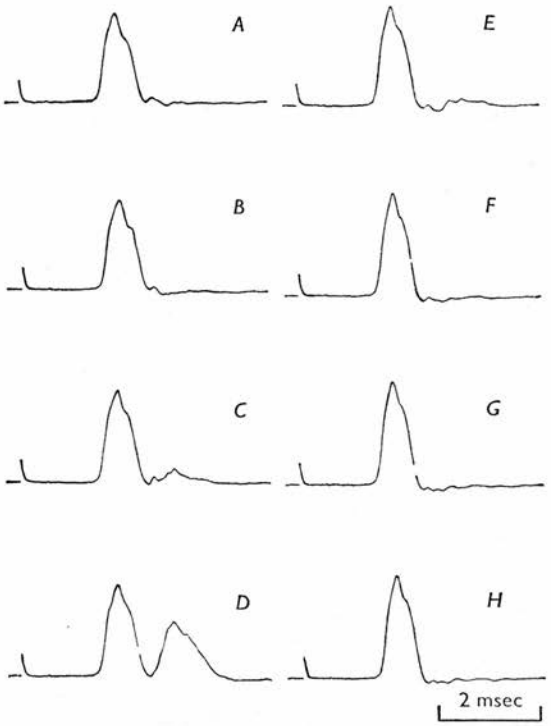

Fig. 3. Cat, chloralose 60 mg/kg. Compound action potentials recorded from lateral gastrocnemius nerve after intraspinal stimulation. Ventral roots L 4–6 and S 2 sectioned. Micro-electrode about 1 mm into cord at dorsal root entry of L 7. Effect of stimulation of neck muscle nerve *B*, 10 msec; *C*, 20 msec; *D*, 25 msec; *E*, 30 msec; *F*, 40 msec; *G*, 50 msec; *H*, 60 msec before intraspinal stimulation.

dorsal root entry and stimulus strength was adjusted so that the compound action potential recorded on the gastrocnemius nerve showed two elevations. With the electrode about 2 mm into the cord the second elevation may be quite small. When the electrode is advanced into the ventral horn, the two elevations are approximately equal (Figs. 3 and 4). The effects of forepaw or neck muscle nerve stimulation were then tested. At the time that the direct response appears on the ventral root, increase in the second

elevation can be seen coincident with the appearance of the direct response (Fig. 3). The early elevation was unaltered at this time, but when the conditioning interval exceeded 40 msec and when the late elevation had returned to normal, the early elevation was enhanced. The early wave is due to antidromic conduction and the late wave due to conduction in the orthodromic pathway, for, following section of the remaining L7 and S1 ventral roots, the early elevation remains intact but the late elevation is lost (Fig. 4).

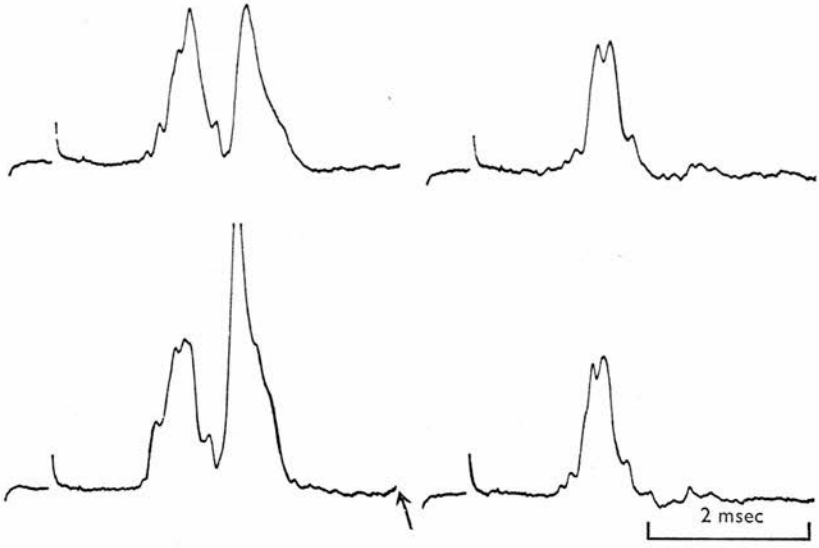

Fig. 4. Cat, chloralose 60 mg/kg. Potentials recorded from lateral gastrocnemius nerve following intraspinal stimulation. Electrode in ventral horn. Top, control stimuli. Bottom, intraspinal stimulation preceded by stimulus to neck muscle nerve (30 msec). Residual ventral roots (L7 and S1) cut at arrow.

These experiments show that forepaw or neck muscle nerve stimulation leads to an increase in excitability in the orthodromic pathway but this is both early and brief and could be either in motoneurones or interneurones. The experiments also show that there are changes in group Ia terminal excitability following stimulation of forepaw or neck muscle nerves, changes which occur at the time when the prolonged monosynaptic facilitation is present. The changes are in the wrong direction, however, and signal the presence of a period of presynaptic inhibition which should signal a reduction in the monosynaptic reflex, not a facilitation.

Although not immediately relevant to studies of the origin of monosynaptic reflex facilitation, the terminal excitability of cutaneous afferents was also tested in the same experiments by recording from the sural nerve.

The effects of the descending system were found to vary among different cutaneous fibre groups. Further, there were differences in the responses elicited by forequarter muscle or skin nerve stimulation. Terminal depolarization in the fastest cutaneous fibres (velocity 60 m/sec) was found as a late response to forepaw nerve stimulation and persisted for up to 120 msec. Such changes were least easily elicited from stimulation of a mixed nerve (but largely cutaneous nerve) such as the superficial radial and were most

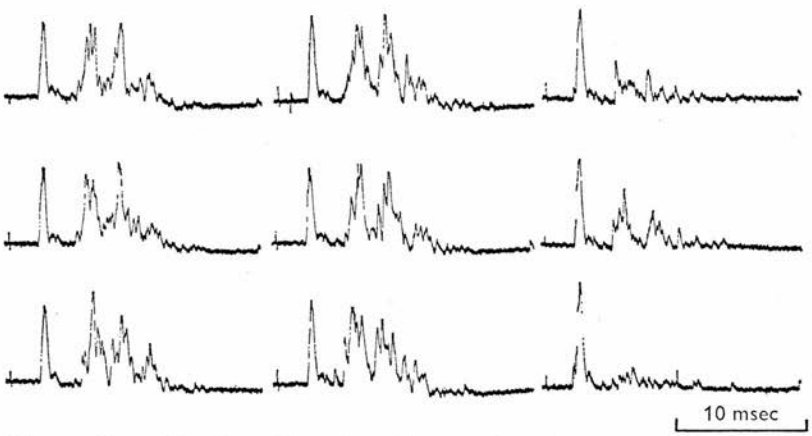

Fig. 5. Cat, chloralose 60 mg/kg. Compound action potentials recorded from sural nerve after intraspinal stimulation. Ventral roots L4 to S2 sectioned. Left panels, control potentials. Middle panels, intraspinal stimulation 30 msec after forequarter nerve stimulation. Right panel, intraspinal stimulation 40 msec after forequarter nerve stimulation. Top records, superficial radial nerve stimulated; middle panel, dorsal interosseus nerve stimulated; bottom panel, biventer cervicis nerve stimulated.

prominent when muscle nerves such as the dorsal interosseus or biventer cervicis were stimulated. When terminal excitability of the slow fibres (15–20 m/sec) was examined there was a period of afferent hyperpolarization, first evident 20–30 msec after a conditioning stimulus and with a duration of 100 msec. This was more readily seen following neck muscle nerve stimulation (Fig. 5).

Dorsal root potentials

The appearance of dorsal root potentials is associated with primary afferent depolarization (presynaptic inhibition) in the spinal cord (Eccles, Magni & Willis, 1962). If the changes in terminal excitability that were found in hind leg group Ia fibres after forepaw nerve stimulation were due to presynaptic inhibition, they should be accompanied by the appearance of dorsal root potentials. In more than thirty experiments such potentials have been consistently recorded from lumbosacral roots after

forepaw or neck muscle nerve stimulation (Fig. 6). The paradoxical rela tionship between segmental reflex excitability and terminal excitability i detailed in Fig. 6. It can be seen that at a time when Group Ia fibres ar apparently inhibited the monosynaptic reflex is still considerably enhanced

The dorsal root potentials recorded on an L7 and S1 rootlet and whic follow forepaw nerve stimulation are of long latency, ranging from 25 t

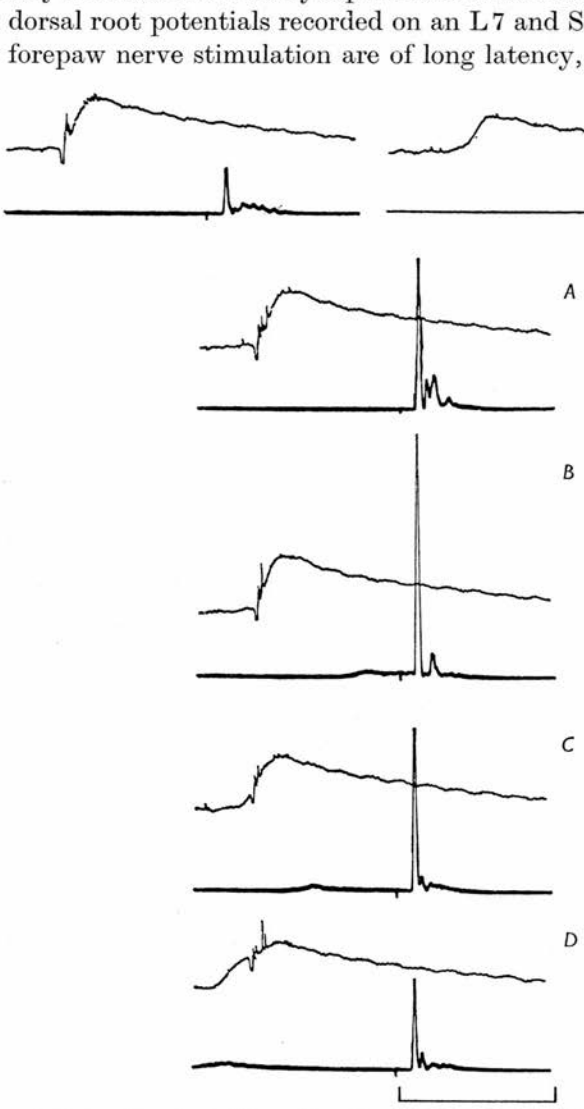

Fig. 6. Cat, chloralose 60 mg/kg. Simultaneous recording of dorsal root potentials and lumbosacral reflexes. Top left, response to posterior tibial nerve stimulation. Top right, response to dorsal interosseus nerve stimulation. Effect of dorsal interosseus nerve stimulation on reflex *A*, 20 msec; *B*, 30 msec; *C*, 40 msec; *D*, 50 msec before reflex. Note time bar represents 12·5 msec for lower (reflex) records, and 50 msec for upper (DRP) records.

40 msec and have their peak some 20 msec or so later. Customarily they have a duration of about 150 msec. They consist only of a long negative nerve DR V (Lloyd & McIntyre, 1949). Following the elicitation of a dorsal root potential from forepaw nerve stimulation, no dorsal root potential can be obtained from stimulation of a hind leg nerve for 2–400 msec and vice versa. The early components of a dorsal root potential elicited from hind leg nerve stimulation DRPs I–IV (Lloyd & McIntyre, 1949) may also be reduced by prior stimulation of a forequarter nerve.

Conduction velocity of fibre groups in forepaw nerves initiating cervico-lumbar reflex interactions

In seven experiments the conduction velocities of the fibre groups in the dorsal interosseus, superficial radial and biventer cervicis that initiated descending interactions were measured. In both skin and muscle nerves it was found that descending effects including SBS loss, direct response, reflex interaction and DRP are present at threshold when only the most rapidly conducting fibres were excited. In the biventer cervicis nerve these fibres conduct at 110–150 m/sec, in dorsal interosseus 90–140 m/sec, and in the superficial radial nerve 80–140 m/sec. As stimulus strength was increased to activate fibres conducting at 50–70 m/sec, the strength of the descending effects increased to a maximum.

Effects of restricted cortical lesions on descending spino-spinal interactions

Evidence has been obtained that the effects of restricted lesions of the frontal cortex are exerted unequally on differing spinal mechanisms. Lesions were made in fourteen cats in the anterior pole of the contralateral suprasylvian and ectosylvian gyrus. Lesions in the region of the anterior syprasylvian gyrus always had some effect on descending effects exerted on the lumbosacral cord. The inhibition of the SBS reflex and monosynaptic reflex facilitation was most readily affected by a small cortical lesion and these effects were almost invariably reduced or abolished by such a lesion. The direct discharge over the ventral roots and its associated brief facilitation of both monosynaptic and polysynaptic reflexes was less affected and may persist even though the later prolonged monosynaptic facilitation was lost. The direct discharge following superficial radial nerve stimulation was least affected by cortical lesions, and often persisted after loss of interactions and direct discharges following biventer cervicis and dorsal interosseus stimulation. The dorsal root potential, although often reduced by cortical lesions, was only rarely abolished. All these effects were associated only with damage to the anterior pole of the suprasylvian gyrus. Lesions of similar size placed more posteriorly in the ectosylvian gyrus were without effect on interactions, direct discharges or dorsal root potentials.

DISCUSSION

In the cat anaesthetized with chloralose, spino-spinal interactions are dependent upon pathways that loop supra-spinally (Adrian & Moruzzi, 1939; Alvord & Fuortes, 1954; Devanandan *et al.* 1969*b*; Abrahams & Falchetto, 1969). The anatomical details of the looping pathway are not known, although there is strong evidence of involvement of the cerebral cortex. Adrian & Moruzzi (1939) found that ablation of the motor cortex eliminated the pyramidal tract discharge that normally follows skin stimulation. Evidence for cortical involvement was also found in an examination of descending spino-spinal systems originating in neck muscle nerves (Abrahams & Falchetto, 1969). In the latter experiments it was found that lesions of the anterior pole of the suprasylvian gyrus, a cortical area receiving input from neck muscles (Landgren & Silfvenius, 1968), abolished descending interactions in acute experiments (Abrahams, 1970*a*). The interactions are not irreversibly abolished and can be demonstrated in animals several days after the placing of a lesion on the anterior suprasylvian gyrus at aseptic operation (Abrahams, 1970*b*).

The cerebral cortex seems able to exert a powerful modulating effect on descending spino-spinal systems, but the cerebral cortex does not appear to be the essential link in an anatomical pathway. In experiments on the role of the cerebral cortex in spinal discharge following sensory stimulus, Ascher *et al.* (1963) found that electrical or chemical stimulation of the appropriate cortical receiving area enhanced the spinal ventral root discharge that follows sensory stimulus. They also found that the topical application of KCl to the appropriate receiving region abolished the ventral root discharge. Despite the loss of response after KCl depression of the cortex, Ascher *et al.* (1963) regard the cortical role as a modulator for they were still able to demonstrate ventral root discharges following ablation of appropriate cortical receiving areas. Further, Buser, St Laurent & Menini (1966) found ventral root discharges following click stimulation to survive removal of the whole neocortex. Our experiments constitute an extension of the findings of Ascher *et al.* (1963) and Buser *et al.* (1966) in that they demonstrate that areas of the cortex are involved in descending spino-spinal interactions. It is also apparent that individual components of the descending interactions may be dependent on the integrity of different cortical areas.

The present experiments, however, have been less concerned with the details of the pathways involved than with the nature of the events that take place in the lumbosacral cord following skin or muscle nerve stimulation in the forequarters. The existence of the chloralose jerk and its electrical analogue, the ventral root discharge, is well known and the intra-

cellular studies of Devanandan *et al.* (1969*a*, *b*) leave little doubt that this discharge is associated with a brief period of EPSP activity in lumbosacral motoneurones. The present experiments have amply confirmed the increased excitability that occurs in this period. A generalized increase in motoneurone excitability could presumably explain the simultaneous facilitation of monosynaptic and polysynaptic reflexes that is seen during the ventral root discharge. The prolonged period of monosynaptic reflex excitability that follows the direct discharge (Alvord & Fuortes, 1954; Abrahams & Falchetto, 1969) is less readily explained. The present experiments fail to show any heightened motoneurone excitability at this time, and indeed the experiments of Devanandan *et al.* (1969*a*) suggest that the reverse happens, for they report that a prolonged period of motoneurone hyperpolarization with decreased synaptic activity follows the EPSP activity. The selective and prolonged monosynaptic reflex facilitation cannot be explained by a period of pre-synaptic hyper-excitability of group Ia fibre terminals. At the time of the prolonged monosynaptic reflex facilitation, evidence from experiments on terminal excitability and from the recording of dorsal root potentials demonstrate a period of terminal depolarization and thus a period of presynaptic inhibition.

Perhaps the simplest explanation of the selective facilitation of the monosynaptic reflex comes from the hypothesis of Frank (1959) and which suggests that a remote (dendritic) facilitation may occur on the dendrite. It seems well established that the majority of synapses including those of Group 1A fibres are not on the motoneurone soma, but are on the motoneurone dendrites (Terzuolo & Llinas, 1965). It is quite possible that descending fibre terminations lie in close proximity to the Ia dendritic terminals and by a local membrane action can modulate the synaptic action of the Ia terminal. This could readily explain the enhanced monosynaptic reflex at a time when the motoneurone is not exhibiting enhanced exictability and the presynaptic fibre is inhibited. It might be expected that the existence of such a system should manifest itself by increased dendritic excitability and thus by increased activity in the orthodromic pathway during intraspinal stimulation. This has not been seen but this too is not unexpected. The dendritic fibres of lumbo-sacral motoneurones are dispersed freely through the cord (Sprague & Ha, 1964). The passage of a current through the stimulating micro-electrode, large enough to excite an appreciable population of dendrites, will simultaneously excite a large population of motoneurones. Activity originating in the dendrites could be masked and thus preclude the ability to distinguish activation of dendritic origin.

No attempt has been made in the present series of experiments to analyse the prolonged inhibition of the SBS reflex, although the existence

of such prolonged effects emphasizes another property of the nervous system of the cat anaesthetized with chloralose. It is a characteristic that responses to sensory stimulation are reduced or eliminated when the stimulus is repeated at brief intervals (Alvord & Fuortes, 1954). The effects on the SBS reflex are in this category and it seems likely that in the cat anaesthetized with chloralose powerful inhibitory systems may be activated by sensory stimuli. The nature of the operation of these systems should itself be of considerable physiological interest.

The present findings suggest that consequences of stimulus application to a cat anaesthetized with chloralose are the near simultaneous activation of a number of what may be independent systems, all with different actions within the spinal cord. There has been some previous attempts to give these phenomena a functional interpretation. Alvord & Fuortes (1954) thought that the mechanism of the chloralose jerk might have some relationship to the mechanisms of the startle reaction. However, it seems likely that following almost any sensory stimulus that the same events take place in the spinal cord, the only obvious differences that occur being in the detail of the timing and the magnitude of the response. Ascher *et al.* (1963) found essentially the same sequence of events in the lumbosacral cord following visual, auditory and cutaneous stimulation. Recently we have found that vestibular nerve stimulation (Abrahams, 1971) leads to a sequence of events in the lumbosacral cord that closely parallels the events that follow stimulation of a forepaw nerve.

It seems likely then that the nervous system of the cat anaesthetized with chloralose is one in which all spinal mechanisms that can be activated by sensory stimulation are nearly simultaneously activated. The response to sensory stimulation is neither appropriate nor graded and normally appears as the well known 'chloralose jerk'. These findings underline the easy access to motor systems that all sensory systems must have, for all sensory systems are inevitably concerned with posture and with movement. Presumably when the response is appropriate and graded it may involve all or only one of the systems described here to an appropriate extent. Direct access to motoneurones (as evidenced by the ventral root discharge) is obviously essential for the direct control of movement. The ability to initiate prolonged periods of inhibition of one or another system is also an obvious asset to the system for it enables irrelevant reflexes to be temporarily suspended. Access to presynaptic or remote systems offers a discrete method of manipulation of individual spinal reflexes, and by specifically being able to manipulate the monosynaptic reflex. It is easy for the body to be stabilized against load fluctuations. The chloralose-anaesthetized animal reveals the existence of a wide range of neurophysiological substrate, which is perhaps the potential armentarium to

sensory inputs whereby they can manipulate the mechanism of posture and movement.

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Some effects on vestibulo-spinal outflow at lumbosacral levels from neck muscle afferents

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In the decerebrate cat a motor discharge is initiated at all levels of the spinal cord by stimulation of the vestibular nerve (Gernandt, Iranyi & Livingston, 1959). Head ventroflexion strongly inhibits the vestibular induced discharge at cervical levels but only weakly affects discharge at lumbosacral levels (Gernandt & Gilman, 1959). This inhibiting effect of head position was attributed to the activation of receptors in the dorsal muscles of the neck. Descending effects from afferents from the dorsal muscles of the neck on the lumbosacral cord were found to be powerful in the cat anaesthetized with chloralose, but absent in the spinal or decerebrate cat (Abrahams & Falchetto, 1969; Abrahams, 1970*a*). Presumably, this is because descending spinal effects from neck muscle afferents are dependent on the integrity of supratentorial structures. In particular they are dependent on the integrity of regions of the cerebral cortex in the anterior pole of the suprasylvian gyrus which receive input from neck muscle proprioceptors (Landgren & Sylfvenius, 1968; Abrahams, 1970*a*).

It now seems apparent that in common with other descending effects from neck muscle nerves, supratentorial structures are necessary for neck muscle afferents to affect vestibulo-spinal output at lumbosacral levels. In the chloralose-anaesthetized cat, stimulation of the cut central end of the nerve to the neck muscle, biventer cervicis, inhibits vestibulo-spinal output in L7 and S1 ventral roots for 200–400 msec. Stimulation of the vestibular nerve in the chloralose-anaesthetized cat also leads to a cycle of presynaptic and post-synaptic excitability changes in the lumbosacral cord resembling those that follow stimulation of skin and muscle nerves entering the cervical cord (Alvord & Fuortes, 1954; Abrahams, 1970*b*). Decerebration abolishes the ability of neck muscle nerves to affect lumbosacral outflow and reduces or abolishes the effects of vestibular nerve stimulation on lumbosacral excitability.

Neck muscle effects on vestibulo-spinal outflow are unlikely to be dependent on the same cortical areas that are involved in other descending effects from neck muscles (Abrahams, 1970*a*). In six out of eight experiments the prolonged inhibition of vestibulo-spinal outflow which follows neck muscle nerve stimulation not only survived excision of the contralateral anterior pole of the suprasylvian gyrus, but also survived excision of the neighbouring region of the ectosylvian gyrus which receives input from vestibular nerves (Walzl & Mountcastle, 1949; Landgren, Silfvenius & Wolsk, 1967*b*). It is concluded that neck muscle afferents can affect vestibulo-spinal output at lumbosacral as well as at cervical levels. For these effects to be readily demonstrated, supratentorial structures are necessary.

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Can. Fed. Biol. Soc., 15, (1972), #732.

732

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Some characteristics of the neck muscle proprioceptive input to the superior colliculi of the cat.

There is an accumulation of evidence pointing to a central role of the superior colliculi in the organisation of head and eye movements. Such movements are based on an ordered output which goes first to the muscles that move the head and then to the extraocular muscles. For such an output to be accurately generated, information must be available as to the position of the head with respect to the trunk. We have now demonstrated that such information is relayed to the superior colliculi. Of 102 cells examined, 60 were fired by stimulation of the neck muscle nerve, biventer cervicis. Many of these cells (47%) also received visual input. Measurement of conduction velocity shows that the fibres projecting to the superior colliculi conduct at 40 m/sec, identifying them as Group II. This indicates that the superior colliculus is provided with a continuous flow of information giving the length of neck muscles. Such information gives the position of the head with respect to the trunk and would be of prime importance in ensuring that an appropriate output is generated for the production of any movement of head and eyes.

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693

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Site of fixation in the spinal cord of the cat.

Fixation may be defined as a semi-permanent functional assymetry induced in the spinal cord and which persists in the surgically isolated cord. The phenomenon has been previously described in rats and guinea pigs following unilateral labyrinthectomy or ablation of one lateral cerebellar hemisphere. We have now demonstrated the phenomenon in the isolated spinal cord of the cat 6 to 8 weeks after unilateral labyrinthectomy. The phenomenon may range from a frank curvature of the trunk with one limb rigidly extended and one flexed to a less evident unilateral mild extension of one hind limb. Preliminary experiments on the mechanisms underlying fixation have tended to rule out the gamma motor system from playing a major role, and no change in motoneurone excitability has been found. It is therefore postulated that fixation is due to a prolonged change of interneurones such that extensor and flexor motoneurones are differentially activated.

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PROPRIOCEPTIVE CONNECTIONS TO THE SUPERIOR COLLICULI OF THE CAT. V. C. Abrahams and P. K. Rose[A]. Department of Physiology, Queen's University, Kingston, Ontario, Canada.

The superior colliculi play a major role in the organisation of head and eye movement in the cat. Such movements must be integrated with other body movements and we now postulate that this integrating function takes place in the superior colliculi. We have found that the superior colliculi receive abundant proprioceptive connection. In cats anaesthetised with chloralose or pentobarbitone 40% of the units sampled in the superior colliculi received both visual and proprioceptive input, and of the remainder, half were activated by proprioceptors alone and half by visual stimulation only. All units responding to proprioceptive stimulation were activated by neck muscle proprioceptors. Many units were activated from the hind legs. The superior colliculi therefore constitute a major projection for proprioceptive systems. This projection may provide the sensory input necessary for the postural adjustments which may be necessitated by or accompany movement of the head and eyes. (Supported by the Medical Research Council of Canada.)

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NECK MUSCLE PROPRIOCEPTORS AND A ROLE OF THE CEREBRAL CORTEX IN POSTURAL REFLEXES IN SUBPRIMATES[1]

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ABSTRACT — It has been found that section of the nerve to a neck extensor muscle may lead to profound disturbances of gait. In attempting to find the origin of this phenomenon descending spino-spinal systems have been found which may be dependant on supra-tentorial loops and which involve the cerebral cortex in the anterior suprasylvian gyrus.

In the last ten years there has been an obvious shift away from older concepts of the regulation of posture. Originally formed by the experiments of Sherrington, Magnus, De Kleijn, Rademaker and their colleagues in the early part of the century, the dominant concept was of a system consisting of "an equilibrium of reflexes". This description was applied by Jung and Hassler[35] who also pointed out, the "... conception (*sic*) that normal body posture is the result of an equilibrium of reflexes is certainly too narrow and its experimental basis obtained by brain-stem transection at different levels in rabbits seems insecure. ... Magnus overrates the reflex conception of peripheral regulation mechanisms from labyrinthine and muscular receptors and neglects the spontaneous innate co-ordinate activity of the cerebral system ...". Much recent work on posture has been concerned with an analysis of the intracerebral connections and functions that could be labelled "innate co-ordinate processes". Work has revealed a neurophysiological substrate with infinitely more complex interconnections and interactions than could have been imagined in the early part of this century. For a brief summary of present work the proceedings of the meeting at Seton College in 1968[21] are worth reading. The experiments described here may seem to be regressive, for they were initially related to the older concepts of reflex regulation of posture. However, the experiments may have some relevance in the interpretation of the role played by the cerebral cortex in the regulation of posture.

In the "equilibrium of reflexes" that Jung and Hassler[35] have described were a class of reflexes that were thought due to activity in neck proprioceptors. De Kleijn[26] demonstrated that in the decerebrate animal, movement of the neck alone could elicit a characteristic set of postural reflexes. These reflexes were labelled "tonic neck reflexes", and were shown to be independent and separate from the labyrinthine reflexes. Although it was recognized that the receptors initiating these reflexes sent fibres into the spinal cord in the upper cervical roots, the nature and location of the receptors

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remained unknown until 1951. Then McCouch, Deering and Ling[45], in experiments to locate the receptors were able to exclude muscle receptors from playing a role, for they found that section of the muscle mass of the neck did not abolish tonic neck reflexes. They ultimately identified the receptive site as lying in vertebral structures, for they found that section of a small branch of the upper cervical dorsal roots serving the vertebrae, abolished tonic neck reflexes. Although these experiments seemed to finally locate the position of receptors underlying tonic neck receptors, they also raised a new question, for proprioceptors are densely distributed in neck muscles[59]. There is also a long history of experiments and clinical observations which show that damage to neck muscles can lead to disorders of posture which cannot be due simply to loss of contractile function in those muscles. Much of this literature was summarised last year by Biemond and De Jong[19]. In 1845 Longet[43] reported that deficits of gait followed interference with neck muscles. The disorder resembled cerebellar ataxia and was observed in a wide range of species including dogs, cats, rabbits, horses and copybaras. A little later, Claude Bernard[17] described an accident which led to similar findings to those of Longet. According to Bernard[17] "Magendie once made investigations on the uses of the cerebrospinal fluid and was led to the conclusion that removing this fluid produces a kind of titubation in animals and a characteristic disturbance in their motions. Indeed, after uncovering the occipito-atloidian membrane, if we pierce it to let the cerebrospinal fluid run out, we notice that the animal is seized with peculiar motor disturbances. Apparently nothing could be simpler or more natural than the influence on their motions of removal of the cerebrospinal fluid; yet this was an error, and Magendie told me how another experimenter chanced to find it. After cutting the neck muscles, this experimenter was interrupted in his experiment at the moment when he had just laid bare the occipito-atloidian membrane. Now when he came back, to go on with his experiment, he saw that the simple preliminary operation had produced the same titubation, though the cephalorachidian fluid had not been removed. What was merely the result of severing the neck muscles had therefore been attributed to removal of the cerebrospinal fluid". Bernard[17] cited this experiment primarily to draw attention to the need for proper controls in experimentation. However the strange phenomenon of ataxia following neck muscle damage itself warrants careful examination and it is this phenomenon which led to the experiments reported here.

The existence of a generalised ataxia following damage to neck muscles was not only seen in animals, but there are occasional clinical reports spread over many years. Reference was made to a syndrome called cervical nystagmus and to one called cervical ataxia[15,25,62]. The origin of both of these phenomena is still in considerable doubt and has only received occasional laboratory investigation. In one set of experiments Cohen[24] investigated the effects of infiltration of the upper three cervical dorsal roots of monkeys with local anaesthesia. The effects of this were profound and the monkeys walked with an unsteadiness like a very drunken man. The monkey were unable to locate points in space, and they showed severe disorientation and imbalance, particularly when walking. In the rabbit, a prominent feature of interference with neck structures was found to be a nystagmus. Biemond and

De Jong[19], were able to exclude skin receptors from playing a role in the origin of the nystagmus, but found that nystagmus was readily provoked by the injection of procaine into the muscle mass of the neck. In further experiments to locate receptor sites they unilaterally denervated the neck and this led to a wide range of postural disorders. Their experiments reinforce the fact that the integrity of neck muscle structures is essential to the maintenance of normal posture. In some way, the nervous system deprived of this information is no longer able to regulate posture in a normal co-ordinated fashion, and in particular there is a severe deficit of the normal mechanisms of oculomotor control.

Our involvement and our own experiments in this field originated with some entirely fortuitous observations. Dr. Falchetto and I were engaged in a systematic analysis of sensory relays to the superior colliculi. In the course of this we discovered a rich proprioceptive relay from the dorsal muscles of the neck to the superior colliculi[3]. At about the same time, Sprague and Meikle[57] reported that bilateral lesions of the superior colliculi of the cat led to deficits of head position. These deficits were quite unusual, and included a loss of the ability to elevate the head. We began to wonder if these two sets of findings were in any way related, whether the superior colliculus, long regarded as essential to the "visual grasp reflex"[34], had a wider role in the regulation of head position.

METHODS

All experiments were performed on cats. When electrical recording was employed anaesthesia was maintained with chloralose (60 mg/kg. i. v. after induction with ether. For the purposes of aseptic operation, or to maintain anaesthesia whilst decerebrating or making a spinal section, Metofane (Pitman-Moore) was employed. All reflexes were recorded electrically in conventional fashion, although averaging of 25 consecutive reflexes was used in searching for small differences. Histological controls were used to determine the extent of cortical lesions.

RESULTS

Information about the location of the motoneurones of neck muscles was sought by sectioning the biventer cervicis nerve, at aseptic operation with the intention of examining the spinal cord for retrograde degeneration. As was expected, the operation led to a defect of upward head movement, but in addition (not being aware of the relevant literature), many of the operated animals also showed gross deficits of posture, particularly affecting the hind legs. This was not due to such obvious factors as the after effects of anaesthesia. Animals given sham operations in which the biventer cervicis nerves were exposed and manipulated, but not cut, had no ataxia. On systematic study[4] it was found that a range of defects of gait were common among cats following biventer cervicis nerve section. After nerve section one third of the animals had such a severe deficit that they resemble cats after bilateral labyrinthectomy[23]. Immediately following operation these animals were relatively immobile. If persuaded to stand, they did so on a wide base with the hind legs spread particularly wide apart (Fig. 1). Any attempt to walk or run led to a fall. This phase of acute disorder lasted 3 or 4 days. Thereafter the animals become more active and began to walk although in very unsteady fashion with frequent falls, particularly when attempting to turn. The locomotor defects slowly disappeared, so that 21 days after operation the animals no longer had any obvious defect. How-

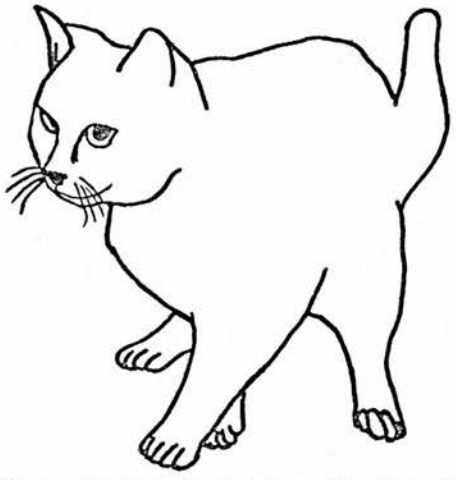

FIG. 1 : Posture of cat 3 days following unilateral section of nerves to biventer cervicis. Note wide separation of hind legs.

ever, even at this time, if an animal jumped or tried to execute a rapid movement, the task was done badly, and the animal frequently fell.

Another third of the animals subjected to section of the biventer cervicis nerves, although grossly affected, had less severe defects than the group just described. This second group was quite mobile immediately after operation, but there were obvious abnormalities of gait. The hind legs were moved with a wide sweeping motion and the cats would often fall when trying to turn. These deficits cleared up rapidly, and within 5 to 7 days of operation the only obvious defect was an instability when turning. In neither of these groups was it possible to show any interference with reflexes that are usually associated with neck afferents. High speed cinematography showed that reflex righting reflexes[44] were unaffected by the nerve section. It was commonly observed that on landing from a fall animals were graceless, and quite uncoordinated.

The remaining one third of the animals subjected to unilateral section of the biventer cervicis nerve were little affected by the operation and only had the slight interference with upward head movement, which was common to all the operated animals. Just as nystagmus may be produced in rabbits by the injection of local anaesthetic into the muscles of the neck[19], so may the locomotor effects of biventer cervicis nerve section be readily reproduced by injections of local anaesthetic into neck muscles. One ml of Xylocaine injected unilaterally into biventer cervicis immediately leads to a gross defect of gait with a marked ataxia and difficulty in turning. At no time has the injection produced a recognizable nystagmus in the cat, nor has biventer cervicis nerve section.

What mechanisms might explain these deficits ? The experiments of McCouch *et al.*[45] seem to eliminate any possible role for neck muscle receptors in tonic neck reflexes. Yet Gernandt and Gilman in 1959 and 1961[32,33], in the course of experiments on the vestibulo-spinal output, showed that altering head position could modulate the output from the cord at cervical levels following stimulation of vestibular nuclei. In an attempt to locate the receptors involved, Gilman and Gernandt[32] found that squeezing dorsal neck muscles affected vestibular output, but squeezing the skin of the neck or muscles other than neck muscles was without effect. It was therefore suggested that receptors in neck muscles could affect vestibulospinal output. However, in these and later experiments on neck position and outflow from the cerebral cortex[33], they found that neck position, whilst having a strong effect on output at cervical levels, had only a weak effect on lumbosacral output. Another instance in which neck position affects

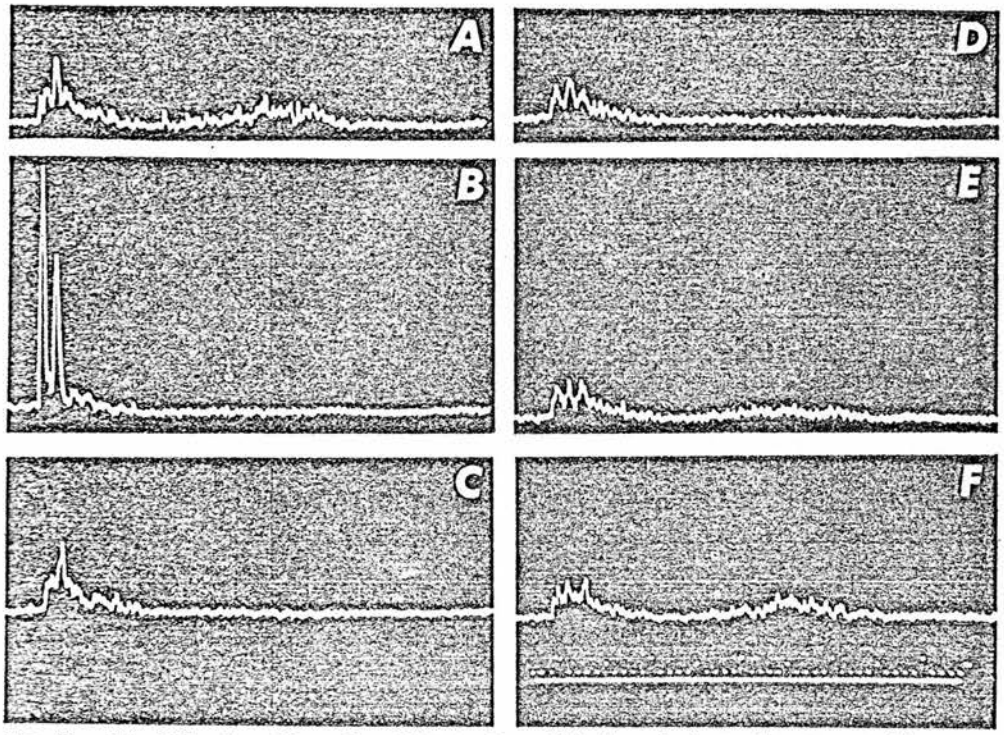

FIG. 2 : Cat. Chloralose 60 mg/kg. Effect of conditioning shock to biventer cervicis nerve on segmental and spino-bulbo-spinal reflexes. Posterior tibial nerve stimulated. Reflex recorded from L7 ventral root. Time marker 1 msec. A, control; B, 30 msec interval; C, 100 msec interval; D, 130 msec interval; E, 150 msec interval; F, 170 msec interval.

cord output was demonstrated by Shimamura and Akert[55] who found head position to affect the amplitude of the SBS reflex[55].

If we are to understand why section of neck muscle nerves had such profound effects on the hind legs we must know something about descending propriospinal systems taking origin in neck muscle nerves. Intraspinal descending propriospinal systems have been known since the experiments of Sherrington and Laslett[54] and a detailed analysis of these intraspinal systems was made by Lloyd[41] and by Lloyd and MacIntyre[42]. Their experiments showed that in the isolated spinal cord there is a descending system with patterned effects exerted unevenly on flexor and extensor muscles of the hind legs. Further the nature of the descending effects depend on whether a forelimb skin or muscle nerve was stimulated. In 10 spinal cats prepared under Metofane, we sought evidence for the existence of a similar descending spinal system taking origin in the nerves of the biventer cervicis muscle. In these preparations there was little difficulty in reproducing the results of Lloyd[41] and Lloyd and MacIntyre[42] and demonstrating patterned effects following forepaw nerve stimulation, but it proved impossible to elicit any effects at lumbosacral level from stimulation of biventer cervicis nerve.

It is well known however, that in preparations with supraspinal structures present and in particular under

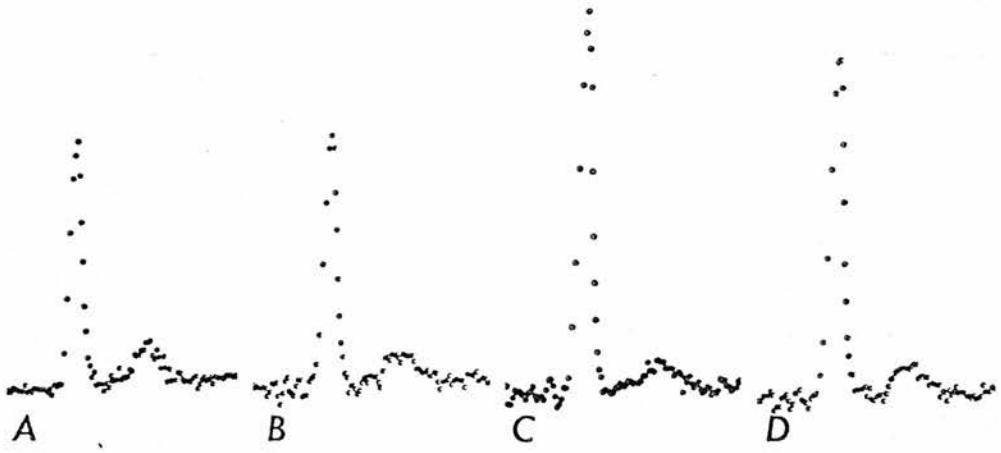

FIG. 3 : Decerebrate cat. Reflexes recorded from L7 ventral root following stimulation of PT nerve. Each record average of 25 consecutive reflexes. A, control reflex; B, reflex preceded by BC nerve stimulation; C, reflex preceded by DI nerve stimulation; D, reflex preceded by SR stimulation. Conditioning interval, 30 msec.

chloralose anaesthesia, that descending spino-spinal systems exist which utilize supraspinal structures[5,6,27,55]. In such preparations stimulation of forepaw nerves leads to a discharge in lumbosacral ventral roots after about 20 msec. This discharge lasts for 8-20 msec and has an amplitude of 1-200 uv and segmental reflex excitability goes through a characteristic cycle during and after such stimulation. In chloralose anaesthetised cats we had no difficulty in showing that effects similar to those obtained from forepaw nerves follow biventer cervicis nerve stimulation. Stimulation leads to a discharge over the ventral roots with a latency of 15-25 msec a duration of 8-20 msec, and an amplitude of about 1-200 uv. Biventer cervicis nerve stimulation affects lumbosacral reflex excitability (Fig. 2). The SBS reflex[56] which is prominent in chloralose anaesthetized cats is suppressed within 10 msec and remains suppressed for 300-400 msecs. When the interval between biventer cervicis nerve stimulation and the elicitation of segmental reflexes was such that the segmental reflex occurs during the ventral root discharge, both monosynaptic and polysynaptic reflex excitability was substantially enhanced. When the interval was extended to 40 or 50 msec the monosynaptic reflex usually continued grossly facilitated but the polysynaptic reflex was reduced below control levels or abolished. This selective facilitation of monosynaptic reflex excitability continued until the conditioning-test interval was about 100 msec. Then both monosynaptic reflex and the polysynaptic reflex continue depressed for a further 100 msec or so. Thereafter the reflex excitability return to control levels.

These experiments provided clear evidence that proprioceptive input from neck muscles could affect lumbosacral reflex excitability and that the presence of supraspinal structures is essential for these effects to be demonstrated. It was easy to show that these structures lie supratentorially. Cats anaesthetized with chloralose were surgically decerebrated at the inter-collicular level after demons-

trating the presence of descending effects from neck muscle and forepaw nerves. The decerebration led to the loss of all descending effects from the neck muscle nerves, but the interactions from forepaw nerves, although reduced were still present (Fig. 3). This result was not unique to the cat anaesthetised with chloralose. Experiments were performed on non-anaesthetized decerebrate cats prepared under Metofane. After allowing time for the anaesthetic to wear off, it was again quite easy to demonstrate descending interactions from forepaw nerves, but once again, there was no evidence of interactions from neck muscle nerves. In these experiments, so that small differences could be found, average responses of spinal reflexes were constructed with an averaging computer and these were used as a basis for comparison. Further confirmation that the action of chloralose was not affecting the responses of the decerebrate cat was obtained by giving an anaesthetic dose of chloralose to the decerebrate cats. This did not modify patterns of descending interaction, although it altered the whole level of reflex excitability.

What supratentorial system might be involved in descending interaction from neck muscles ? The work of Adrian and Moruzzi[5] focussed attention on the cerebral cortex. In their experiments they had shown that ablation of the motor areas abolished the convulsive response that normally followed sensory stimulation. Further, there is overwhelming evidence of proprioceptive connection to the cerebral cortex[7, 31, 37, 38, 40, 46, 48, 49, 50] and specifically a relay from the neck muscle nerves to the anterior pole of the suprasylvian gyrus has recently been demonstrated[40]. We stimulated the frontal pole of the cerebral cortex, testing for changes in lumbosacral reflex excitability following single pulse stimuli of the cortex. Such stimulation produces a wide range of effects on lumbosacral motoneurones, interneurones and afferent terminals. The gross effects[18, 58] of such stimulation at the lumbosacral cord were to be expected from previous experiments, [18, 58] and closely resembles the effects seen in our experiments after forequarter nerve stimulation. There was an early inconstant discharge over the ventral roots, a period of monosynaptic reflex facilitation, and a prolonged inhibition of the SBS reflex (Fig. 4). These effects could be obtained from stimulation of the major proprioceptive receiving areas in the somato-sensory cortex, and also obtained from other regions of the cerebral cortex receiving proprioceptive input, including the post-

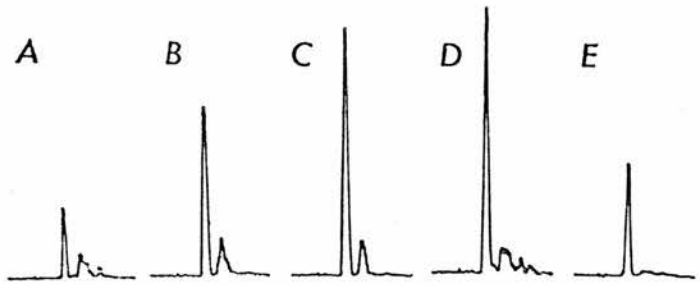

FIG. 4 : Cat. Chloralose 50 mg/kg. Effect of single shock stimulation of the PCD on lumbosacral reflexes. A, control reflex; B, cortical stimulation 10 msec; C, 20 msec; D, 30 msec; E, 40 msec prior to eliciting reflex.

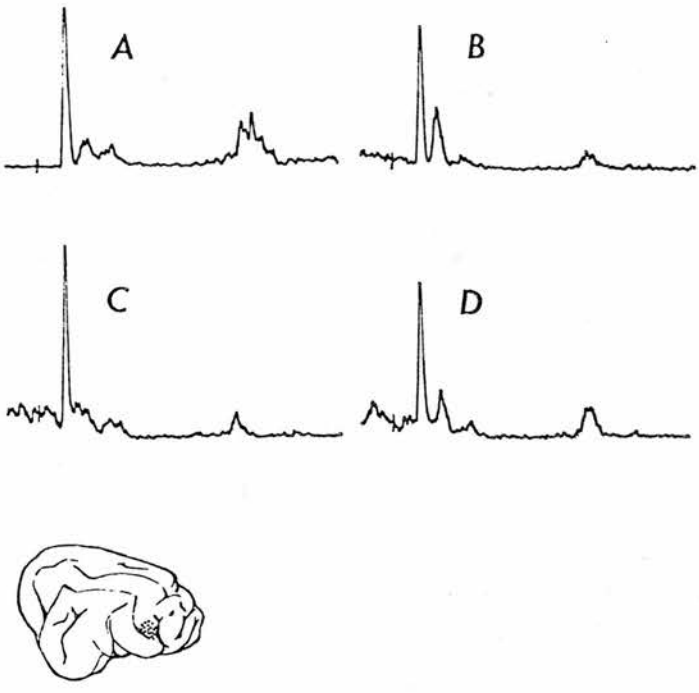

FIG. 5 : Cat. Chloralose 60 mg/kg. Loss of interactions after restricted lesion of suprasylvian gyrus. A, control reflex; B, prior BC stimulation; C, prior SR stimulation; D, prior DI stimulation. Extent of lesion shown on inset.

cruciate dimple[49,50], the second somato-sensory area[31,48] and that region of the anterior suprasylvian gyrus that receives proprioceptive input from the muscles of the neck[37,39,40].

All these cortical regions then are presumably connected directly or indirectly to structures that when stimulated can produce similar effects on the lumbosacral cord to those produced by forequarter nerve stimulation. Ablation experiments were therefore performed to investigate whether the facilitation of monosynaptic reflexes that occurs after a conditioning shock to a neck muscle or forepaw nerve[1] is dependant on cortical structure. The frontal lobes were progressively ablated by suction starting anteriorly at the orbital sulcus. Ablation of the anterior poles of the frontal lobe led to a transient depression of the monosynaptic facilitation, but only for 10 or 15 minutes. Only when the lesion was extended to include the anterior pole of the suprasylvian gyrus was there any lasting loss of the monosynaptic reflex potentiation. In the next series of experiments, restricted lesions of one or another proprioceptive receiving areas were produced. Lesions in the region of the post-cruciate dimple, the region receiving proprioceptive input from forepaw muscle nerves[50], reduced interactions from forepaw nerves without affecting interactions from neck muscle nerves. When a small region embracing the anterior pole of the suprasylvian gyrus was removed then there was usually a prompt (and within the time course of an acute experiment, irreversible) loss of interactions from the biventer cervicis nerve (Fig. 5). In 18 experiments such lesions

reduced or abolished biventer cervicis interactions, whilst having varying effects on descending interactions from forepaw nerves[1]. The ascending pathway, from the neck proprioceptors to the anterior suprasylvian gyrus is evidently crossed, for the effects of a cortical lesion are only to abolish the responses to contralateral nerve stimulation. The descending pathway exerts its effects bilaterally, and abolishes the ability of biventer cervicis nerve stimulation to affect reflex excitability on both sides of the cord.

The particular dependence of interactions from neck muscle nerves on this restricted region of the cerebral cortex led us to the conclusion that was something quite special related to the input from neck muscle nerves. One explanation could be that this is a reflection of the organisation of the regulation of posture. The region of the anterior suprasylvian gyrus that is necessary for the elecitation of descending interactions, is a region which receives input from the vestibular system and from the visual, auditory and cutaneous systems[8,36,37,38,39,47,61]). It seemed possible that this reflected some critical role in the integration of proprioceptive afferents.

What specific role is this region of cortex likely to be playing or, that matter, any region of the cerebral cortex ? If the development of ideas with respect to physiology of the cerebral cortex is followed it is clear that the physiologist for many years has regarded the role of the cerebral cortex of the sub-primate as minimal in the reflex regulation of posture. As previously mentioned, this is due to the careful and thorough analysis of reflexes of postural regulation as an equilibrium of reflexes. The reflex activities and capability of animals following the removal of substantial amounts of brain were described and categorised. It was possible to show an increasing hierarchy of reflexes as more brain was intact, and the experiments and descriptions of this time still form the basis of most textbook accounts of the regulation of posture and leave very little, apart from specific reflexes, for the domain of the cerebral cortex.

Experiments on the decorticate cat or dog were powerful evidence for this concept, for it was said that the decorticate had little malfunction in its progression movements and that the deficits that were observed related to deficiencies of static posture[29,51,53]. On the basis of these accounts and others as summarized by Bard[12], it would seem that the decorticate cat or dog cannot sustain a stable static posture. Slowly and inexorably its legs slide to new and often incongruous postures without any correction on the part of the animals. Thus the decorticate animal may assume and sustain the most bizarre position at rest. As Bard[12] showed, the loss of hopping and placing reflexes which seems to underly this deficit is associated specifically with the loss of the somatomotor area of the cerebral cortex. This was deemed to be the key postural reflex under cortical control and it was considered that all the neurophysiological machinery for the movements of walking and running lay intact in subcortical structures.

Our experiments on ataxia were leading to the view that there was a cortical role in progression movements in the cat. Examination of published accounts do in fact make it quite clear that despite the widely held views to the contrary, postural deficits of the chronic decorticate cat or dog are not restricted to static posture, but there are also well marked abnormalities of

gait[10,11,13,14,43] and of ability to resist a load[52] and further, the decorticate cat has additional deficits that are not present in the cat solely with pericruciate cortex ablated, including the adoption of an abnormal crouched posture with the head held low[12]. This evidence suggests that not only does the cortex of the cat have a greater role in static posture than is commonly believed, but that the cortex must also play some role in the coordination of progression movements. Certainly, as has previously been mentioned, the proprioceptive connections to the cerebral cortex are rich, to regions whose functions are not understood, and which might well be involved in progression movements.

Accordingly, a series of experiments was initiated on the chronic effects of lesions restricted to the anterior pole of the suprasylvian gyrus. As yet unfinished, the experiments have given unexpected results. Although both unilateral or bilateral ablation of the anterior suprasylvian gyrus affects normal walking or running, the effects have never been as devastating as biventer cervicis nerve section. In about half the operated animals there have been defects of movement, but always small and transient, and lasting no more than 3 days. The deficits did not appear in control sham operated animals in which skull and dura were opened. Like the animals after biventer cervicis section, the operated animals show some instability of gait of the hind leg with difficulties most pronounced when turning. Watching and filming cats walking is not in our opinion an adequate method for noting deficiencies of postural mechanisms and we may simply have missed more subtle defects. Further investigation must await the development of more subtle devices such as the controlled pertubation platform of Brookhart[2] or the unstable platform of Begbie[16] for such testing.

The fact that chronic lesions of the anterior suprasylvian gyrus do not have such devastating effects as section of the biventer cervicis nerves strongly suggests that spino-spinal loops involving the cerebral cortex are not a simple single-channel pathway. Rather, as Ascher, Jassik-Gerschenfeld and Buser[9] and Buser, St. Laurent and Menini[22] have showed in their experiments, the cortical role may be that of a modulator. Ascher *et al.*[9] were concerned with the long latency discharge which can be recorded from ventral roots following almost all forms of sensory stimulation. They showed that it could be enhanced by chemical or electrical excitation of the appropriate cortical receiving area, or inhibited if that cortical receiving area was depressed by potassium chloride. However, the ventral root discharge persisted after decortication. Within the conditions of our ablation experiment it is conceivable that the involvement of the anterior pole of the suprasylvian gyrus is in such a modulating capacity and that the loss of response in acute experiments is a transient phenomenon. This has indeed proven to be the case and we find that several days after a chronic lesion of the anterior pole of the anterior or suprasylvian gyrus, when gait has returned to normal, descending cervico-lumbar reflex interactions are demonstrable at terminal acute experiments.

If the cortex has the job of modulating events in the lumbosacral cord, the question now is what events ? In all our experiments up to this point we had been concerned with the prominent facilitation of the monosynaptic reflex for this is the most prominent and (next to the inhibition of the

SBS reflex) the most consistently observed phenomenon.

However what happens in the lumbosacral cord after a stimulus to a nerve entering the cord at cervical levels is not a single event. The most obvious consequence is the direct discharge, which is synonymous with the chloralose jerk. If reflex excitability is tested during the chloralose jerk, both monosynaptic and polysynaptic reflexes are facilitated. We would have little hesitation in assigning that to a generalised increase in motoneurone excitability, and indeed this has been well demonstrated in intracellular studies[27,28]. We do not find, as these authors do, that such effects are most consistently obtained on flexor motoneurones, as we have found facilitation to be equally prominent in monosynaptic reflexes of both flexor and extensor origin[3].

During the period of monosynaptic and polysynaptic reflex facilitation, the SBS reflex is inhibited. This is an early effect and is first seen within 10 msec of stimulation of a forepaw nerve. It is also a prolonged effect and may exceed 400 msec. After the direct discharge has ended, testing of segmental reflex excitability has revealed a rather unusual phenomenon. The monosynaptic reflex normally stays enhanced, but the polysynaptic reflex is usually reduced and frequently completely abolished (at the gain settings commonly used). There is therefore a selective facilitation of monosynaptic reflexes. This would suggest that one descending effect is facilitation, or disinhibition in a pathway terminating pre-synaptically and able to selectively control the Gp. 1A afferent excitability.

To test for such a pre-synaptic action we have employed the test of terminal excitability of Wall[60]. In this test all ventral roots are cut, and an antidromically propagated compound action potential recorded in a peripheral nerve following intraspinal stimulation. Any change in that action potential is considered due to changes in the excitability of the nerve terminals entering the cord. At no time in our experiments have we obtained any evidence for changes in Gp. 1A terminal excitability that would explain an enhanced monosynaptic reflex[2]. On the contrary, we have consistently found evidence for pre-synaptic inhibition with a peak 40-50 msec after forepaw stimulation (Fig. 6). The existence of such a primary afferent depolarization has been confirmed by the

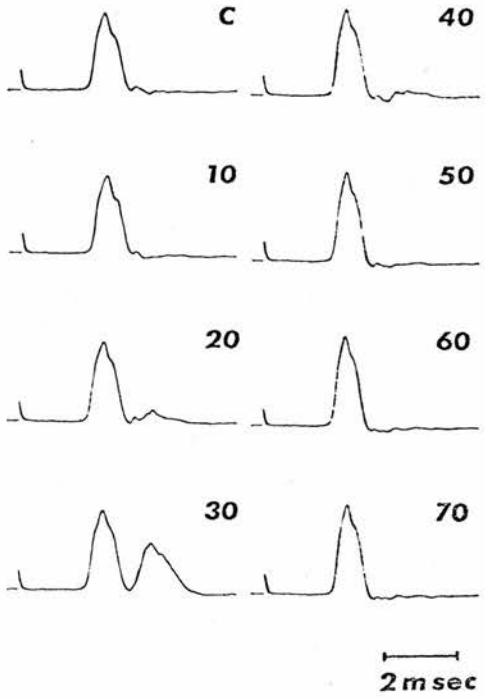

FIG. 6 : Cat. Chloralose 60 mg/kg. Compound action potentials recorded from lateral gastrocnemius nerve following stimulation in root entry zone of L7 segment. Ventral roots L4, L5 and L6 and S2 cut. Effect of conditioning shock to biventer cervicis nerve at stated intervals (in msec).

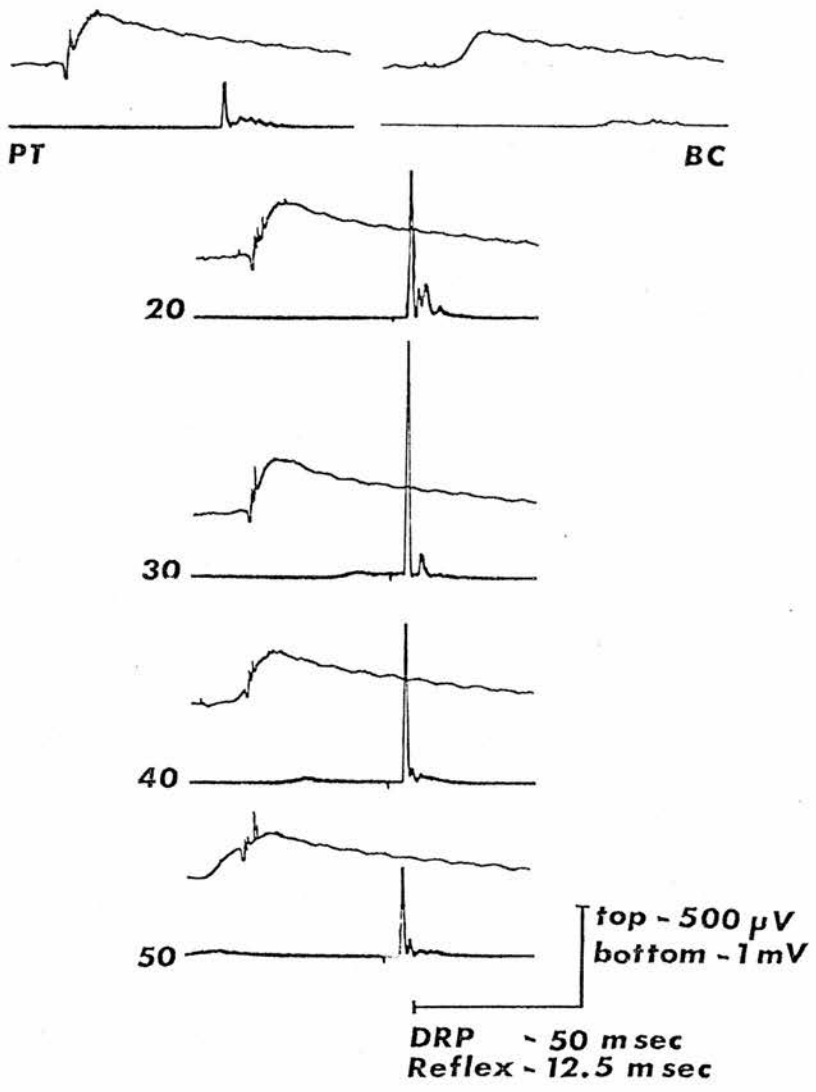

FIG. 7 : Cat. Chloralose 60 mg/kg. Dorsal root potentials and ventral root potentials recorded from stimulation of posterior tibial nerve (PT) and biventer cervicis nerve (BC). Biventer cervicis stimulation precedes posterior tibial nerve stimulation by the interval indicated (in msec). Note different sweep speeds employed for dorsal and ventral root potentials.

consistent appearance of dorsal root potentials following forepaw nerve stimulation (Fig. 7). We are thus left with the paradoxical situation of monosynaptic reflex facilitation at a time when the Gp. 1A endings are partially depolarized and releasing reduced amounts of transmitter. At this time the most attractive hypothesis to explain this is to accept Frank's[30] concept of remote action and postulate that the Gp. 1A terminals at dendritic sites are closely approximated with descending terminals whose alteration in local excitability are sufficient to overcome the inhibition usually asso-

ciated with reduced transmitter release.

Be that as it may, there is evidence then for at least 4 separate actions in the lumbosacral cord as a result of input at cervical levels. These are respectively direct excitation of motoneurones, inhibition of SBS reflexes, pre-synaptic inhibition in 1A terminals and an unknown mechanism causing prolonged facilitation of monosynaptic reflexes. We also have performed experiments which suggest that these are all due to the operation of independent pathways. Decerebration has only minor effects on the appearance of dorsal root potentials suggesting that the pathway for primary afferent depolarisation is infratentorial. Further selective lesions of the cortex interfere unequally with the direct discharge, and the monosynaptic reflex facilitation and also may spare descending effects from one nerve whilst abolishing effects from another nerve.

DISCUSSION

These experiments open questions with regard to the role of neck structures in posture, the role of the cerebral cortex and also the organization of the nervous system of the chloralose anaesthetized cat.

Our experiments suggest that information from neck muscle proprioceptors can be used to help determine output at many levels of the spinal cord, and that this balance of effects involves fairly high levels of the brain. The information from neck muscles is used as an aid and is not essential, for compensation for loss of this information occurs quickly. In the same way man or an animal can ultimately compensate for loss of the vestibular apparatus although here the magnitude and duration of defects is much greater.

At least 4 systems are affected by neck muscle afferents. There is one system whereby EPSP's and action potentials can be generated in motoneurones. This system can presumably initiate and conduct movement. There is a pathway for regulating presynaptic terminal excitability. By this action the myotatic reflex can rapidly compensate for altered loading in a way frequently associated with fusimotor activity. The significance of the effects on the SBS reflexes are not clear, except to suggest that whatever this system is doing, it is disabled by higher centres for the period in which the system is active. These experiments thus lay bare a set of physiological functions, all of which are affected by neck muscle afferents and all of which may be part and parcel of the mechanism that control posture.

If an outrageous simile is permitted, the experimenter working on the chloralose anaesthetized animal resembles an archaeologist excavating a pipe organ. As each pipe is disinterred, it is played and its single note discovered. Then, in ignorance, the whole is put together and all pipes sounded simultaneously. The resultant cacophony bears no resemblance to the music that the organ is capable of producing. So in our chloralose anaesthetized animal, the response to a stimulus is a jerk, a convulsive movement, but it is created by the same systems which under other circumstances create the fluidity and grace of movement.

Perhaps all we have succeeded in confirming what would be obvious to an early naturalist. All sensory systems are of necessity connected to motor systems and motor activity is an end point of activity in the whole complex of brain and spinal cord.

ABRÉGÉ — **Les récepteurs proprioceptifs au niveau des muscles de la nuque et le rôle du cortex cérébral dans les réflexes de posture.** La section du nerf d'un muscle extenseur de la nuque peut donner des troubles de la démarche. Ce phénomène serait relié à des systèmes descendants interspinaux qui dépendraient de boucles sus-tentorielles et qui impliqueraient le cortex cérébral dans la région de la circonvolution suprasylvienne antérieure.

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Neck Muscle Proprioceptors and Vestibulospinal Outflow at Lumbosacral Levels

V. C. Abrahams

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Neck Muscle Proprioceptors and Vestibulospinal Outflow at Lumbosacral Levels

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ABRAHAMS, V. C. 1972. Neck muscle proprioceptors and vestibulospinal outflow at lumbosacral levels. Can. J. Physiol. Pharmacol. **50**, 17–21.

It has been shown that neck muscle proprioceptors can inhibit vestibulospinal outflow at lumbosacral levels of the cord. The inhibition was abolished by decerebration, but was unaffected by ablation of the contiguous regions of the ectosylvian and suprasylvian gyri receiving vestibular projections. Descending vestibular effects on segmental lumbosacral reflexes were also examined. One effect only, a prolonged facilitation of the monosynaptic reflex, was abolished by decerebration. Unlike a similar effect obtainable from neck muscle proprioceptors, the facilitation survived acute ablation of the anterior poles of the suprasylvian and ectosylvian gyri.

ABRAHAMS, V. C. 1972. Neck muscle proprioceptors and vestibulospinal outflow at lumbosacral levels. Can. J. Physiol. Pharmacol. **50**, 17–21.

Les récepteurs proprioceptifs de muscles du cou inhibent l'activité vestibulo-spinale au niveau lombro-sacré de la moelle épinière. Cet effet est éliminé par décérébration mais n'est pas affecté par l'ablation des régions contiguës des gyri ecto et suprasylviens, lesquelles reçoivent des projections vestibulaires. Nous avons également examiné l'effet de la stimulation du nerf vestibulaire sur les reflexes segmentaires lombo-sacré. Seule la facilitation du reflexe monosynaptique est éliminée par décérébration. Contrairement à un effet similaire obtenu à partir des propriocepteurs des muscles du cou, cette facilitation persiste après ablation aiguë des pôles antérieurs des gyri ecto et suprasylviens.

Introduction

Alterations of gaze, involving movement of both head and eyes, require considerable integration of visual, vestibular, and neck proprioceptive systems. It is therefore not unexpected to find that in the decerebrate cat the vestibulospinal output can be modified by activity in neck muscle proprioceptors (Gernandt and Gilman 1959; Gernandt and Proler 1965). What is unexpected is that these effects are largely confined to the cervical levels of the cord, and are weak or absent at lumbosacral levels.

In the intact cat, neck muscle receptors exert a wide range of effects on the lumbosacral cord which are abolished by high spinal section, decerebration, and also, in acute preparations, by cortical lesions (Abrahams and Falchetto 1969; Abrahams 1970, 1971) and this may involve looping systems. It seemed likely that a similar looping mechanism might be involved in interactions between neck muscle proprioceptors and vestibulospinal output. This has been examined in the present experiments.

Methods

Experiments were successfully completed on 16 cats. Anesthesia was induced with ethyl chloride and preliminary surgical procedures were completed under ether. Anesthesia was then sustained with a single intravenous dose of chloralose (60 mg/kg). Branches of the vestibular nerve were prepared for stimulation as described by Andersson and Gernandt (1954). Electrodes were secured in place on nerve branches with low melting point paraffin and the leads secured with acrylic cement to the bone of the middle ear. In three experiments the electrodes were not placed on a nerve branch, but were thrust into the substance of the membranous labyrinth. Stimulation through these electrodes gave results identical to those of nerve stimulation. Records of reflex activity were made from electrodes placed on exposed ventral roots. Stimulation, recording, and display were by conventional electrophysiological techniques. Reflexes were initiated by stimulation of nerves exposed in the popliteal fossa. The animal's condition was monitored with the aid of blood pressure, respiration, and temperature transducers.

When the effect of a brain lesion was to be tested, all surgical procedures except the placing of the lesion were completed, and the reflexes and evoked responses tested immediately prior to the making of the lesion. The extent of the lesion was determined postmortem in formalin-fixed material.

Results

Effects of Neck Muscle Nerve Stimulation on Lumbosacral Vestibulospinal Discharge

Stimulation of any branch of the vestibular nerve in the chloralose-anesthetized cat regularly led to a discharge in L7 and S1 ventral roots. The discharge had a latency of about 15 ms and a duration of about 20 ms. A similar discharge followed stimulation of the cut central end of the neck muscle nerve biventer cervicis. When biventer cervicis nerve was stimulated prior to vestibular nerve stimulation the predominant effect was a prolonged inhibition of the vestibular discharge (Fig. 1). First apparent when the interval between stimuli was 10 ms, the effect was prolonged, invariably persisting for at least 200 ms, and generally for as long as 400 ms. When the interval between stimuli was 20 ms a brief facilitation was present. When the order of stimulation was reversed, and the effect of prior vestibular nerve stimulation examined on the discharge due to biventer cervicis nerve stimulation, then vestibular stimulation inhibited the biventer cervicis discharge. Apparent at an interval of 10 ms, the inhibition was of limited duration and was absent after 60 ms.

Effects of Vestibular Nerve Stimulation on Lumbosacral Reflexes in the Chloralose-Anesthetized Cat

Fig. 2 illustrates the effects that can be brought about on lumbosacral segmental and looping reflexes by prior stimulation of the vestibular nerve. At short intervals, when the vestibular nerve stimulation produces ventral root discharge, it can be seen that monosynaptic and polysynaptic reflexes are facilitated. When the interval is increased beyond 30 ms and up to 100 ms there is a prolonged and selective increase in the monosynaptic reflex accompanied by depressed or absent polysynaptic reflexes. The looping reflex, the spino–bulbo–spinal (S.B.S.) reflex,

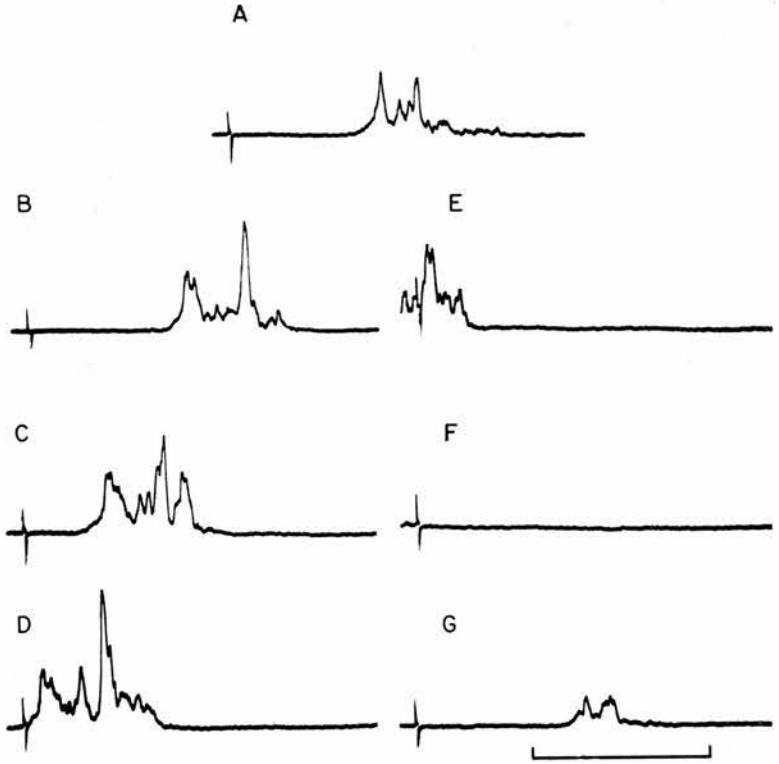

FIG. 1. Cat, chloralose 60 mg/kg. Discharges recorded from L7 ventral root after (A) vestibular nerve stimulation and (B) biventer cervicis nerve stimulation. Effects of biventer cervicis nerve stimulation preceding vestibular nerve stimulation at intervals of (C) 10 ms, (D) 20 ms, (E) 30 ms, (F) 40 ms, (G) 300 ms. Time marker 25 ms.

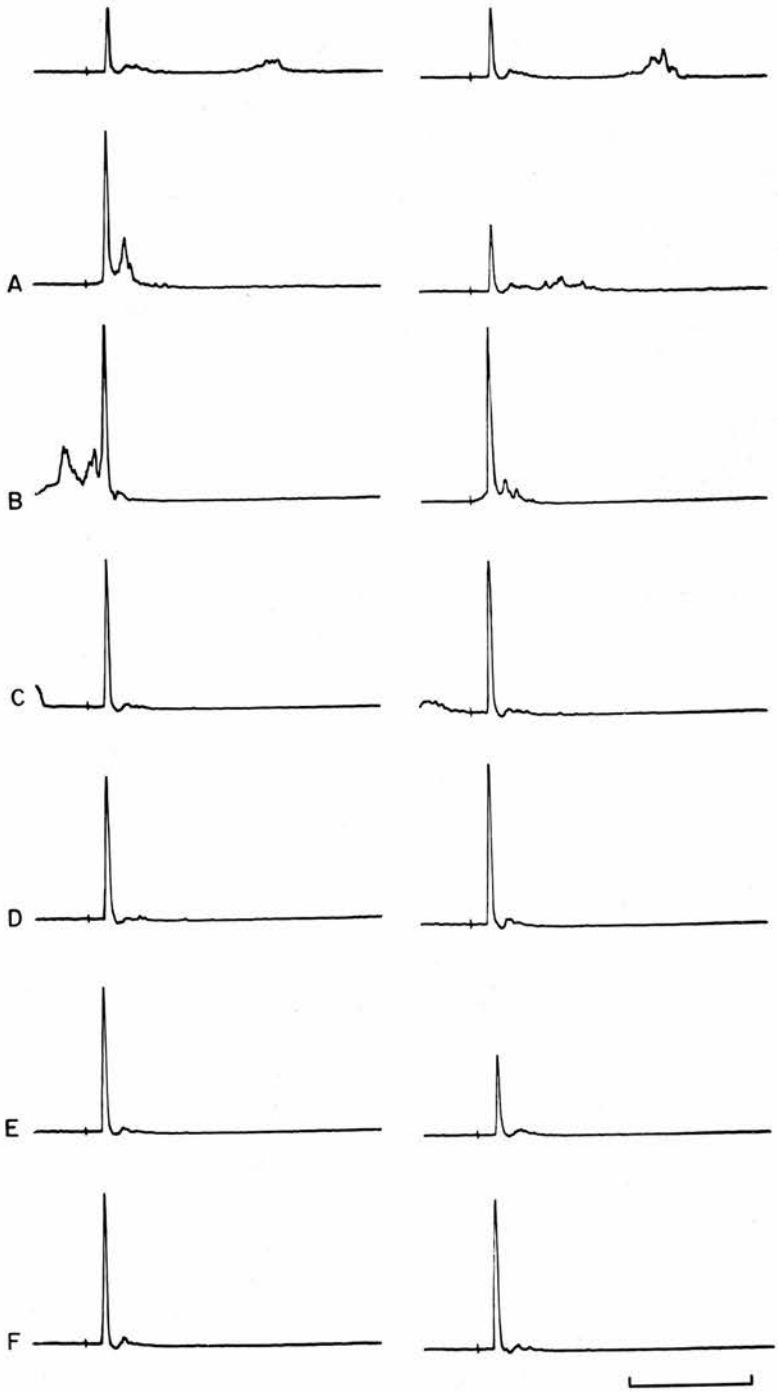

FIG. 2. Cat, chloralose 60 mg/kg. Segmental reflexes recorded from L7 ventral root following posterior tibial nerve stimulation. Effects on segmental reflexes of vestibular nerve (left column) and biventer cervicis nerve stimulation (right column). Top, control. Intervals: (A) 10 ms, (B) 20 ms, (C) 30 ms, (D) 40 ms, (E) 50 ms, (F) 60 ms. Time marker 20 ms.

is abolished within 10 ms of the vestibular nerve stimulation and only returns after 400 ms or so. These descending effects are virtually identical with those that follow forepaw and neck muscle nerve stimulation (Abrahams 1971), the only difference being that in the same animal vestibular nerve stimulation produces its effects about 10 ms earlier than forequarter nerve stimulation (Fig. 2).

Effects of Decerebration on Descending Effects from the Vestibular Nerve

In six cats, after establishing the presence of the descending effects from the vestibular nerve the animals were decerebrated. This procedure slightly reduced the vestibulospinal discharge, and this reduced discharge was now unaffected by prior biventer cervicis nerve stimulation. The effect of vestibular nerve stimulation on segmental reflexes was largely unaffected by decerebration except that the prolonged facilitation of lumbosacral monosynaptic reflexes could no longer be demonstrated.

Effect of Restricted Cortical Lesions in the Anterior Suprasylvian and Ectosylvian Gyri

After establishing the presence of descending interactions from both the vestibular and biventer cervicis nerves, small lesions were placed in eight cats in those regions of the suprasylvian and ectosylvian gyri which are contiguous and which receive input from neck muscle proprioceptors and the vestibular apparatus (Walzl and Mountcastle 1949; Kempinsky 1951; Landgren *et al.* 1967; Massopust and Daigle 1960). In the first two experiments these lesions were followed by the loss of all descending interactions. It was not possible to repeat this, and a further six experiments were performed in which the lesions were completely without effect on any descending interaction from the vestibular nerve. Further, in these six experiments stimulation of the biventer cervicis nerve still produced a prolonged inhibition of vestibulospinal discharge.

Discussion

These experiments show that vestibulospinal outflow at lumbosacral levels can be substantially affected by neck muscle proprioceptive activity. It is also evident that the direct interaction cannot be demonstrated after decerebration. Output from the vestibular nuclei may follow a number of routes, including the vestibulospinal tract, the medial longitudinal fasciculus, and the reticulospinal system (Brodal *et al.* 1962; Gernandt *et al.* 1957; Nyberg-Hansen 1964; Nyberg-Hansen and Mascitti 1964; Pompeiano and Brodal 1957; Thulin 1953; Ladpli and Brodal 1968). A total inhibition of vestibulospinal discharge following biventer cervicis stimulation would imply either a spinal or medullary effect, or a mixture of both. The known events in the cord following biventer cervicis stimulation (Abrahams 1971) could, in part, account for some features, particularly the brief enhancement seen 20 ms or so after biventer cervicis nerve stimulation. However, apart from a weak monosynaptic connection to motoneurones (Lund and Pompeiano 1965; Grillner *et al.* 1970), the bulk of vestibulospinal fibers terminate on interneurones (Erulkar *et al.* 1966), and although there is evidence of an inhibitory action of biventer cervicis stimulation on polysynaptic reflexes (Abrahams 1971), this neither occurs early enough, nor is it of sufficient duration to adequately explain our present observations. It seems more likely that the strong inhibitory effect of biventer cervicis output on vestibulospinal output is due to a central action. There is anatomical evidence that fibers entering the cord at upper cervical levels terminate in the descending vestibular nuclei (Corbin and Hinsey 1935; Yee and Corbin 1939; Escolar 1948). There is no evidence to suggest that these fibers have their origin in neck muscle proprioceptors and their density of termination does not seem adequate to explain inhibition of all three major descending systems from the vestibular nuclei. For the moment it seems more likely that supratentorial structures are responsible for initiating a widespread inhibition at medullary levels.

In previous acute experiments it was found that lesions of the cortical receiving areas for neck muscle proprioceptors could abolish descending spinal interactions from neck muscle proprioceptors (Abrahams 1970). This region is contiguous with a vestibulocortical relay whose function is not understood. The possibility that this vestibulocortical relay

was involved in descending vestibulospinal effects has not been supported. The consistent inability to modify descending vestibulospinal effects in the latter six consecutive experiments argues strongly against such a role for these cortical projections. There is circumstantial evidence for supratentorial structures, including the cortex, being involved in descending effects from neck muscle proprioceptors, and there is evidence that supratentorial structures participate in a limited way in the modulation of vestibulospinal output, but there is no evidence to suggest that the vestibulocortical relay to the ectosylvian gyrus is involved in either of these processes.

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Soc. Neurosc., 2nd Ann. Meeting, 1972, p. 204.

43.1 FIXATION OF INHIBITION OF A SPINAL REFLEX BY ELECTRICAL STIMULATION OF THE MEDULLA. Vivian C. Abrahams and Jenny Daynes*. Cerebral Functions Group, University College, London, and Dept. of Physiol., Queen's Univ., Kingston, Ontario, Canada.

Most descending spinal effects are brief, enduring for a few milliseconds, or a few seconds at most. We now report on a descending inhibition of the plantar reflex which may persist for hours following its initiation. This prolonged inhibition resembles the phenomenon of fixation, since once established it will persist after cord section. The plantar reflex was recorded bilaterally from the ventral roots of Dial anaesthetised cats. The spinal cord was partially sectioned at the mid-thoracic level leaving only one dorso-lateral quadrant intact. A stimulating micro-electrode was then inserted into the medulla to a site whose excitation produced inhibition of the ipsilateral plantar reflex. Stimulation through the electrode with brief trains every 3 seconds for one hour either abolished the ipsilateral plantar reflex or reduced it to below 40% of its control value. The contralateral reflex was largely unaffected. Despite the loss of the ipsilateral plantar reflex, other reflexes from that spinal root were normal. When the residual dorso-lateral cord was sectioned the reduced ipsilateral plantar reflex continued unchanged in 5 experiments, but there was a transient return of the reflex in 2 experiments. The "fixation" of inhibition of the ipsilateral plantar reflex persisted for the remaining 2-4 hours of the experiment, whilst the contralateral reflex was still normal.

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ATPase Distribution in Dorsal Neck Muscles of the Cat

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ATPase Distribution in Dorsal Neck Muscles of the Cat

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ABRAHAMS, V. C., and RANCIER, F. 1973. ATPase distribution in dorsal neck muscles of the cat. Can. J. Physiol. Pharmacol. **51**, 549–552.

An examination of the histochemical distribution of ATPase has been made in the synergistic dorsal muscles of the cat neck that move the head. On the basis of this distribution, three types of muscle are described: one pair predominately composed of fibers rich in ATPase, one pair predominately composed of fibers poor in ATPase, and a single muscle with characteristics between the others. The dorsal muscles of the neck are therefore interpreted as comprising three functional groups: one pair of fast muscles, one pair of slow muscles, and a single muscle with intermediate properties.

ABRAHAMS, V. C., et RANCIER, F. 1973. ATPase distribution in dorsal neck muscles of the cat. Can. J. Physiol. Pharmacol. **51**, 549–552.

A l'aide d'une méthode histochimique, nous avons étudié la distribution de l'ATPase dans les muscles dorsaux synergistes du cou contrôlant les mouvements de la tête du chat. D'après la distribution de l'ATPase, trois types de muscles peuvent être distingués: une paire composée principalement de fibres riches en ATPase, une paire composée principalement de fibres pauvres en ATPase et un muscle ayant des caractéristiques intermédiaires. Les muscles dorsaux du cou peuvent donc être classés en trois groupes fonctionnels: une paire de muscles rapides, une paire de muscles lents et un muscle aux propriétés intermédiaires.

[Traduit par le journal]

Introduction

The control of head and eye movement has been intensively studied, yet there has been little study of the muscles that directly move the head and which form an important part of this specialized motor system. It is all the more surprising since the central control of these muscles is closely integrated with that of the extraocular muscles (Bizzi *et al.* 1971). The experiments described here are concerned with one muscle group of particular importance in the organization of head movement in the cat, the dorsal muscles of the neck. These comprise a group of at least five pairs of synergist muscles, all of which serve to elevate or turn the head. What has been done has been to provide a more comprehensive description than that presently available, and to use the technique of muscle typing based on myofibrillar ATPase content (Padykula and Herman 1955; Burke *et al.* 1971) to give a functional classification to individual muscles within the grouping.

The muscles studied were those called rectus capitis posterior major (RC) biventer cervicis (BC), complexus (CM), occipitoscapularis (OS), and splenius (SP) in the nomenclature of Elliott (1934). All are attached to the lamboidal ridge of the skull and all but one are inserted on cervical vertebrae; the exception is OS, which is inserted into the scapula.

Methods

Cats were anesthetized with a single intraperitoneal injection of 30 mg/kg pentobarbitone sodium (Nembutal, Abbott). In one series of experiments the dorsal neck muscles were widely dissected to investigate their innervation. They were then dissected free and weighed. In the second series of experiments the dissection was restricted so that the muscles were separated at about the C3 level. The belly of each muscle was then freed and a complete segment of each muscle, about 5 mm wide, was removed and dropped into saline at 4 °C. Samples were kept in a refrigerator at this temperature until the last muscle sample was removed (about 30 min after the first sample). The pieces of muscle were then frozen and 15 μ sections cut on a freezing microtome. The sections were then stained for ATPase by the technique of Padykula and Herman (1955). All the fibers in individual sections of each muscle were then counted and assigned to either a "dark" or "light" category. The accuracy of counts on the same

TABLE 1. Weights of neck muscles in the cat and the ratio of dark to light ATPase staining in individual fibres of the muscles

	Muscle weights, g				Dark/Light		
	Mean	*n*	S.E.M.	% total mass	Mean	*n*	S.E.M.
Occipitoscapularis	0.672	6	0.12	6	0.69	8	0.06
Splenius	3.66	6	0.60	34	2.64	8	0.19
Biventer cervicis	2.74	6	0.40	25	0.717	10	0.05
Complexus	2.90	6	0.42	27	1.26	7	0.026
Rectus capitis	0.913	6	0.14	8	2.68	7	0.19

sections was verified by having three individuals make separate counts.

The ratio of dark and light fibers in each muscle appeared to fall into three categories. The statistical validity of this classification was tested using Duncan's multiple-range test (Duncan 1955).

Results

Inspection and weighing shows that the dorsal muscles of the neck fall into two types. BC, SP, and CM form one type and OS and RC a second. The first type of muscle is relatively large and has three motor nerves, each taking origin in a different cervical segment, either C1–C3 or C2–C4. Between them these three muscles comprise more than 85% of the total dorsal muscle mass (Table 1). The second type of muscle is small, each comprising less than 10% of the dorsal muscle mass (Table 1), and is each innervated by a single motor nerve.

Table 1 also contains the data relating to the distribution of myofibrillar ATPase in each of the five muscles. The results are expressed as a "dark–light" ratio (D.L.R.), calculated by dividing the number of heavily stained fibers in a muscle by the lightly staining fibers. The average number of fibers counted per muscle section was 904, the average count ranging from 814 for SP to 1060 for BC. As Fig. 1 shows the differences between the two types of fiber are clear. On the basis of the D.L.R., SP and RC are seen to form a pair of predominately light muscles, and BC and OS to form a pair of predominately dark muscles. Complexus appears to be intermediate between the other two types. Statistical evaluation of these data by Duncan's multiple-range test confirms the impression gained by inspection and further shows that the classification into three types is based on differences in the D.L.R. significant at the 1% level.

In two animals the muscle spindles present in all five muscles were examined. A total of 104 chain fibers were seen, of which all but two were darkly stained. These two were both aberrant in that one was about one-quarter the diameter of the other chain fibers and the other was larger than the other chain fibers. Of the 51 nuclear bag fibers examined, 26 exhibited dense staining, and 25 were lightly stained.

Discussion

The data presented here show that the five dorsal muscles of the neck, all capable of initiating similar head movement, fall into three categories. To make this classification the association between ATPase content and muscle function demonstrated in the hind leg of the cat (Burke *et al.* 1971) has been applied to the neck muscles, that is, darkly stained fibers are regarded as functionally fast fibers, lightly stained fibers as functionally slow. Each of the three large muscles then, represents a different functional type, and the slow (BC) and fast (SP) large muscle are each supported by a small muscle with similar characteristics. An insight into what might be the functional organization of the muscle types has been given by the experiments of Anderson *et al.* (1971). The superior colliculus of the cat has long been implicated in head and eye movement and its stimulation leads to movements of both head and eyes (Hess *et al.* 1946). Anderson *et al.* (1971) recorded intracellular potentials from neck muscle motoneurones following stimulation of the deep layers of the superior colliculus. Some evidence for differential control of SP and BC

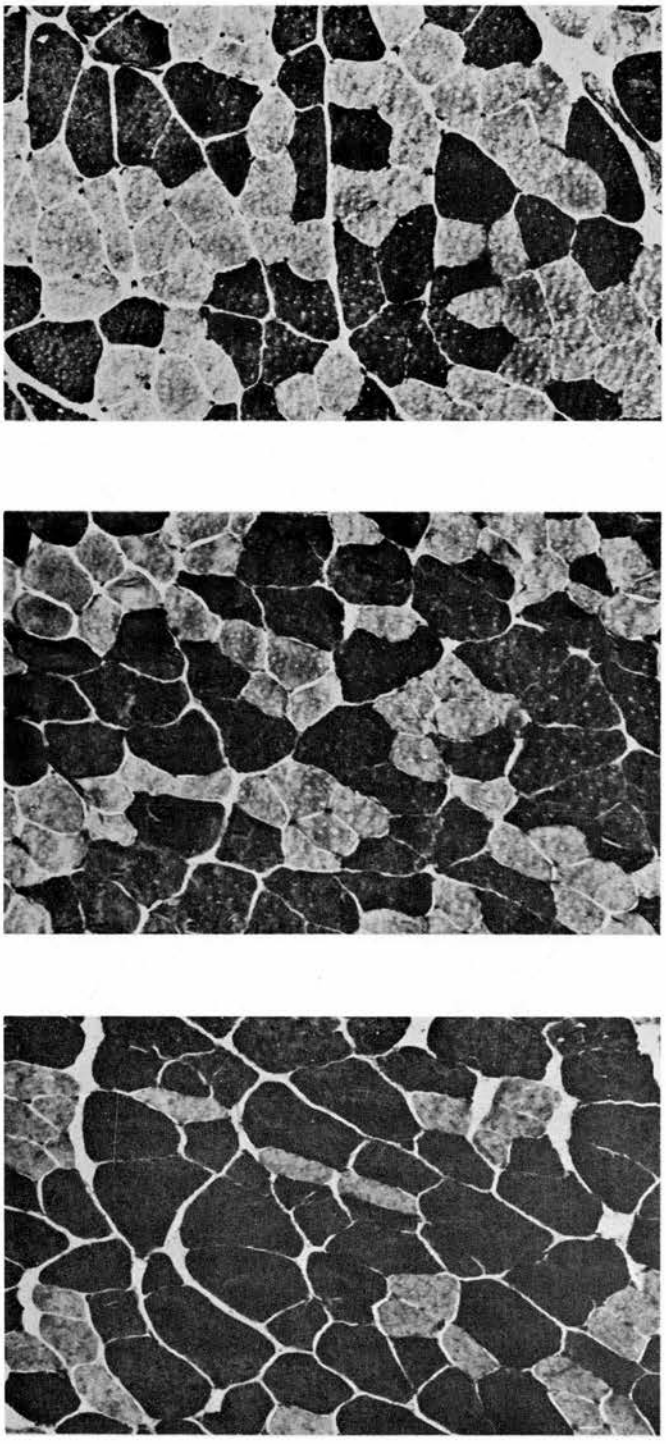

FIG. 1. Sections through biventer cervicis (top), complexus (middle), and splenius (bottom) to show differences in ATPase distribution. Magnification ×100.

and CM motoneurones emerges from this work. Anderson *et al.* (1971) found that stimulation of one colliculus generated mainly ipsilateral excitatory postsynaptic potentials (E.P.S.P.) in SP motoneurones but ipsilateral inhibitory postsynaptic potentials (I.P.S.P.) in BC and CM motoneurones. This would suggest that the SP, the fast muscle, is used in the turning movement, whereas the slow and intermediate muscles, BC and CM, are inhibited. The implication is that these latter muscles are involved in the tonic activity needed to maintain a head position, and that the execution of rapid movements is done using SP.

The extraocular muscle system is composed of a variety of muscle fiber types (Hess 1961; Bach-y-Rita and Ito 1966; Cheng-Minoda *et al.* 1968; Peachey 1971) and their motoneurones are of at least two types (Schubert and Bornschein 1962; Schaefer 1965). The neck muscle system has now been shown to comprise a variety of muscle types. Presumably we should find associated with this a variety of spinal motoneurone types. An examination of the various motoneurone types and the systems of descending control of the various motoneurones should shed further light on the central organization of the central mechanisms underlying head and eye movement.

Muscle spindles were not systematically studied in the present experiments and those that were seen did not differ strikingly from those seen elsewhere in muscles of the cat. Their presence is a reminder that they too must participate in the control system for head and eye movement, and their role in the system is also in need of definition.

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AN ELECTROPHYSIOLOGICAL DEMONSTRATION OF EXTRAOCULAR MUSCLE RECEPTOR PROJECTIONS TO THE SUPERIOR COLLICULUS IN THE CAT. P.K. Rose* and V.C. Abrahams, Dept. Physiology, Queen's University, Kingston, Ontario.

Despite the rich distribution of receptors in the extraocular muscles little is known about their central projections. We now report that an extensive projection exists from extraocular muscles to the superior colliculus in the cat. In cats anaesthetised with chloralose explorations were made throughout the various layers of the superior colliculus. Seventy-nine of the eighty-seven units tested were excited by stimulation of the third nerve within the orbit. Of fifty-seven units tested, fifty-two responded to stimulation of the sixth nerve in the orbit. Thus proprioceptive information from both the inferior oblique and lateral rectus muscles is conveyed to the superior colliculus. We have already reported on a rich projection from neck muscles to the superior colliculus; this projection combined with that from the extraocular muscles means that the superior colliculus has access to information concerning the position of the eye in the orbit and the head on the trunk. The existence of a rich afferent connection to the superior colliculus from both neck and extraocular muscles is consistent with a role of the superior colliculus in organization of head and eye turning.

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EXTRAOCULAR MUSCLE PROPRIOCEPTIVE PROJECTION TO THE SUPERIOR COLLICULUS IN THE CAT. P.K. Rose* and V.C. Abrahams. Queen's University, Kingston, Ontario, Canada.

Explorations have been made in the anaesthetised cat to investigate the distribution of projections from extraocular muscle afferents to the superior colliculus. Units activated by intraorbital stimulation of the 3rd or 6th nerves were found widely distributed in the superior colliculus. The richest projection to the superior colliculus so far described is from skeletal muscle afferents. Of the units examined, and which responded to skeletal muscle proprioceptive stimulation, 90% were also activated by extraocular muscle afferents. The extraocular muscle afferents have a distribution similar to that of skeletal muscle afferents, with short latency responses predominating in deep collicular layers, long latency responses predominating in more superficial layers. There was no apparent topographical distribution of this projection. This data shows that the layers of the superior colliculus receiving a retinal input also receive a long latency input from the extraocular muscle proprioceptors and those layers which generate output to extraocular and neck muscles receive a short latency input from extraocular muscle proprioceptors. These findings are consistent with the view that the superior colliculus plays an important role in the organization of combined head and eye movement. (Supported by the M.R.C. of Canada).

Soc. Neurosc., 3rd Ann. Meeting, 1973, 336.

53.7 AFFERENT CONNECTIONS OF CELLS IN THE SUPERIOR COLLICULUS OF THE CAT GIVING RISE TO THE TECTOSPINAL TRACT. V.C. Abrahams, P.K. Rose*.
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The tectospinal tract is one of two disynaptic pathways from the superior colliculus of the cat to motoneurones in the upper cervical cord supplying neck muscles. These pathways constitute the most direct route whereby the superior colliculus can control head movement. The neurones of origin of this pathway have been identified within the superior colliculus by antidromic activation. The cells were widely dispersed in the deep and intermediate layers of the superior colliculus. More than 40% of the neurones could be excited by both visual and neck muscle afferent stimuli and a further 30% could be activated by one or another stimulus. Since there is almost total convergence between extraocular and neck muscle afferents in the superior colliculus, it is likely that the tectospinal cells also receive a substantial extraocular muscle input. These experiments identify the tectospinal tract as one whose activity may be influenced by visual information as well as proprioceptive information from eye and neck muscles.

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On the Induction of Prolonged Change in the Functional State of the Spinal Cord

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There is general agreement that the perception of pain by an individual may be greatly affected by the circumstances under which the pain is suffered. One frequently cited example of this phenomenon of dissociation between pain perception and stimulus intensity is that referred to by Beecher (1) of the American soldiers wounded at Anzio who "denied pain from their extensive wounds." Such a dissociation between pain perception and stimulus intensity is sometimes cited as evidence of the many psychological variables that enter into the perception of pain (2). But such psychological variables must make use of physiological substrates. Variability of pain thresholds and the ability to suppress the sensation of pain must, like the mechanism of pain itself, be sought within the normal framework of the physiology of the nervous system.

One physiological substrate that must be of great importance in modifying perceptive thresholds are the connections to primary afferents and interneurons that can alter synaptic transmission in cutaneous pathways. Descending systems make such connections in abundance and their influence has been repeatedly demonstrated on cutaneous afferents at spinal levels (3–20), in the dorsal column nuclei (21–25), and in the trigeminal system (26–30).

One of the earlier electrophysiological demonstrations that activity in descending spinal systems might affect transmission in ascending systems was made by Hagbarth and Kerr (3). They recorded from electrodes placed in the dorsal and anterolateral columns of the lumbosacral spinal cord of the cat. The electrical record obtained from the dorsal column following dorsal root stimulation comprised an initial spike (associated with conduction in the dorsal column pathway) and a late wave (associated with interneuronal activity in the dorsal horn). Hagbarth and Kerr (3) consistently found depression of the late dorsal column wave and the anterolateral wave to follow repetitive stimulation of the bulbar and mesencephalic reticular formation, the vermis of the cerebellum, and both the motor and sensory cortex. The inference from these experiments was that synaptic effective-

ness within the spinal cord could be reduced by activity in a variety of descending pathways. In view of the experiments reported in this chapter, it is worth noting that Hagbarth and Kerr (3) found inhibition of test responses in their experiments and no evidence of facilitation. Thus in these sensory pathways an inhibitory action could be injected between the first- and second-order neurons. Because Hagbarth and Kerr (3) stimulated whole dorsal roots and not cutaneous nerves, there was no direct evidence of an inhibitory action exerted specifically on cutaneous afferents. In later experiments in the cat, Andersen, Eccles, and Sears (8), and Carpenter, Lundberg, and Norrsell (9) specifically showed that stimulation of descending tracts originating in the cortical somatosensory areas I and II could reduce the synaptic efficacy of cutaneous primary afferents in the cord. Andersen et al. (8) impaled the primary afferents and showed that cortical stimulation caused depolarization, an action which reduced synaptic efficacy.

The ability to reduce the effectiveness of synaptic transfer in the spinal cord from cutaneous afferents is widespread in descending systems and is not confined to the corticospinal tract. Hongo and Jankowska (13), and Hongo, Jankowska, and Lundberg (31) found that the rubrospinal system caused primary afferent depolarization (P.A.D.) from low-threshold skin receptors. The vestibulospinal and reticulospinal systems can also cause P.A.D. in large cutaneous afferents (12, 32, 33). P.A.D. thus is used by many descending systems to manipulate directly the inflow of information from cutaneous receptors. But this is not the only mechanism available to alter sensory inflow. Descending systems have an abundance of connections on interneurons in the cord and can control afferent ascending sensory transmission by altering excitability in these interneurons (14–19, 34, 39, 40, 41, 43). By the combination of effects centered on primary afferents and interneurons, transmission in all major ascending pathways can be regulated by descending systems.

The most obvious form of regulation is by the reduction or suppression of input, but alterations in receptive fields and receptive field properties can also be attributed to descending systems. In an analysis of the receptive fields of spinocervical units, Taub (11) showed that cutaneous fields can be altered by reticulospinal, rubrospinal, and cerebellospinal mechanisms. In similar experiments Wall (14) and Hillman and Wall (34) have shown descending systems to affect the degree of convergence of sensory afferents on spinal interneurons.

Thus there exists a substantial neurophysiological substrate which can influence ascending cutaneous afferent transmission and which potentially can affect pain perception.

The experimental data presented in this chapter indicates that another action of descending systems may need to be added to those already described, namely the ability of a descending system to produce prolonged

effects which persist for hours after the stimulus has been terminated. The fact that such a spinal mechanism exists should interest those concerned with the manner in which periods of protracted pain of central origin can occur as well as those concerned with the alleviation of pain. The findings were made in the course of experiments on descending systems that inhibit the plantar reflex. The plantar reflex in the cat consists of toe extension following stimulation of the plantar pad (34). Although its functional role is uncertain (35), it is of interest to neurophysiologists since its neuronal substrate in the lumbosacral cord is limited. Egger and Wall (36) coined the term oligosynaptic to describe the reflex, for in its minimum configuration it involves no more than two serially connected interneurons and a motoneuron. Thus the number of sites for descending control of this reflex is limited, and it presents a very useful model for the examination of the interplay of descending spinal systems in spinal reflex function.

The experiments were performed on cats. In order to adhere as closely as possible to the procedures of Egger and Wall (36), most experiments were performed under Dial anesthesia (0.5 ml/kg, i.p.). This anesthetic was not available initially and early experiments were performed using a pentobarbital sodium (15 mg/kg) and urethane (1.2 g/kg) mixture given i.p. A few experiments were performed under chloralose (60 mg/kg, i.v.). The results do not appear to be affected by the anesthetic used. The plantar reflex was recorded electrically from an S1 ventral root, amplified, and displayed in the conventional manner. For the observation of prolonged effects the reflex was initially displayed on one beam of a storage oscilloscope using a very slow time base. In later experiments the amplified electrical signal was fed to a gate-controlled integrator whose output was similarly displayed as a vertical line on a storage oscilloscope. The vertical line was proportional to the total area under the signal. In all experiments the dorsal columns were sectioned at about T10 to prevent antidromic invasion of dorsal root ganglion cells. Microstimulation of brain and spinal cord was through a glass-insulated tungsten microelectrode (38), and the current intensity of the stimulus pulse was controlled either by a Grass (CCU-1) or Tektronix (Model 2620) constant current unit. All electrode placements were verified by histological examination of frozen sections. Tests were conducted on the plantar reflex at 3-sec intervals. To elicit the reflex 0.5-msec pulses were delivered to the medial plantar pad through a pair of 25 s.w.g. stimulus steel needles. The stimulus intensity was adjusted prior to ventral root section to give a plantar reflex with no flexor reflex contamination. The plantar reflex was routinely rested 1 msec after the end of a conditioning stimulus train at 100 impulses/sec.

In our experiments we found trains of stimuli lasting 100 msec or more delivered to either the rubrospinal, corticospinal, or dorsal inhibitory reticulospinal systems (39, 40) strongly inhibitory to the plantar reflex. In the course of these experiments we found that inhibition of the plantar reflex

from stimulation of any of these systems could be progressive and cumulative. Figure 1 illustrates the manner in which succeeding trains of stimuli spaced 3 sec apart may lead to a progressive increase in inhibition of the plantar reflex. This type of phenomenon is not uncommon and was reported by Hagbarth and Kerr (3) in their experiments. In our experiments, like theirs, the phenomenon was not seen regularly.

In the course of other experiments on the plantar reflex, repeated stimulus applications were required. The phenomenon of a progressive and cumulative inhibition then ceased to be a curiosity and instead became a severe handicap, for when periods of stimulation were prolonged, recovery from inhibition was often incomplete. This is illustrated in Fig. 2. In some experiments a reflex amplitude at the commencement of the experiment of 800 μV could be reduced to 50 μV within 3 or 4 hr. Such an extreme and persistent inhibition, once induced, would force an experiment to a premature conclusion.

The phenomenon of cumulative inhibition was examined in a series of experiments in which the plantar reflex was simultaneously elicited on both sides of the cord. In these experiments inhibitory brain sites were stimulated. In order to restrict the descending pathways activated, as well as

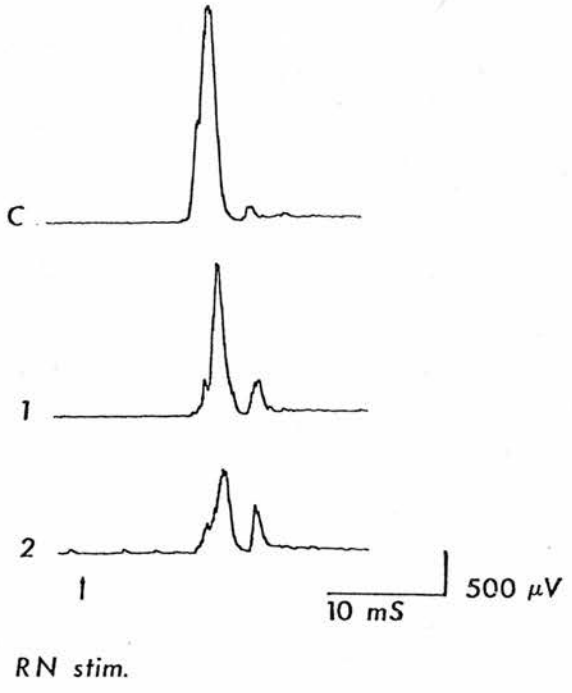

FIG. 1. Cumulative inhibition of the plantar reflex by stimulation of the red nucleus. C = control plantar reflex. 1 = plantar reflex after first stimulus to red nucleus. 2 = plantar reflex after second stimulus to red nucleus.

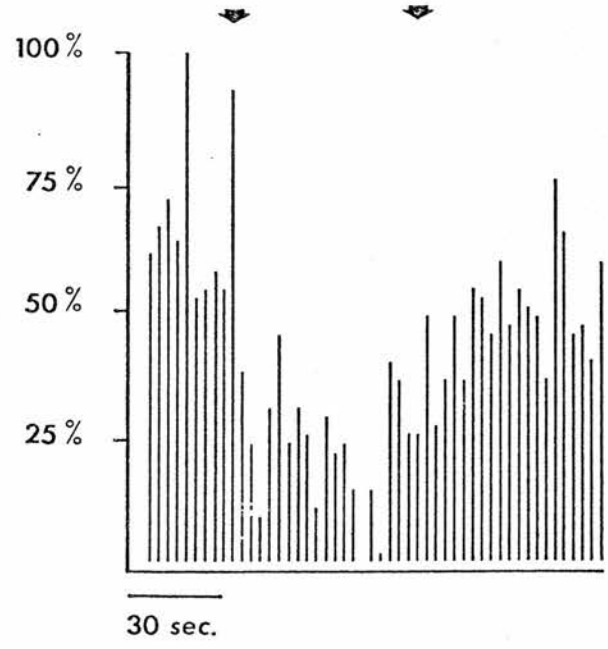

FIG. 2. Prolonged inhibition of the plantar reflex. The record consists of a plot of the peak amplitude of the reflex before, during, and after stimulation in the ipsilateral dorsolateral columns. A 200-msec train (100/sec, 0.2 msec, 50 μA pulses) preceded each reflex test in the period between the arrows.

transecting the dorsal columns, the cord was hemisected at about T10 and the ventral cord at this level was then further sectioned to prevent conduction in the ventral quadrant. Control experiments showed that from about 15 min after such a spinal section the reflexes on both sides were normal and stable for long periods. The rubrospinal and corticospinal tracts, and dorsal reticulospinal system are predominantly (but not completely) uncrossed at low-thoracic levels, so that the inhibitory effects of their stimulation were largely (but not entirely) confined to the ipsilateral reflex. Inhibition was then produced once every 3 sec for 1 hr by stimulation of appropriate brain sites at high bulbar levels. Eight such experiments were performed, and in keeping with the uncertain nature of the phenomenon, ipsilateral inhibition was seen in five. The reflex on the ipsilateral side was abolished by the prolonged period of stimulation, but the other reflex was still substantial. In a subsequent 2-hr observation period no recovery was seen. At this time the remaining cord remnant at the midthoracic level was cut. In the subsequent 2 hr the reflex still did not return. For this entire 4-hr period following the cessation of inhibitory stimulation, the control reflex remained stable. During the last 2 hr of the experiment, segmental reflexes were tested from both left and right S1 roots following stimulation of muscle and mixed hindleg nerves. Such reflexes were always present

on both sides, although an impression was gained that control side polysynaptic reflexes were larger than on the other side (Fig. 3). These experiments demonstrate that the progressive onset of inhibition is associated with the activation of an inhibitory system which is not only powerful, but whose action within the cord is also prolonged and, since it survives cord transection, is due to a local change in cord function.

Because of the oligosynaptic organization of the reflex, the sites at which inhibition might occur are restricted. The most likely cause would be P.A.D., for both the rubrospinal and corticospinal systems are known to produce P.A.D. in large cutaneous afferents (6, 8, 13, 31). This assumption was not borne out by experiment. Dorsal root potentials (D.R.P.'s) are generally regarded as resulting from P.A.D. (8). D.R.P.'s were readily obtained from one anterior S1 rootlet by rubrospinal or corticospinal stimulation. When the strength of such rubrospinal or corticospinal stimulation was gradually reduced, it was found that D.R.P. disappeared at low current strength which could still cause strong inhibition of the plantar reflex.

The afferent fibers that leave the plantar pad and whose excitation is responsible for the plantar reflex form a distinct group with conduction velocities of about 64 m/sec (36). In six experiments the technique of Wall (37) was used to test the terminal excitability of these fibers during and after stimulation of the corticospinal and rubrospinal tract. In five out of six experiments stimulation did not affect terminal excitability. In one experiment a small change in terminal excitability of 20% was seen.

These experiments make it unlikely that plantar reflex inhibition from

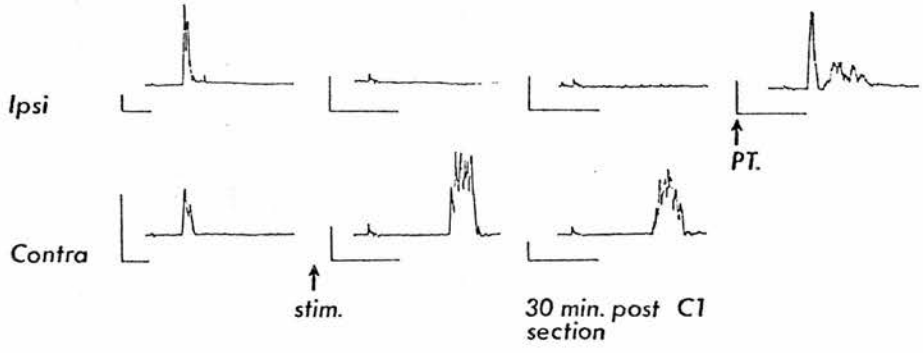

FIG. 3. Persistent inhibition of the plantar reflex following prolonged intermittent bulbar stimulation. Extreme left, control plantar reflexes. Middle left, plantar reflexes after stimulating bulbar reticular nuclei every 3 sec (200 msec, 100/sec, 0.2 msec, 200 μA) for 1 hr. Middle right, plantar reflexes 30 min after C1 section. Top right, segmental reflex following posterior tibial nerve stimulation.

rubrospinal or corticospinal inhibition is due to P.A.D. The plantar motoneuron was also ruled out as the target of inhibitory connections. Using monosynaptic reflex testing of plantar motoneurons, we have consistently found, as has been previously reported (35,41), that the plantar motoneurons are facilitated, not inhibited, by stimulation of the rubrospinal or the corticospinal system. By exclusion, the source of plantar reflex inhibition is most probably due to an action of the descending pathways on spinal interneurons. In five experiments recordings were made from interneurons that are monosynaptically and disynaptically activated by plantar pad stimulation and are thought to be the interneuronal substrate of the reflex (36). In this small series of experiments no inhibitory effects were found on neurons monosynaptically activated by plantar stimulation. On several occasions, units were found which (by their latencies) appear to be disynaptically activated from the plantar pad and which ceased their discharge following spinal or rubrospinal stimulation inhibitory to the plantar reflex. Such units were inhibited for 10 to 15 sec when the inhibitory stimulus was repeated three or four times at 3-sec intervals. As yet, the histology of these experiments is incomplete, but from previous findings of Egger and Wall (36) the units probably lie in Rexed's (42) lamina IV and V.

The simplest way of explaining the plantar reflex inhibition is by direct action on interneurons. The lamina IV and V region of the spinal cord is of considerable interest to sensory physiologists, for it is here that there are cell bodies that give rise to the spinocervical tract (14, 43). It is difficult to conceive that the descending fibers are confined to interneurons of the plantar reflex and the spread of such inhibitory effects within lamina IV and V could cause interference with ascending sensory systems. It also seems likely that P.A.D. may spread within fiber systems (44). A wider effect on afferent systems must, for the moment, remain pure speculation, for it is as yet unknown whether the mechanism of prolonged inhibition of the plantar reflex is necessarily the same as the mechanism of short-term inhibition. Yet it may not be entirely idle speculation and it could have substantial implications for these mechanisms that bring about prolonged alterations in pain threshold. Although there is evidence that identifies the spinal neurons that are directly connected to strictly nociceptive receptors as lying only in lamina I (45), one of the most potent ways of blocking pain, namely general anesthesia, is accompanied by an inhibition of dorsal horn units in both lamina IV and V (46–49).

What then of the mechanism of prolonged change? Descending tracts contain a very high percentage of small fibers. In the human pyramid more than 90% of the fibers have diameters of between 1 and 4μ. As such fibers branch and terminate they become even smaller. In this situation their surface area becomes a very considerable proportion of their mass, and a situation is created where loss of material from the fiber during activity can become a significant factor in altering the extracellular environment of the

terminal area. An example of this has recently been given by Krnjevic and Morris (51), who have shown that D.R.P.'s are associated with an increase in extracellular [K+] and this may be the reason for the long P.A.D. that accompanied a D.R.P. During protracted activity in the small fibers of descending systems such a loss of material could be a significant factor in altering the functional state of interneurons and if the loss of material is sufficiently large, such phenomenon as depolarization block could occur.

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NECK MUSCLE AND EXTRAOCULAR RECEPTORS AND THEIR RELATIONSHIP TO THE TECTO-SPINAL TRACT

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Afferents from extraocular and neck muscles constitute the richest projection to the superior colliculus. Sixty percent of cells of origin of the tectospinal tract within the superior colliculus are excited by this muscle afferent input. The superior colliculus thus constitutes a site where a variety of influences can be brought to bear on the regulation of head movement.

The regulation of head movement constitutes a special case of postural control, for head movement must be integrated with eye movement. Evidence has been obtained by Bizzi, Kalil and Tagliasco (1971) that some combined head and eye movements are integrated in such a way as to suggest that a central mechanism develops patterned motor output simultaneously to neck muscles and extraocular muscles, minimally controlled and corrected during the execution of the movement. We now provide evidence of connections within the superior colliculus that suggest that the organization of such combined head and eye movement might be a collicular function.

The extraocular muscles of the cat's eye are well provided with receptors giving information about muscle length and load (Bach-y-Rita and Ito, 1966). There has been little concrete evidence as to what use might be made of such information by the brain. There is no stretch reflex in extraocular muscles and

most theories propose that these receptors are involved in "subconscious nervous control of muscle contraction" (Bach-y-Rita, 1971). The neck muscles of the cat are rich in muscle spindles. In our own examination of rectus capitis major (one of the dorsal muscles of the neck) spindle densities exceeding 57/gm have been found, a density only exceeded in the interossei. Originally assigned only a minor role in the regulation of posture, evidence has been accumulating for more than a century that neck muscle receptors may play a more important role, for damage or interference with neck muscles or their nerves can lead to widespread postural deficiencies extending beyond interference with head movement (for review, see Abrahams, 1972a).

In experiments on anaesthetized cats we have found that neck muscle afferents relay to the superior colliculus and constitute the single richest relay to this structure. More units in the superior colliculus can be activated by stimulation of neck muscle nerves than by a wide variety of visual stimuli (Abrahams and Rose, 1972). We have further found that the projection of extraocular muscle afferents to the superior colliculus is also particularly rich, and such connections are as abundant as neck muscle projections (Rose and Abrahams, 1973). The evidence that these projections play a role in the organization of head movement comes from an examination of the afferent connections of those units to cells of origin of the tectospinal tract.

Anderson, Yoshida and Wilson (1971) identified two pathways from the superior colliculus to the motoneurones of the neck, the tectospinal pathway terminating on interneurones in the ventral cervical cord, and the tectoreticulospinal which terminates directly on motoneurones after synapsing in reticular nuclei. Both pathways are disynaptic. Using the usual criteria of antidromic invasion we have identified cells of origin of the tectospinal tract within the superior colliculi. We have found that about 60% of these cells can be fired by neck muscle or extraocular muscle afferents. These experiments demonstrate that at the level of the superior colliculus eye and head muscle afferent activity can influence output to neck motoneurones via the tectospinal pathway. What information concerning the head and the eyes is conveyed to the superior colliculus is as yet unknown, but we have found the projection to the superior colliculus from neck muscles to be exclusively from afferents conducting with a group II velocity.

Vestibulospinal connections to neck motoneurones are monosynaptic (Wilson and Yoshida, 1969), and thus might appear to be pre-emptive. Receptors in neck muscles can inhibit or excite vestibulospinal output at cervical levels (Gernandt and Gilman, 1959; Gernandt and Proler, 1965) and can suppress vestibulospinal output at lumbosacral levels for several hundred msec. (Abrahams, 1972b). A central organization thus exists which could suspend the dominant control of head position by labyrinthine structures and allow the systems taking origin in extraocular and neck muscle afferents to exert an extensive role in the control of head movement.

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MUSCLE SPINDLES IN THE DORSAL NECK MUSCLES OF THE CAT. F. Richmond* and V.C. Abrahams, Department of Physiology, Queen's University at Kingston, Ontario.

Afferents from the dorsal muscles of the neck constitute an important sensory system in the cat. They operate through collicular loops to participate in the control of motoneurons of neck muscles and also contribute to the integration of motor activity in the lumbosacral cord. So far no study has been made of these receptors although the uniquely high density of muscle spindles in neck muscles has caused comment. We have now systematically examined the spindles of neck muscles in serial paraffin sections cut at 15μ, and stained with Holmes silver stain.

Five dorsal muscles of the neck from each of three cats has been examined. All the muscles insert on the lamboidal crest. Four, biventer cervicis, splenius, complexus, and rectus capitis major, have their origin on cervical vertebrae. A fifth, occipitoscapularis, has its origin on the scapula. With the exception of occipitoscapularis, the spindle index (spindles/gram tissue) has been found to be very high, with a range of 40-96. Such high densities have only previously been observed in such small specialized muscles as the extraocular muscles, or the small muscles of the hand. Because of the relatively high weights of the neck muscles, the total numbers of spindles in neck muscles were extraordinarily high, and in one biventer cervicis, were found to exceed 170 (this compares with reported figures of 55 in soleus and 100 in rectus femoris). Occipitoscapularis was found to have a spindle index of 13, an index similar to that reported in the large muscles of the leg.

The spindles had varying distribution patterns. In thin sheet-like muscles such as splenius, the spindles were found quite evenly across the core of the muscle. In muscles such as biventer cervicis, the spindles were unevenly distributed, being abundant in some muscle fibre bundles and absent from other bundles. Various kinds of morphological attachments were observed between spindles, including tandem arrangements of two or more spindles, occasional parallel spindles and frequent mechanical contact of spindle capsules.

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AFFERENT CONNECTIONS TO SYSTEMS DESCENDING TO NECK MOTONEURONS.

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Two pathways take origin in the superior colliculus and terminate on the motoneurons of the neck. One, the tectospinal tract, has an intermediate synapse on a spinal interneuron, and the second, the tecto-reticulospinal pathway has an intermediate synapse in the pontine and medullary reticular system. We have identified cells of origin of the tectospinal system within the superior colliculus by antidromic activation and by the collision technique. More than 50% of the cells of origin of the tectospinal tract have afferent connections from visual, neck muscle and extraocular muscle afferents. Thus, a loop exists which originates from neck muscle afferents, projects to the superior colliculus, and terminates in neck muscle motoneurons via the tecto-spinal tract. Activity in the loop may be modified in the SC by input from the retina and from receptors capable of giving information with respect to eye position. Cells have also been identified in the superior colliculus which are antidromically activated by stimulation in pontine and medullary reticular nuclei and thus might be cells of origin of the tecto-reticulospinal system. The connections to these cells also include retinal, extraocular muscle and neck muscle afferent projections. So far, there appear to be no differences in the afferent connection of the 2 pathways from the superior colliculus and the important differences seem confined to their output routes, namely their intermediate synaptic arrangements.

The spinal synapse in the tectospinal system will be available only to intraspinal systems and to those descending systems that terminate in the upper cervical cord upon appropriate interneurons. The tecto-reticulospinal input is accessible to the wide variety of reticular afferent connection, but protected from much of the intraspinal connections. Thus this dual system allows the superior colliculus to either act essentially as a final common pathway for control of neck motoneurons through the tectospinal tract, or to bring influence to bear on neck motoneurons only after further modification within pontine and medullary reticular nuclei.

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Proprioceptive and retinal afferent projections to the superior colliculus of the cat and their connexions to the tectospinal tract

By V. C. Abrahams, F. Richmond and P. K. Rose. *Department of Physiology, Queen's University, Kingston, Ontario, Canada K7L 3N6*

Muscle receptors are assumed to play a role in the execution of most movements. One exception is combined head and eye movement which appear to be controlled by the visual and vestibular systems. Evidence is now provided that muscle receptors in the cat may play a vital role in co-ordinating head and eye movement.

The dorsal muscles of the cat neck have a remarkable spindle content. We have found the average spindle index (spindles/gramme) to range from 57 to 90 compared with 10–25 for other large skeletal muscle. Output from these spindles connects to one descending tract uniquely concerned with head movement, the tectospinal tract. Taking origin in the superior colliculus, the tract is distributed to the upper cervical cord. Cells of origin of the tectospinal tract were identified by antidromic stimulation. More than 60 % of these cells could be excited by electrical stimulation of neck muscle afferents at strengths appropriate to spindle afferent excitation. Seventy-five per cent of these same cells received retinal afferent connexion. Thus, the superior colliculus contains the output cells of a loop to neck motoneurones taking origin in neck muscle receptors and the retina.

This looping collicular system is also under the influence of extraocular muscle receptors. Electrical stimulation of extraocular muscle nerves excited 80 % of tectospinal cells receiving visual and neck muscle afferent input. Some insight into the possible role of this system was obtained in experiments in which controlled stretches were applied to the lateral rectus muscle or to the eye. Unit activity recorded in the superior colliculus was found to signal that the eye was approaching or had reached the normal limits of its travel. These characteristics of the extraocular projection system together with the known characteristics of the retinal projection suggests that the superior colliculus acting through the tectospinal tract can initiate head movement when the eye is at its own movement limit.

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BASIC PHYSIOLOGY OF THE HEAD–EYE MOVEMENT SYSTEM†

V. C. Abrahams, F. Richmond and P. K. Rose

To a substantial degree the head-movement system is synergistic to and integrated with the oculomotor system. While the oculomotor system has been thoroughly examined, the head movement system has been relatively neglected. We have examined the physiology of some of the component parts of the head movement system in the cat. This includes typing the muscles most directly concerned with head movement, examining the morphology and distribution of the receptors of these muscles, and examining the ascending projections from these muscle receptors and their relationship to descending systems to the motoneurons of the neck.

Five pairs of dorsal neck muscles insert on the lamboidal crest in the cat and serve to elevate and turn the head. Using the histochemical technique for ATPase (Padykula and Herman, 1955), we found that the ratio of dark (fast) to light (slow) staining fibres varied within the five muscles (Abrahams and Rancier, 1973). Table 1 illustrates this data. More extensive histochemical testing has shown that as in other cat muscles, fast fibres of neck muscle can be further divided into two types, implying that three functional types of neck motoneurons exist (Burke *et al.*, 1971; Burke *et al.*, 1973).

Table 1. Weights of Neck Muscles in the Cat and the Ratio of Dark to Light ATPase Staining in Individual Fibres of the Muscles

	Muscle weights, g				Dark/Light		
	Mean	*n*	S.E.M.	% total mass	Mean	*n*	S.E.M.
Occipitoscapularis	0.672	6	0.12	6	0.69	8	0.06
Splenius	3.66	6	0.60	34	2.64	8	0.19
Biventer cervicis	2.74	6	0.40	25	0.717	10	0.05
Complexus	2.90	6	0.42	27	1.26	7	0.026
Rectus Captis major	0.913	6	0.14	8	2.68	7	0.19

Neck muscles have long been known to be extraordinarily rich in their spindle density. Data obtained from human material by Voss (1958), and by Cooper and Daniel (1963), showed that neck muscles have the highest density of muscle spindles in the body. We have examined neck-muscle spindles in serial sections cut from the five dorsal neck muscles of the cat previously referred to. Except in occipitoscapularis, spindle density is very high, ranging from 46 to 106/g. Occipitoscapularis has a density

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comparable to hind leg muscles in the cat of 13–18/g (Table 2). Spindle types found include single and tandem spindles, multiple arrays, and dyads with both spindles and Golgi organs in apposition.

TABLE 2. SPINDLES OF DORSAL NECK MUSCLES. EACH PAIR OF FIGURES WAS TAKEN FROM AN INDIVIDUAL MUSCLE

Muscle	Absolute number of spindles	Density (spindles/g)
Rectus capitis major	33	53.6
	44	58.8
	38	61.0
	57	83.8
Splenius	148	46.6
	189	63.1
	172	66.4
Complexus	190	71.5
	254	106.8
Biventer cervicis	68	74.8
	180	74.4
	173	95.9
Occipitoscapularis	6	13.2
	11	18.7
	15	16.2

The spinal and medullary course of Gp I and II afferents from neck muscles has been examined in chloralose anaesthetized cat. Fibres were found to enter the dorsolateral part of the dorsal column system as predicted by the known anatomy (Imai and Kusama, 1969; Kerr, 1972). Group I and II primary afferents were sparsely distributed in the ventral portion of the cuneate nucleus, and also in a narrow strip between the spinal tract and the spinal nucleus of the trigeminal nerve. They were most abundant in the area of subnucleus gelatinosa of the spinal nucleus of the trigeminal nerve where they synapse within 2 or 3 mm of the obex.

While the ascending course of neck muscle afferent system beyond the spinal nucleus of the trigeminal nerve is not entirely clear, one major central projection is to the superior colliculus (Abrahams and Rose, 1975).

Like the visual projection to the superior colliculus, the neck muscle afferent projection is distributed to all layers of the superior colliculus and most units excited by neck muscle afferents may also be excited by a brief flash of light. These units are also excited by extraocular muscle afferent projections. The muscle afferent projections from the neck and extraocular system do not constitute the sole proprioceptive projections to the superior colliculus and a less dense projection exists from both the fore and hind leg.

Both the tectospinal tract and the tectoreticular tract are disynaptically connected to neck motoneurons (Anderson *et al.*, 1971). Antidromic excitation has been used to identify cells of origin of the tectospinal tract within the superior colliculus and the sensory input to these cells have been examined. Table 3 shows that the majority of tectospinal cells receive input from neck and extraocular muscle afferents and from the retina, as do cells of origin of the tectoreticular system.

The head-movement system therefore includes a loop with afferents from neck muscle, extraocular muscle and the retina converging on the superior colliculus where they excite cells disynaptically connected to motoneurons of the neck.

TABLE 3. CONVERGENCE ON CELLS OF ORIGIN OF TECTOSPINAL TRACT

Trimodal	Extraocular Neck Visual	27
Bimodal	Extraocular and visual Neck and visual	3 2
Unimodal	Extraocular Visual	2 5
Unresponsive to orthodromic stimuli		18
	Total number of units	57

This collicular system can alter neck motoneuron excitability as a result of the eye movement and position. The eyes of chloralose anaesthetized cats have been passively moved using a shaker controlled by a function generator. Such movements activate units in the superior colliculus which regularly fire in a brief burst when the eye reaches a certain displacement. Providing a certain minimal velocity is exceeded, the discharge occurs regularly at a fixed eye displacement. A histogram (Fig. 1) relates unit firing to the minimum deflection from the centre of gaze and shows that the system tends to signal large eye excursions. The previous experiments would suggest

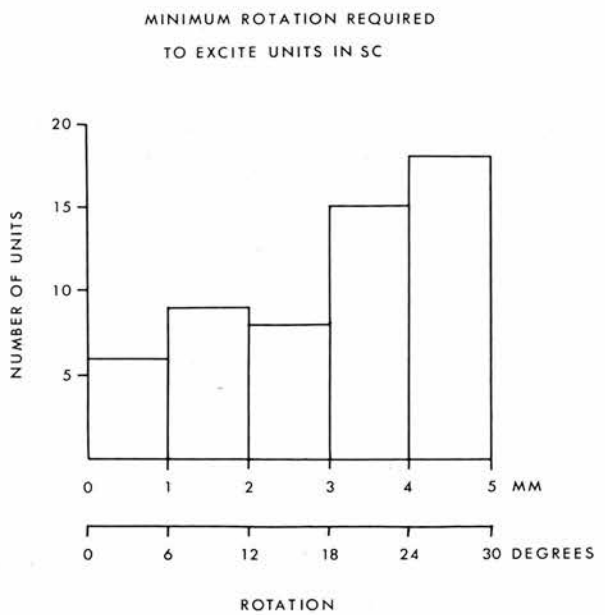

FIG. 1. Histogram showing eye displacement required to excite individual units of the superior colliculus. Velocity of displacement 100 mm/s.

that some of the cells responding to eye movement are cells of origin of the tectospinal and tectoreticulospinal tracts. The superior colliculus of the cat thus has the potential for the impulse traffic to neck motoneurons to be initiated when the eye reaches a new position. Since it is well known that the preferred visual stimulus is an object moving away from the fovea, it would seem that the superior colliculus can initiate head movements when eye movement is no longer able to secure foveation. The role of the neck muscle afferents may be in the control of such movements once initiated.

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409

F. RICHMOND* and V.C. ABRAHAMS. Department of Physiology, Queen's University, Kingston, Ontario. Enzyme distribution in extrafusal fibres of cat dorsal neck muscles.

Head movements involve specialized central and peripheral mechanisms. To date, however, the functional characteristics of muscles most directly concerned with head movement have not yet been systematically examined. We found previously that the ratio of fast/slow fibres in five dorsal pairs of muscles of the cat varied so that individual muscles fell into one of three categories. Serial sections have now been examined from the same five muscles, stained for each of 5 enzymes - SDH, DPNH, ATPase, acid stable ATPase and alkali stable ATPase. The histochemical profile so obtained shows only three individual fibre types, identical with those found by Burke in cat gastrocnemius. Accepting Burke's data for the correlation of histochemical profile and functional type, Type B are slow fibres, C are fast, fatigue resistant (FR) fibres and A are fast rapidly fatigued (FF) fibres. The ratio of fast/slow fibres was consistent and agreed well with previous data. In all muscles, FF fibres usually outnumbered FR fibres and in splenius and rectus capitis major they were the most common fibre type. However, the ratios of the FF/FR fibres was highly variable in individual muscles of the same type.

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Fore- and hind-limb muscle afferent projections to the superior colliculus of the cat

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We have recently shown that muscle afferent input from neck muscles and from extraocular muscles constitutes a substantial relay to the superior colliculus of the cat (Abrahams & Rose, 1974).

A further proprioceptive projection to the superior colliculus of the cat takes origin in fore- and hind-limb muscles. In cats anaesthetized with chloralose (60 mg/kg I.V.), electrical excitation of forelimb flexor muscle nerves (deep radial nerve), hind-limb flexor muscle nerves (flexor digitorium longus) and extensor muscle nerves (medial gastrocnemius nerve) all lead to the appearance of unit discharge in the superior colliculus. Typically, the responses show substantial habituation and for a consistent response to be elicited in the superior colliculus the frequency of stimulus presentation must be restricted. Short latency (10–40 msec) responses were found in the deep layers of the superior colliculus and the underlying tegmentum, and faithfully followed stimulation at once a second. Long latency (70–160 msec) projections were distributed predominantly to the superficial and intermediate layers of the superior colliculus and usually failed when stimulation exceeded once every 4 sec.

Stimulus thresholds required to induce unit responses fell when repetitive rather than single pulse stimuli were used. For 500/sec trains the fall in threshold was complete after five stimuli. The threshold strength of these stimulus trains necessary to elicit superior collicular unit responses was sufficient to excite Group II afferents.

The spinal pathway followed by limb muscle afferent projections to the superior colliculus was traced by examining the effects of partial spinal sections on unit responsiveness in the superior colliculus. These experiments identify the spinal course of ascending limb muscle afferent connexions to the superior colliculus as the spinoreticular projection to the lateral reticular nucleus and the lateral funiculus climbing fibre spino-cerebellar pathway (Lundberg & Oscarsson, 1962; Larson, Miller & Oscarsson, 1969). These pathways receive input from all four limbs as do most units within the superior colliculus.

Many of the units responding to fore- and hind-limb muscle afferent input also responded to a flash of light and input from neck muscle afferents, but the limb muscle afferent projection to the superior colliculus is less abundant than either the retinal or neck muscle afferent connexions. The presence of such connexions to the superior colliculus is consistent with a role of the superior colliculus in visually guided movement requiring substantial bodily movement involving redirection of the head and trunk.

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5 MEDULLARY PROJECTIONS OF GROUP I AND II AFFERENTS FROM NECK MUSCLE IN THE CAT. V.C. Abrahams and P.K. Rose* Department of Physiology, Queen's University, Kingston, Ontario, Canada K7L 3N6.

The medullary course of Group I and II afferents from the neck muscle, biventer cervicis, were examined using conventional microelectrode techniques. The most abundant medullary projection is to the spinal nucleus of the trigeminal nerve. Primary fibres ascend dorso-laterally, turn ventrally and then terminate among cells with connections from cutaneous receptors of the face. The second order cells from neck muscle do not appear to have facial fields. Primary afferents also are present in narrow bands between the external cuneate nucleus and the spinal nucleus of the trigeminal nerve and in the ventral part of the cuneate nucleus. In one experiment, substantial termination of Group I and II primary afferents was found in the ventral cuneate nucleus adjacent to units activated by light touch of the skin of the neck. Primary afferents from neck muscle terminate within 2-3 mm of the obex and do not appear to travel higher in the brain stem. Thus, neck muscle afferents like forelimb muscle afferents have a cuneate projection, but unlike other lower body proprioceptive projections also have a substantial primary input to the spinal trigeminal nucleus.

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Projections of Extraocular, Neck Muscle, and Retinal Afferents to Superior Colliculus in the Cat: Their Connections to Cells of Origin of Tectospinal Tract

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THE TECTOSPINAL TRACT of the cat only projects to the upper cervical segments (29, 30), at which level connection is made to motoneurons via interneurons (5). Consequently, the tectospinal tract is one pathway whereby the superior colliculus can participate in the control of head movement.

The control of tectospinal tract cells is of considerable interest for the understanding of the control of head movement. Control of tectospinal tract cells will depend on the nature of the projections to the superior colliculus as well as the intrinsic organization of the superior colliculus. The experiments reported here describe some projections to the superior colliculus that excite tectospinal tract cells. One projection is that from extraocular muscle afferents, a projection which was first described in goat (15), but which is also present in cat (16) and sheep (25). The role of afferents from extraocular muscles is not clear. Their excitation does not give rise to a conventional moytatic reflex (22, 26), but to a weak inverse myotatic reflex (7, 31). Their central projections are only partially known. As well as the superior colliculus projection, a cerebellar projection exists (9, 18) which may be involved in control of saccadic eye movement (18).

Like extraocular muscle afferents, neck muscle afferents are something of a curiosity. Receptors in neck muscle do not play a role in the well-known tonic neck and compensatory eye reflexes of the decerebrate cat (13, 27). Nonetheless, damage to neck muscles or their nerves produce generalized motor defects not confined to the execution of head movement (for reviews, see ref 2, 10), and neck muscle afferents are the source of powerful spinospinal interactions (1). It has now been found that neck muscle afferents give rise to a major projection to the superior colliculus where, together with afferents from extraocular muscles and the retina, they terminate on cells of origin of the tectospinal tract.

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METHODS

Experiments were performed on 59 adult cats weighing 2.5–3.8 kg; 50 were anesthetized with chloralose (60 mg/kg administered intravenously after the induction of anesthesia by ethyl chloride and ether), and 9 were anesthetized with sodium thiopental (Pentothal, Abbott), with the initial dose of 40–50 mg/kg administered intraperitoneally and supplemented as required. Just prior to electrical recording the animals were paralyzed with gallamine triethiodide (Flaxedil, Poulenc). The animal was then artificially ventilated and a bilateral pneumothorax made. In early experiments, blood pressure was continuously monitored by a Statham gauge (P23d) connected to a femoral arterial catheter and end-tidal P_{CO_2} was also monitored. The temperature of the animal was maintained between 37 and 39°C using either a heating blanket or a heated operating table. The eyes were protected with a +2 diopter contact lens and focused on a projection screen placed from 30 to 100 cm in front of the cat (17).

In all experiments, after branches of the C_2 and C_3 nerves to the neck muscle, biventer cervicis, were exposed, fine bipolar platinum hook stimulating electrodes were placed in position on the nerves and secured with low-melting point (39°C) paraffin wax. In 22 experiments, the branch of the IIIrd nerve, which innervates the inferior oblique muscle, and the branch of the VIth nerve, which innervates the lateral rectus muscle, were exposed in the orbit after enucleating one eye. Bipolar platinum stimulat-

ing electrodes were placed under each nerve, and secured with the low-melting point paraffin wax. A laminectomy was performed between C_1 and C_4 in 28 experiments so that the tectospinal tract could be stimulated.

Stimuli to the neck and extraocular muscle nerves were single rectangular pulses 0.05 ms in duration and usually of 5–10 V in magnitude, although the nerve to the inferior oblique often required voltages above this for its excitation. The ventral cervical cord was stimulated through either a monopolar tungsten microelectrode (Haer, type 25-10-3) or a bipolar stainless steel electrode (David Kopf, type SCE-100) using a constant-current unit (Tektronix, type 2620). When monopolar stimulation was used, an indifferent electrode was clipped to the muscles of the neck adjacent to the tungsten electrode.

Various types of visual stimuli were used. In some experiments moving shapes were projected onto the screen. Rectangular shapes (slits, etc.) were formed by a rectangular diaphragm placed in a Leitz projector; circular shapes were projected from 2 inch x 2 inch slides. Movement was produced by first projecting the images onto a flat mirror mounted on a pen motor driven by a function generator (Krohn-Hite, model 5100A). Thus, the velocity and position of the moving image reflected onto the screen was readily controlled. Another visual stimulus that was used consisted of 2-ms flashes generated with a prefocused bulb subtending 2° of arc and carried on a mount which could be placed anywhere in the visual field. If at any time the flash alone was regarded as an inadequate stimulus, a flashlight pointer was used to produce moving visual stimuli. The room lighting was subdued during visual stimulation. Because responses rapidly attenuated, the frequency of stimulus presentation was kept in the range of 4–15/min.

The conduction velocity of neck muscle afferents relaying to the superior colliculus was measured by recording compound action potentials at the entry point of the appropriate cervical dorsal root with a tungsten microelectrode. Extracellular unit activity within the superior colliculus was recorded using tungsten microelectrodes (Haer, type 25-10-3) introduced stereotaxically. Conventional amplification and display techniques were used. Since spontaneous activity was rarely recorded, the microelectrode was advanced into the superior colliculus during repetitive (15/min) presentation of a visual stimulus. When the electrode entered the deep layers of the superior colliculus and the tegmentum, where visual responses became less common, muscle nerve stimulus was alternated with the visual stimulus. Each unit encountered was tested for responsiveness to visual stimuli and to stimulation of muscle nerves. On completion of each electrode penetration, the deepest position was marked by a small lesion made by passing 50–100 μA through the recording electrode for 1–2 s.

In 22 experiments units antidromically activated from the cervical cord were examined. A recording microelectrode was advanced through the superior colliculus while the contralateral ventral cervical cord was stimulated once a second. The criteria of antidromic activation employed included short constant latency, ability to follow paired stimuli at 2-ms intervals, and extinction by collision with orthodromic impulses.

At the end of each experiment the brain was perfused with normal saline, and then with 25% formaldehyde in saline. Serial sections, 30 μm in thickness, were cut on a freezing microtome and stained for fibers and cells (23). The location of each recording site was established by referral to the lesion identifying the terminal point of each track. The location of the stimulating electrode in the ventral cervical cord was also identified histologically. Units were assigned to three major divisions of the superior colliculus; the superficial (stratum zonale, superficiale, and opticum), intermediate (stratum griseum intermediale and album intermediale), and deep layers (stratum profundum), using the anatomical criteria of Ramón y Cajal (12) and Szekely (35). Since the colliculotegmental border cannot be clearly defined in the cat (32), units which were ventral to a line extending horizontally from the lateralmost margin of the periaqueductal gray were arbitrarily assigned to the tegmentum.

RESULTS

In the first 20 experiments with chloralose anesthesia and in the 9 experiments with Pentothal anesthesia, it was found that any unit that was activated by a moving visual target could also be activated by a 2-ms flash in the appropriate part of the visual field. The flash was employed as a visual stimulus in the remainder of the experiments.

Neck muscle nerve stimulation is more effective in exciting superior collicular units than a visual stimulus. In a series of 28 experiments on chloralose-anesthetized cats, 145 units were tested with both visual and neck muscle afferent stimulation. The visual stimulus activated 107 units (74%), but 124 (86%) responded to biventer cervicis nerve stimulation. In a further 13 experiments, 93 units were tested for their respon-

siveness to extraocular muscle nerve stimulation as well as to visual and neck muscle nerve stimulation. Extraocular muscle afferents proved slightly more effective in activating superior collicular units than neck muscle afferent stimulation. Extraocular muscle afferent stimulation excited 91 units, neck muscle afferents 85 units. As Table 1 shows, there is considerable convergence on superior collicular units and 61 of the 93 tested (66%) responded to neck and extraocular muscle nerve stimulation and to visual stimulation. When a unit responded to only two kinds of stimuli, these were almost always both types of muscle afferents. No units in this series were found that responded only to neck muscle and retinal stimulation.

Convergence was also found within each type of muscle afferent stimulation. Of 66 units tested, 54 (82%) responded to both inferior oblique and lateral rectus nerve stimulation. The biventer cervicis muscle, like the other large dorsal muscles of the neck, has multiple innervation, with branches going to the same muscle from two or more cervical segments (3). Stimulation of each of these branches always activated the same superior collicular unit. The neck muscle projection is largely bilateral and 108 of 124 units (87%) responded to both ipsilateral and contralateral nerve stimulation. The remaining 16 units in this series responded only to unilateral stimulation, 9 being ipsilaterally activated and 7 being contralaterally activated.

Units responding to biventer cervicis muscle afferent stimulation fell into three groups. Some units had a short latency (10–50 ms), some a long latency (80–140 ms), and the remainder fired twice (Fig. 1*A*, *B*, and *C*). As Fig. 1 shows, paired firing appeared to be a combination of an early and a late discharge. Short-latency units faithfully followed stimulus frequencies up to a maximum of 1/s, but the long-latency responses only followed reliably when stimulus presentation did not occur more often than 15/min. The short-latency response in paired firing appeared following stimulation at 1/s, but the late response then dropped out and only appeared regularly when stimulus-repetition intervals were increased to 4 s or more.

TABLE 1. *Convergence of retinal, extraocular, and neck muscle afferents on units in superior colliculus*

No. of Stimulus Types		No. of Units
3	Extraocular Neck Visual	61
2	Extraocular and visual	3
	Neck and extraocular	23
	Visual and neck	0
1	Extraocular	4
	Neck	1
	Visual	1
Total no. of units		93

Units responsive to neck and extraocular muscle nerve stimulation are widely distributed throughout the superior colliculus. No orderly pattern could be demonstrated within the superior colliculus linking stimulus to recording site except when the distribution of units was examined on the basis of their firing patterns and latencies. Units responding to biventer cervicis nerve stimulation then showed a clear spatial patterning. Long-latency units predominated in the superficial layers and were very rare in the tegmentum, and short-latency units predominated in the tegmentum (Fig. 2). Units responding with paired discharge were fairly evenly distributed throughout the superior colliculus but were less common in the tegmentum. Units activated by extraocular muscle afferents were mainly found in the various layers of the superior colliculus and only 7% were found in the tegmentum. Extraocular muscle afferent stimulation normally produced discharge after long latencies and the small proportion of short-latency units were evenly distributed in the superior colliculus (Fig. 2*C* and *D*). Paired firing to extraocular muscle nerve stimulation was evenly distributed except that lateral rectus-activated units were more common in the superficial layers of the superior colliculus.

The tectospinal tract projects exclusively to the contralateral ventromedial upper cervical cord (29, 30). Stimulation of this region of ventromedial cervical cord led to the appearance of antidromic unit potentials distributed in the intermediate and deep layers of the superior colliculus and

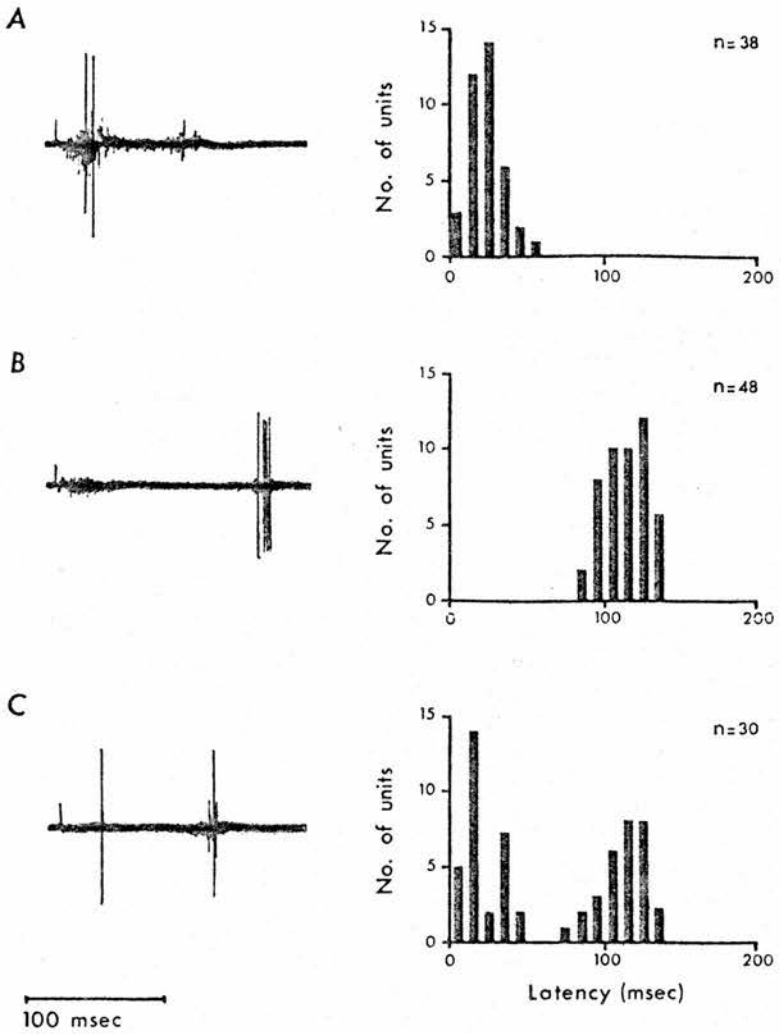

FIG. 1. Firing patterns and latency histograms of 116 units responding to biventer cervicis muscle stimulation. *A:* early discharge; *B:* late discharge; *C:* paired firing.

tegmentum. The potentials were virtually identical with those described by Bishop et al. (11) in the lateral geniculate body following optic radiation stimulation. As the microelectrode was advanced into the superior colliculus, a low-amplitude field potential was recorded. This would abruptly give way to a unitary potential of several hundred microvolts (Fig. 3). These unitary potentials consisted of an early stable negative potential (the initial segment potential), and a biphasic wave of variable latency (11).

A total of 218 tectospinal tract units were examined in 22 experiments; 130 units (60%) were located with the stimulating electrode between the first and second cervical roots, 88 units (40%) with the electrode in the third cervical segment. Latencies from C_1 segment ranged from 0.40 to 4.50 ms, with a mean of 0.92 ± 0.05 ms (SEM). Assuming a conduction distance of 5 cm, the mean conduction velocity is 54 m/s, with a range of 11–125 m/s. Latencies from the C_3 segment were 1.22 ± 0.07 ms (SEM), with a range from 0.50 to 3.90 ms. Assuming a 1-cm additional conduction distance, the mean conduction velocity is 49 m/s, range 15–120 m/s. Cells of origin of the tectospinal tract were widely distributed through the superior colliculus from the anterior to the posterior borders (Fig. 4). Most cells

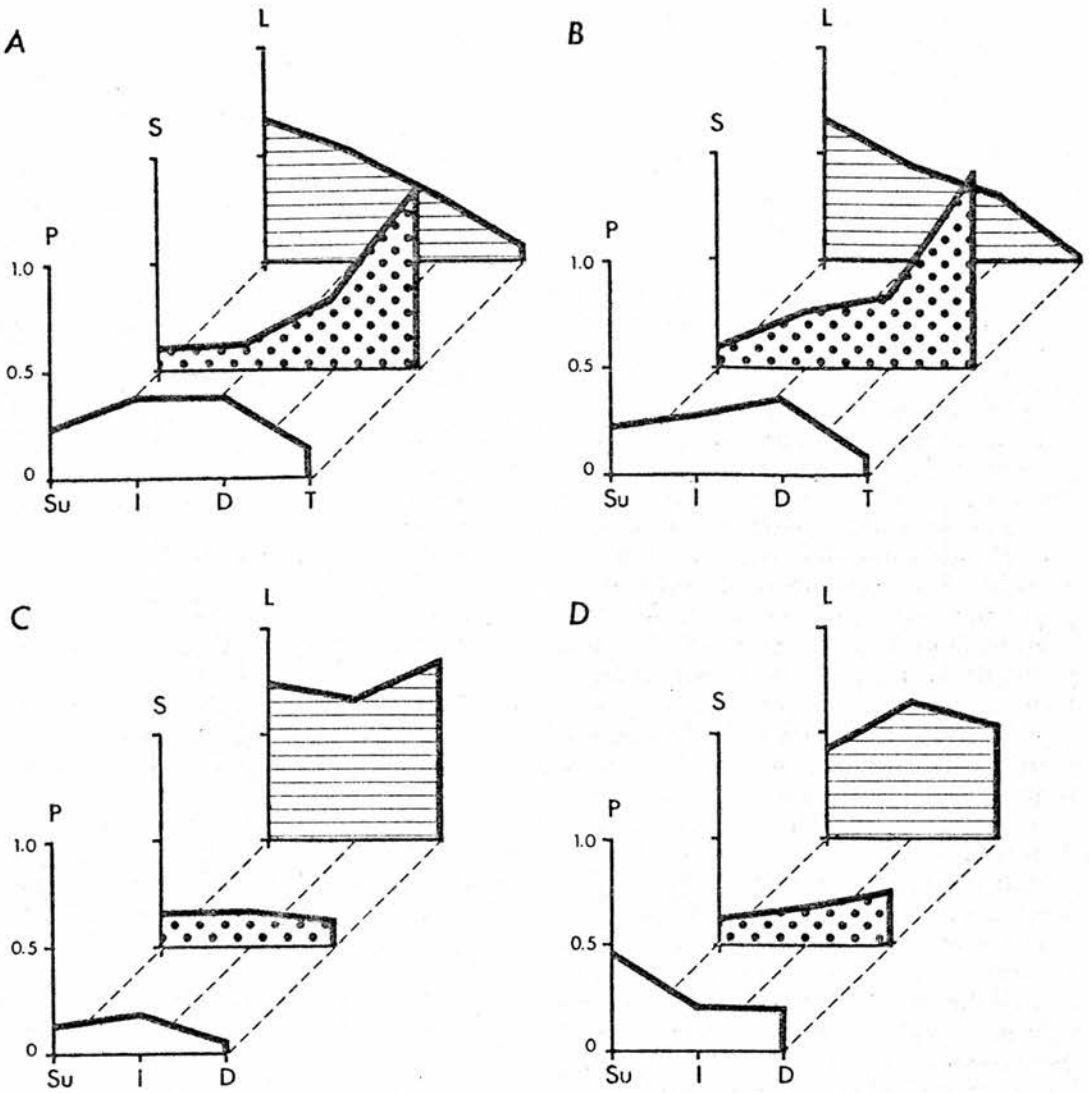

FIG. 2. Plot to illustrate distribution of unit discharge in superior colliculus with respect to latency, modality, and recording site. *A:* ipsilateral neck muscle nerve stimulation (127 units); *B:* contralateral neck muscle nerve stimulation (116 units); *C:* ipsilateral inferior oblique nerve stimulation (67 units); *D:* ipsilateral lateral rectus nerve stimulation (70 units). L, long-latency responses; S, short-latency responses; P, paired responses. Su, superficial layers of superior colliculus; I, intermediate layers of superior colliculus; D, deep layers of superior colliculus; T, tegmentum. The ordinate scale has been adjusted so that 1.0 = 100% of units in that anatomically defined layer responding to the stimulus modality.

(88%) were evenly distributed in the intermediate and deep layers; 10 cells (5%) were in the superficial layers and 16 cells (7%) in the tegmentum.

Of 218 tectospinal tract cells, 160 were examined for input from visual and neck muscle afferents; 99 (62%) could be driven by neck muscle afferent stimulation and 89 (56%) by visual stimuli. As usual there was considerable convergence and, 75 units (47%) were activated by both visual and neck muscle afferent stimuli; 24% of units tested responded only to one of the two stimuli, 24 (15%) to neck muscle afferent stimulation, and 14 (9%) to flash. A substantial number of tectospinal tract cells responded to neither neck muscle nerve stimulation nor visual stimuli.

Fifty-seven tectospinal tract cells were tested with all three types of stimuli em-

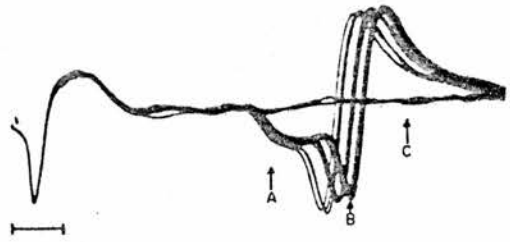

FIG. 3. Antidromic response in superior colliculus following stimulation of ventromedial funiculus of upper cervical spinal cord. Repetition rate 50/s. Response consists of an early negative potential whose latency is consistent (A) and a late biphasic negative positive potential whose latency varies (B). Even at these relatively low repetitive rates, antidromic firing is not alway seen (C). Time mark, 0.1 ms.

ployed in these experiments, and 32% of these units proved inexcitable. As shown in Table 2, convergence is considerable and 27 (47%) of these cells responded to all three stimulus types. The firing patterns of tectospinal tract cells showed the same three patterns of response as other superior collicular units, and their adaptation patterns also paralleled other superior collicular units.

The neck muscle projection to the superior colliculus was examined in nine cats anesthetized with Pentothal. The characteristics of the relay were similar in many respects to those found in chloralose-anesthetized cats. Of 49 units found in these experiments, 35 (71%) could be excited by stimulation of biventer cervicis muscle nerves, 32 (65%) responding to bilateral stimulation and 3 (6%) to ipsilateral stimulation. One major difference was found between the chloralose-anesthetized cat and the Pentothal-anesthetized cat. In the deep layers of the chloralose-anesthetized cat there is equal distribution of long- and short-latency units; under Pentothal 90% of units were short latency.

The conduction velocity of biventer cervicis muscle afferents projecting to the superior colliculus was estimated in six experiments in chloralose-anesthetized cats. In all six experiments, when superior collicular unit activity followed biventer cervicis nerve stimulation it was of sufficient strength to activate fibers conducting with a velocity (at a nerve temperature of 34°C) of 30–70 m/s.

DISCUSSION

The present experiments show that in the cat afferent projections from neck and extraocular muscles to the superior colliculus are more extensive than visual projections. In stating this it should be remembered that the experimental protocol contains a built-in bias in favor of retinally activated cells, for the normal stimulus used in exploration was a visual stimulus. This means that some units inexcitable to a visual stimulus but excitable by a muscle afferent stimulus would be missed. It should also be emphasized that the flash stimulus, while producing a less vigorous superior collicular discharge than an appropriate moving stimulus (28, 33, 34) was, in our experience,

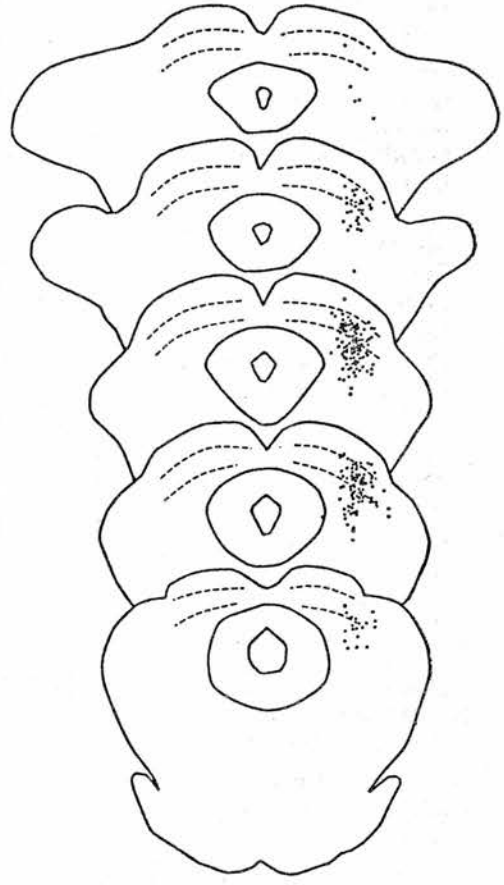

FIG. 4. Series of representative sections through the superior colliculus to show the location of cells of origin of the tectospinal tract, identified by antidromic excitation. Top dotted line indicates boundary between superficial and intermediate layer; bottom dotted line, the boundary between intermediate and deep layers.

TABLE 2. *Convergence on cells of origin of tectospinal tract*

No. of Stimulus Types		No. of Units
3	Extraocular Neck Visual	27
2	Extraocular and visual	3
	Neck and visual	2
1	Extraocular	2
	Visual	5
Unresponsive to orthodromic stimuli		18
Total no. of units		57

capable of activating every unit that could be activated by a moving stimulus. It is unlikely that the sample of visually activated cells was reduced by restricting the visual stimuli in later experiments to a flash. Other details in the conduct of this type of experiment will substantially affect the sample of units obtained. The choice of anesthetic agent is important, as exemplified by the fact that units responding at a long latency following neck muscle afferent stimuli were uncommon in the deep layers in Pentothal-anesthetized animals. The details of the protocol too will affect the sample, for stimulus presentations more frequent than 15/min will reduce the observed population of long-latency responses.

There seems little doubt that the neck muscle afferent projection to the superior colliculus follows two pathways, a relatively short-latency pathway projecting primarily (but not exclusively) to the deep layers of the superior colliculus and the neighboring tegmentum and a long-latency pathway predominantly projecting to the superficial layers. Cells responding with paired discharge act as though innervated by both pathways. Responses to cutaneous stimulation recorded in the superior colliculus also occur at either short or long latencies (20, 21). Evidence has been obtained that the long-latency cutaneous pathway is dependent on the integrity of the ipsilateral visual cortex. Long-latency cutaneous responses in the superior colliculus are lost following ablation of the ipsilateral visual cortex or the topical application of KCl to the visual cortex (29). Neck muscle afferents do project to the cortex (24), and the long-latency pathway to the superior colliculus may reflect a long intracerebral route. Extraocular muscle afferent projections are more uniformly long latency and evenly distributed through the superior colliculus. The projections from both neck and extraocular muscle show considerable convergence with each other and with retinal projections. The superior colliculus is a synaptic junction in a looping pathway, for among the cells that are excited by these afferent projections are a high proportion of the cells of origin of the tectospinal tract. The distribution of tectospinal tract cells shows that both deep and intermediate layers of the superior colliculus are output layers which have the potential for affecting head movement.

The existence of a rich projection to the superior colliculus from neck and extraocular muscle afferents fits well with the idea that the superior colliculus plays a role in the control of head and eye movement, a view of superior collicular function which has received experimental support for many years (6, 19). Our experiments shed no light on what information might be conveyed to the superior colliculus from muscle afferent systems. Neck muscles in both man (14, 36) and cat (4) are uniquely rich in spindles and contain the largest number of any muscles in the body. We have found the spindle density in dorsal neck muscles of the cat to range from 46 to 106/g, with individual muscles containing more than 250 spindles (4). The conduction-velocity experiments show that unit discharge in the superior colliculus occurs when group II fibers are activated, suggesting that the origin of the neck muscle afferents are spindle secondaries. While there are no muscle spindles in cat extraocular muscle, the receptors that are present have sensory properties similar to both muscle spindles and tendon organs (8), and thus can signal eye position and movement. However, given the long latency and uncertainty of conduction in the projections to the superior colliculus, it is unclear how much of the original signal from muscle receptors is preserved and conveyed to the superior colliculus. The present experiments do however extend the possible range of function of extraocular muscle receptors,

and show that they may play some role in the control of head movement through the tectospinal tract.

SUMMARY

Unit recordings were made in the superior colliculus of cats anesthetized with chloralose and with Pentothal. Electrical stimulation of extraocular muscle afferents and neck muscle afferents excited more units in the superior colliculus than did a variety of moving and stationary visual stimuli.

Units responding to neck muscle afferent stimulation fell into three populations; one population firing with a short latency and following stimulus presentation up to 1/s, a second population with a long latency and following stimulus presentation at frequencies lower than 15/min, and a third population exhibiting paired firing. The latencies and firing patterns of the third population combined the characteristics of each of the first two patterns. It is suggested that these characteristics of unit discharges stem from the existence of two pathways from neck muscle afferents to the superior colliculus. The projection is predominantly bilateral.

Units responding to neck muscle afferent stimulation are distributed throughout the superior colliculus on the basis of their latencies. Long-latency responses predominate in the superficial layers of the superior colliculus and short-latency responses, while more common in the intermediate and deep layers, predominate in the tegmentum.

Extraocular muscle afferent projections to the superior colliculus constitute the single richest projection found in these experiments. While the response patterns and latencies are similar to those of the neck muscle afferents, long-latency responses are the most common and dominate in all collicular regions. Few units in the tegmentum could be excited by extraocular muscle afferents. Both extraocular muscle and neck muscle afferents show considerable convergence with one another and with retinal afferents within the superior colliculus.

Cells of origin of the tectospinal tract were identified within the superior colliculus and tegmentum by antidromic excitation from the upper cervical cord. These cells were distributed predominantly within the intermediate and deep layers of the superior colliculus, and sparsely in the superficial layers and tegmentum. Almost 50% of the cells of origin of the tectospinal tract receive a convergent input from extraocular muscle and neck muscle afferents and from the retina. About 30% of the cells were inexcitable to the stimuli employed in these experiments.

The significance of these projections is discussed with respect to superior collicular function in the cat and in relation to the known functions of extraocular and neck muscles.

ACKNOWLEDGMENTS

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THE SPINAL COURSE AND DISTRIBUTION OF FORE AND HIND LIMB MUSCLE AFFERENT PROJECTIONS TO THE SUPERIOR COLLICULUS OF THE CAT

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SUMMARY

1. Projections to the superior colliculus from fore and hind limb muscle nerves have been examined.

2. Hind leg nerve stimulation at strengths sufficient to excite Group II and III fibres elicited unit discharge in all layers of the superior colliculus and underlying tegmentum.

3. Forelimb nerve stimulation excited units in all layers of the superior colliculus and underlying tegmentum. Twenty per cent were activated by stimulation below Group II threshold, the remainder by stimulation above Group II threshold.

4. Most units activated by limb afferent nerve stimulation were also activated by visual stimuli. There was considerable convergence from both flexor and extensor nerves and most units were excited by stimulation of nerves in more than one limb.

5. Units fired either with a short or long latency or with a paired discharge. Short latency units were most common in deep collicular layers and the tegmentum, long latency units were most common in superficial layers.

6. The spinal pathway of the afferents is mainly in the dorsolateral quadrant contralateral to the recording site. The anatomical characteristics of the pathway are similar to the lateral funiculus climbing fibre-spinocerebellar pathway.

INTRODUCTION

The superior colliculus (SC) of the cat receives input from a number of non-visual systems. These include connexions from extraocular (Cooper,

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Daniel & Whitteridge, 1955; Cooper & Fillenz, 1955; Abrahams, Rancier & Rose, 1973; Abrahams & Rose, 1975), and neck muscle afferents (Abrahams & Rose, 1975), as well as connexions from the auditory (Straschill & Hoffman, 1969; Stein & Arigbede, 1972; Gordon, 1973), and somatosensory systems (Bell, Sierra, Buendia & Segundo, 1964; Jassik-Gerschenfeld, 1965, 1966; Straschill & Hoffman, 1969; Stein & Arigbede, 1972; Gordon, 1973). The extraocular and neck muscle projections constitute one of the richest projections to the SC and electrical excitation of neck muscle and extraocular muscle nerves can be a more effective stimulus to unit discharge in the SC than visual stimulation (Abrahams & Rose, 1975).

The question that has now been examined is whether afferent projections from neck and extraocular muscles to the SC are the only muscle afferent projections to this structure, or whether they are part of a more generalized projection to the SC from muscle afferent systems. Some evidence exists for such a generalized projection as limb movements have been reported to initiate unit discharge in the SC of the cat (Gordon, 1973). Preliminary reports have been given of the present findings (Abrahams & Falchetto, 1966; Abrahams & Rose, 1972).

METHODS

Experiments were performed on forty-nine adult cats weighing 2·3–3·4 kg. All animals were anaesthetized with chloralose (60 mg/kg) I.V. after the induction of anaesthesia with ethyl chloride and ether. The animals were mounted on a stereotaxic frame which enabled visual fields to be explored (La Précision Cinématographique, Asnières, France). The occipital cortex over the SC was exposed to permit the introduction of a tungsten micro-electrode (Haer 25–10–3), and the bony defect was covered with a low melting point (39° C) paraffin wax after the insertion of the electrode into the surface layers of the cerebral cortex. Laminectomies were performed in the lower cervical, midthoracic and lumbar regions and pools were created with skin flaps and filled with Silicone Medical Fluid (no. 360, Dow-Corning). At the termination of surgery the animals were given Flaxedil (gallamine triethiodide, Poulenc) artificially ventilated and a bilateral pneumothorax was performed. Body temperature was maintained with a heating pad and warmed operating table.

The arrangements for visual stimulation were as previously described (Abrahams & Rose, 1975), and consisted of brief (2 msec) flashes delivered through a prefocused incandescent bulb located in the appropriate part of the visual field, 10 in. in front of the cat. The eye was protected from drying by a × 2 D contact lens. The forelimb flexor nerve, the deep radial (DR), the hind limb flexor nerve, flexor digitorium longus (FDL), and the hind limb extensor nerve, medial gastrocnemius (MG) were dissected and bipolar platinum stimulating electrodes placed on the nerves. The electrodes were held in position by low melting point (39° C) paraffin wax. In some experiments the C3 nerves to the biventer cervicis muscle in the neck (BC) were also dissected free on both sides and prepared for stimulation.

Afferent volleys were monitored by bipolar recording electrodes placed on the appropriate lumbar dorsal roots or on the sciatic nerve, and on the dorsal funiculus in the lower cervical region. Stimulation strengths are expressed in multiples of the

threshold voltage (T), necessary to elicit a barely detectable compound action potential at the monitoring electrode.

The micro-electrode was advanced into the SC while presenting visual stimuli or stimulating one of the muscle nerves. Because muscle afferent systems projecting to the SC are characterized by rapid adaptation, stimulus repetition rates were kept low, usually not exceeding 15/min during the search for unit responses. Each unit encountered was tested for its responsiveness to all nerves prepared for stimulation in that experiment. In experiments on the effects of stimulus repetition intervals, a total of ten observations were made at any given stimulus interval. At the end of each penetration the electrode position was marked with a small (about 100 μm) lesion made by passing 50–100 μA for 1 sec with the electrode as cathode.

The path of the hind limb and forelimb projection in the spinal cord was determined by partial sections of the cord (cf. Oscarsson, 1973), at either T10 or C4. The micro-electrode was first placed in the SC and single or multi-unit responses to fore and hind limb stimulation obtained. Sequential transections of the spinal cord were then performed. Sections of the cord were often found to lead to a temporary loss of response. At least 1 hr was therefore allowed to elapse between each lesion and the testing of responses in the SC to limb nerve stimulation. To ensure that any loss of response was not due to functional failure within the SC, visual responses were repeatedly tested and the data only accepted when unit responses to visual stimuli were stable for the duration of the experiment.

Upon completion of micro-electrode recording the brain was perfused with saline and formalin. Serial frozen sections were cut and stained with Luxol fast blue and cresyl violet (Klüver & Barrera, 1953), so that the lesions produced at the end of each track could be located. The extent of the spinal cord section was determined by inspection through a binocular operating microscope after formalin fixation of the cord.

The classification of collicular and tegmental zones is that previously adopted (Abrahams & Rose, 1975). The superficial layer included stratum zonale, superficiale and opticum; the intermediale layer, stratum griseum intermediale and album intermediale; the deep layer, stratum profundum. The colliculo-tegmental border was arbitrarily defined as extending horizontally from the lateral extent of the periaqueductal gray, and units ventral to this line were regarded as tegmental.

RESULTS

Projections from hind limb muscle nerves to the SC

Firing patterns

Unit discharge was analysed in forty experiments following electrical stimulation of hind leg muscle nerves. Such stimulation led to three patterns of unit discharge within the SC and neighbouring tegmentum. Most commonly, responses either had short latencies, ranging from 20–50 msec (Fig. 1 *A*), or long latencies, ranging from 80 to 160 msec (Fig. 1 *B*). The third, and least common pattern of unit discharge (Fig. 1*C*) was a double burst of impulses. The double burst appeared to be a combination of short and long latency responses. Table 1 contains data from 58 units responding to MG stimulation showing that there is a relationship between latency of response and the anatomical loaction of the unit. Short latency

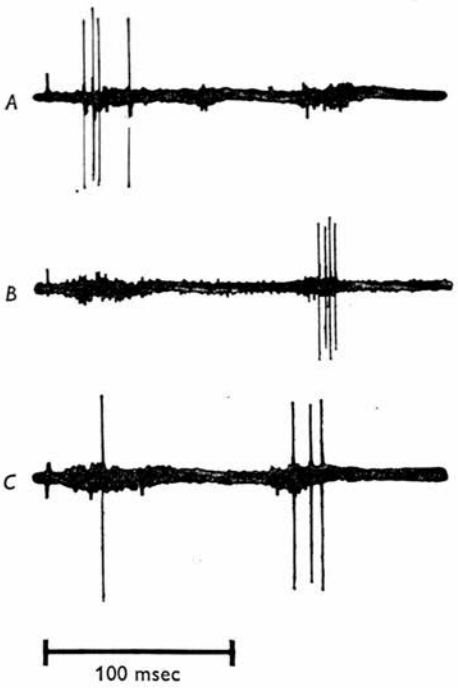

Fig. 1. Patterns of unit discharge recorded from SC following stimulation of limb muscle afferent nerves in chloralose anaesthetized cats. *A*, short latency discharge; *B*, long latency discharge; *C*, paired firing.

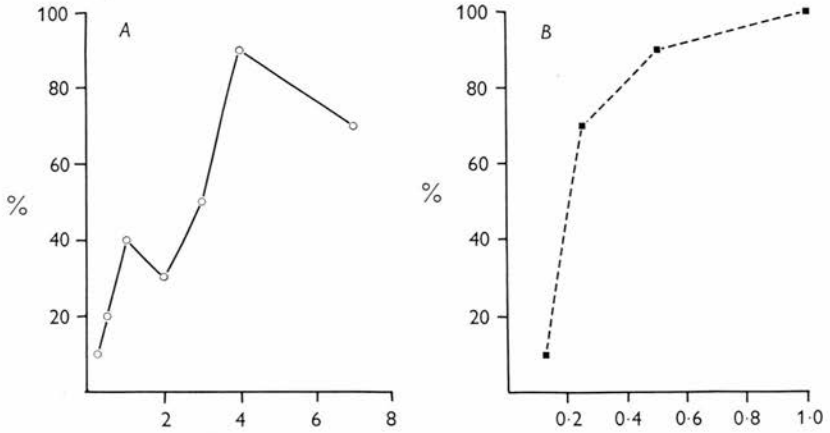

Fig. 2. Effect of stimulus interval on the probability of unit response in the superior colliculus. *A*, long latency response to FDL stimulation; *B*, short latency response to FDL stimulation.

responses predominated in the tegmentum and in the deep layers of the SC and long latency responses predominated in the superficial and intermediate layers of the SC. Paired responses were found in all layers of the SC. Long latency units (and the long latency component of paired responses) reliably followed stimulus presentations at intervals of 4 sec or more and there was a tendency for following to deteriorate at more frequent

TABLE 1. Distribution of unit responses within the superior colliculus on the basis of response latency

A Ipsilateral medial gastrocnemius

Location in SC	Response			
	Short	Long	Paired	Total
Superficial	1	13	1	15
Intermediate	1	10	4	15
Deep	6	4	4	14
Tegmentum	13	—	1	14
Total	21	27	10	58

B Contralateral medial gastrocnemius

Location in SC	Response			
	Short	Long	Paired	Total
Superficial	2	10	2	14
Intermediate	2	7	2	11
Deep	6	4	2	12
Tegmentum	14	—	1	15
Total	24	21	7	52

intervals (Fig. 2). When the interval between stimuli was reduced, the responses became irregular. Short latency responses (and the short latency component of paired responses) reliably followed stimulus presentations down to 1 per sec or less (Fig. 2). These characteristics apply equally to units responding to flexor and extensor nerve stimulation, and indeed most units respond equally well to FDL and MG nerve stimulation. Of fifty-seven units examined, 40 (70 %) received bilateral input.

Stimulus thresholds of fibres from hind leg muscle nerve projection to the SC.

Single shocks to hind leg muscle nerves will activate units in the SC if high stimulus strengths are used. If a brief train of impulses is employed, the threshold rapidly decreases to a low stable level. Fig. 3 illustrates the effect of increasing the length of stimulus trains to FDL at a stimulus

frequency of 500/sec on the threshold required to elicit unit activity in the SC. It can be seen that at train lengths of 10 msec (5 impulses) or more, threshold falls to a minimum. Flexor nerve threshold was lower than the extensor nerve threshold and the minimum value of FDL threshold was in the range of 2–5T and of MG thresholds in the range of 5–20T.

Spinal course of hindlimb muscle afferent projection to the SC.

In thirteen experiments in which stable single or multi-unit responses were recorded in the SC following stimulation of muscle nerves in both hind legs, progressive sections were made in the spinal cord. In nine experiments the sections were made at C4, and in four experiments the sections were made at T10. The cardiovascular effects of the T10 section were minimal, but this benefit was outweighed by the fact that sections at the C4 level allowed simultaneous examination of the forelimb pathway (the results of which are referred to in a separate section).

In all experiments, the first procedure was a complete transection of the dorsal columns. Regardless of whether the units in the SC fired at short or long latency, or were activated by low or high threshold fibres, transection always resulted in a change in the unit firing pattern. Impulse frequency fell and the burst was approximately doubled in duration. There was also a slight increase in latency of approximately 25% (Fig. 4). These effects were the same regardless of the latency of responses or the threshold of the muscle afferent fibres.

In the four animals with cord section at T10, the cord was then hemisectioned ipsilateral to the recording electrode. As Fig. 4*C* shows, hemisection did not abolish conduction, but led to a further dispersal of unit discharge and responses became less certain. Hemisections performed in five animals at the C4 level following dorsal column section had the same results.

In two animals which had C4 hemisection and two animals with a T10 hemisection a further transection was made interrupting the remaining ventral funiculus contralateral to the recording electrode. This was without effect on the pattern of unit activity in the SC. The only portion of the spinal cord in these four animals which was now intact was one dorsolateral quadrant contralateral to the recording micro-electrode. In one of these four animals, a further section was made interrupting the dorsolateral funiculus but sparing part of the lateral funiculus. This section (within the time course of the experiment) irreversibly abolished all unit responses in the SC to hind limb muscle afferent stimulation.

To confirm the importance of the dorsolateral funiculi in conduction of hind limb afferents to the SC, both dorsolateral funiculi were transected in two animals with C4 dorsal column transections. This procedure sharply

reduced the intensity of unit response in the SC, but did not completely abolish it.

These experiments strongly suggest that the spinal pathways from the hind legs travel predominantly in the dorsolateral and lateral funiculi contralateral to the recording electrode. Fig. 5 is a diagram showing the major anatomical characteristics of a pathway which would account for the experimental data.

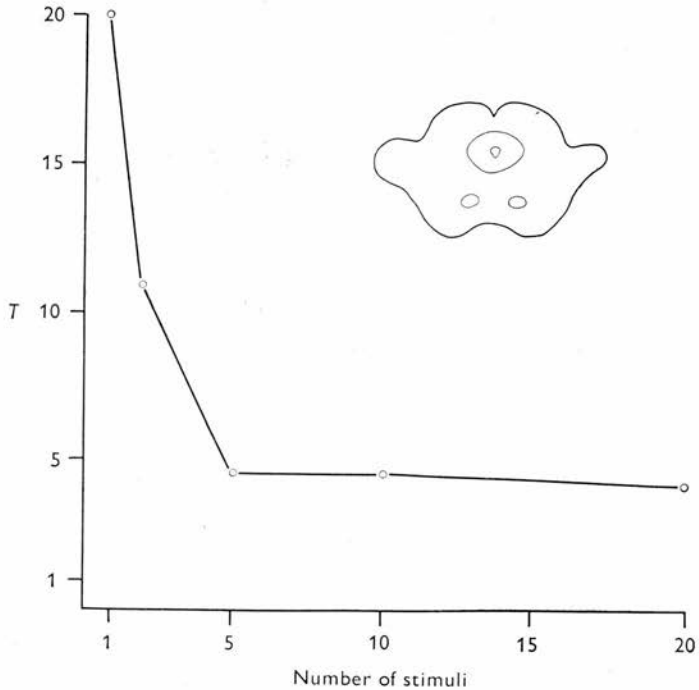

Fig. 3. Effect of stimulus train length on stimulus strength needed to elicit unit responses in the SC. Stimulation applied to FDL. *T*, stimulus strength required to elicit a threshold compound action potential on the sciatic nerve. Pulse frequency, 500/sec. Inset, diagrammatic cross-section of superior colliculi. Dot indicates the recording site.

Forelimb muscle afferent projections to the SC

Firing patterns

In thirty-five experiments 107 units were found in the SC which could be activated by stimulation of the DR nerve. The same three firing patterns followed DR nerve stimulation as followed hind limb muscle nerve stimulation and the anatomical location with respect to latency and firing pattern was also essentially the same. Paired firing was a more common

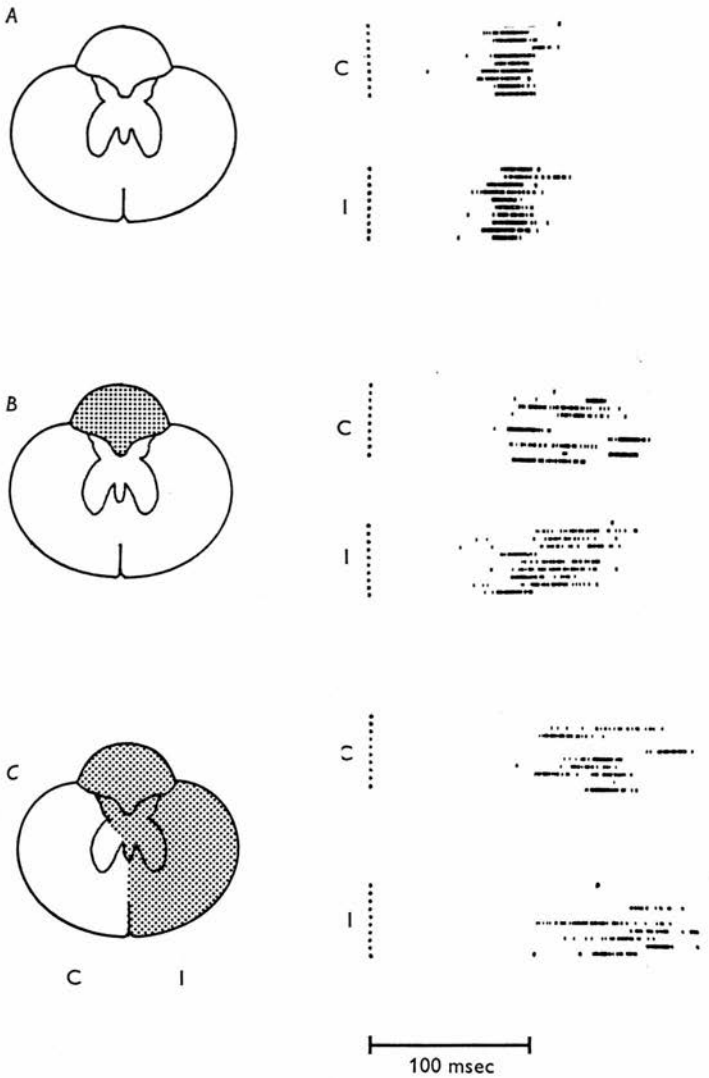

Fig. 4. Effect of partial cord transection on the pattern of unit discharge in the SC following stimulation of FDL ipsilateral and contralateral to the recording electrode. Each set of responses was obtained from ten consecutive stimulus trains (5 impulses/500 per sec) at 10 sec intervals. C, contralateral to recording electrode; I, ipsilateral to recording electrode. Each dot is caused by one unit spike. *A*, control before cord section; *B*, 1 hr after dorsal column section; *C*, 1 hr after hemisection of remaining cord ipsilateral to the recording electrode.

response following DR nerve stimulation than with stimulation of hind limb nerves, particularly when the contralateral nerve was stimulated. As with the hind leg projection short latency responses consistently followed stimulus presentation at 1/sec or less; long latency responses consistently followed stimulation intervals exceeding 4 sec.

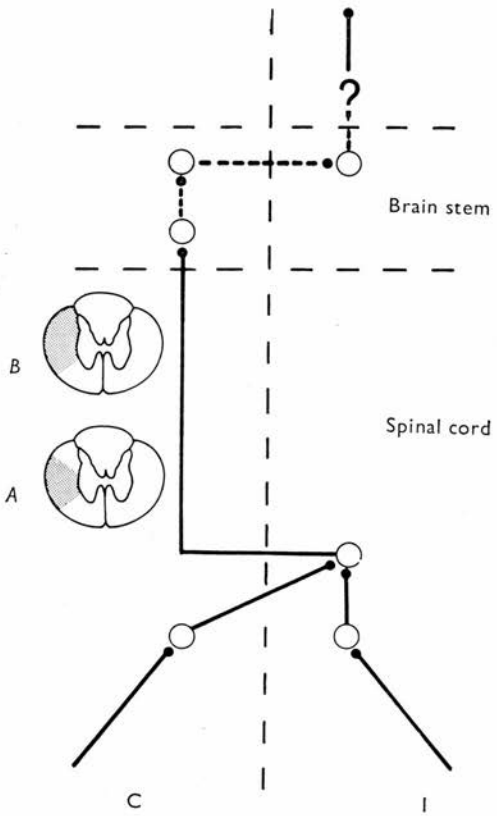

Fig. 5. Diagram to show probable spinal course of limb afferents to superior colliculus. *A*, hind limb ascending pathway. *B*, forelimb ascending pathway. The diagram shows convergence at spinal level and a supraspinal decussation characteristic of limb muscle afferent pathways to the SC. C, contralateral to recording electrode; I, ipsilateral to recording electrode.

The forelimb muscle afferent projections to the SC, like the hind limb limb muscle afferent projections, are bilateral. Eighty-one of the 100 units examined were excited by both deep radial nerves regardless of firing pattern exhibited by the unit or its anatomical location.

Stimulus thresholds of forelimb muscle afferent nerve projection to the SC

As with the hind leg nerves, the use of a stimulus train decreased the intensity of stimulation required to excite SC units but once train length (at 500/sec) exceeded five impulses there was no further decrease in threshold. Thresholds for units excited by DR nerve stimulation were lower than those for the hind leg nerves and ranged between 1 and 5T, with 50 % of the units responding at 2T or less and 20 % firing at 1·5T or less.

Spinal course of forelimb muscle afferent projections to the SC

Nine of the thirteen cats used to study hind limb spinal pathways were also used to study the forelimb pathway to the SC. Responses could be recorded from all animals from stimulation of both DR nerves and included short, paired, and long latency responses and were obtained at both low and high thresholds. Initially, the dorsal columns were transected at C4. This led to a dispersal of unit discharge of the type illustrated in Fig. 4. In five of the animals the cord was then hemisected ipsilateral to the recording electrode, leading to a further dispersal of unit discharge without interruption of conduction. In a sixth animal an incomplete ipsilateral hemisection was performed which spared the ventral funiculus. In this one animal there was an irreversible loss of response to stimulation of the contralateral DR nerve.

In two of the five animals with cord hemisections, the contralateral ventral funiculus was sectioned. This further section was without any effect on responses to forelimb stimulation. In one of these two animals, one dorsolateral funiculus was then sectioned, decreasing the response contralateral to the recording electrode. It is concluded from these experiments that the cervical course of the forelimb muscle afferent projection, like the hind leg projection lies mainly in the dorsolateral and lateral funiculi of the cord contralateral to the recording electrode.

Convergence between fore and hind limb projections and retinal afferents

Convergence between forelimb and hind limb muscle afferent projections and retinal afferents within the SC was examined in thirty experiments. Of seventy-seven units activated by a brief light flash 64 (83 %) were also excited by stimulation of a forelimb muscle nerve. Twenty-nine units were found that were not excited by the light flash, but which were excited by stimulation of either of the DR nerves. Convergence between visually excited units and hind leg muscle afferents was substantial, but not as great as convergence with forelimb nerves and only 33 of 54 (61 %) visually activated units responded to MG or FDL stimulation. Twenty-one units were found in these experiments that could be activated by a flash but

not by limb stimulation, and 18 units were found which were activated by hind limb muscle afferent stimulation alone.

In twenty-five experiments convergence between the fore and hind limb projections was examined. Twenty-five of thirty-one units (81 %) tested received input from all four limbs and a further three units were excited by stimulation of nerves in more than one limb.

Units responding to limb muscle nerve stimulation are less common in the SC than are those responding to visual stimuli or to stimulation of neck or extraocular muscle afferents. This is summarized in Table 2 which provides quantitative information concerning the efficacy of various afferents in eliciting unit discharge in the SC. Data from experiments on extraocular and neck muscle afferents (Abrahams & Rose, 1975) has been included. Almost exactly 75 % of all cells encountered in experiments on muscle afferents are visually excitable. The ratio of cells excited by muscle afferent stimulation over visually excited cells (N_m/N_v) averages 1·17 and only falls below unity (0·95) for cells excited by hind leg muscle afferents (Table 2).

TABLE 2. Efficacy of visual and muscle afferent stimulation in eliciting unit discharges in the superior colliculus (this table includes data from Abrahams & Rose, 1975)

Muscle afferent input	N_t	N_v	N_m	N_v/N_t	N_m/N_v
Extraocular	93	68	91	0·73	1·34
Neck	145	107	124	0·74	1·16
Forelimb	106	77	93	0·73	1·21
Hind limb	72	54	51	0·75	0·95

N_t = total number of units.
N_v = visually excited.
N_m = muscle afferent excited.

DISCUSSION

The SC of the cat has been previously shown to receive muscle afferent input from extraocular muscles (Cooper & Fillenz, 1955; Abrahams & Rose, 1972), and neck muscles (Abrahams & Rose, 1975). To this list of muscle afferent projections must be added projections from both fore and hind limbs. Numerically somewhat weaker than either the extraocular or neck muscle afferent system, the projection is still considerable and shows many characteristics in common with the neck muscle projection. The same firing patterns have been observed, and the distribution within the SC and tegmentum based on response latency is similar to that seen for neck muscle afferents with short latency responses predominating in deep layers, long latency in superficial layers (Abrahams & Rose, 1975).

As with the neck muscle projection, the projection largely originates with fibres of Group II and III thresholds. Group I fibres in the hind leg of the cat are exclusively activated at stimulus strengths from 1 to 1·5T (Perl, 1962). The thresholds in the hind leg in these experiments ranged from 2 to 20T, a stimulus strength adequate to excite both Group II and Group III fibres (Eccles & Lundberg, 1959). There is however a distinct Group I component in the forelimb projection and about 20% of units respond to DR nerve stimulation as a result of Group I fibre activation. The Group I fibres probably include some afferents from joint receptors as well as spindle primaries (Burgess & Clark, 1969).

The afferent projection from limb muscle nerves is characterized by a considerable convergence. Not only do many of the units respond to both visual and muscle afferent stimuli, but they also usually respond to stimuli to all four limbs. Such a degree of convergence could be due to a multiplicity of muscle afferent pathways projecting to the SC. However, most of the characteristics of the muscle afferent projection coincide with those of a single pathway, the lateral funiculus climbing fibre pathway (LF-CF-SCP) described by Larson, Miller & Oscarsson (1969). This pathway takes its origin in all four limbs and is activated by flexor reflex afferents (FRA). Convergence within the pathway is considerable, and its spinal course closely coincides with that reported here, occupying the dorsal quadrant, with bilateral inputs, and a supraspinal decussation. The conduction time in this pathway to the cerebellum is relatively slow, 17–27 msec from fore leg, 23–27 msec from hind leg. Spinal conduction is fast and the slowing is thought to be due to multiple supraspinal synapses (Larson *et al.* 1969). A relay to the SC from an early medullary synapse would be adequate to account for the observed short latency responses to the SC. The long latency responses in the SC have to be explained on the existence of two intracerebral pathways from LF–CF–SCP to the SC.

Objections to considering the LF–CF–SCP as the limb muscle afferent pathway to the SC come from a consideration of the cutaneous projections to the SC. If convergence from FRA within the LF–CF–SCP occurs below the medulla and within the spinal cord then the pathway will carry cutaneous afferents as well as muscle afferents from all four limbs to the SC. Cutaneous projections to the SC are known to exist and units may be activated by stimuli applied to all four limbs and even the whole body surface (Jassik-Gerschenfeld, 1965, 1966; Abrahams & Falchetto, 1966; Straschill & Hoffman, 1969; Stein & Arigbede, 1972). These characteristics would accord with the characteristics of cutaneous projections through LF–CF–SCP but in her experiments, Jassik-Gerschenfeld (1966) reported that section of the ventral funiculi in the lower thoracic cord abolished the hind leg cutaneous afferent projection. Unless convergence between the

cutaneous and muscle afferents of the FRA of the LF–CF–SCP takes place above the level of the thoracic cord, the findings of Jassik-Gerschenfeld (1966) would exclude the LF–CF–SCP from projecting to the SC, and would suggest that spinal conduction to the SC is by separate cutaneous and muscle afferent systems occupying different locations within the spinal cord.

Dorsal column section and ipsilateral hemisections, substantially affected the regularity and nature of the SC unit response to limb muscle afferents. This effect could be due to the interruption of ascending pathways affecting conduction in systems relaying to the SC. Equally likely the disruption of firing patterns may be due to the interruption of descending pathways (including those in the dorsal column) which are known to affect excitability in interneurons giving rise to ascending pathways (Brown, Kirk & Martin, 1973; Oscarsson, 1973).

The role of ascending spinal pathways such as the LF–CF–SCP in which there is convergence not only within modalities, but also between modalities is difficult to interpret. Some experimental evidence suggests that cerebellar and cortical projection of such convergent pathways are concerned with the monitoring of interneurone excitability (Oscarsson, 1973). What role can we see for these convergent pathways to the SC? The SC is clearly involved in motor function of both head and eye. Chemical or electrical stimulation of the SC lead to head and eye movement (Apter, 1946; Hess, Burgi & Bucher, 1946) presumably due to activation of the oligosynaptic connexions from the SC to neck (Anderson, Yoshida & Wilson, 1971) and oculomotor (Carpenter, 1971) motoneurones. We have recently shown that the muscle afferent input from both extraocular and neck muscles terminates on a high percentage of the cells of origin of the tectospinal tract (Abrahams & Rose, 1975), and thus can exert effects on head movement. Whether limb muscle afferents make similar connexions is not yet known, but given the convergence of muscle afferents on units of the superior colliculus, it is highly likely. The inference would be that the extensive limb muscle afferent projections to the SC described here are in part, at least, linked to the head movement system.

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THE EFFECT OF PASSIVE EYE MOVEMENT ON UNIT DISCHARGE IN THE SUPERIOR COLLICULUS OF THE CAT

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SUMMARY

The effects of passive eye rotation on unit discharge in the superior colliculus has been examined in the chloralose anaesthetized cat. Providing that velocity exceeded 50°/sec, passive eye movement led to brief-burst discharges in the superior colliculus. This discharge occurred as the eye traversed a fixed point. The displacement of this point from the primary position has been defined as the displacement threshold for that unit. Displacement thresholds range from 3 to 30 ° and are consistent over a wide range of velocities but become larger at the velocity threshold for the unit. Conduction times to the superior colliculus ranged from 7 to 108 msec.

INTRODUCTION

The central connections of extraocular muscle receptors are extensive and project to pontine and mesencephalic sites, the superior colliculus and cerebellum[1,2,7,10,11,13,14,17]. Extraocular muscles are rich in receptors but the nature of the receptors varies from species to species. Muscle spindles are present in ungulates and primates, but in the cat no spindles are present and the receptors include a number of simpler forms of free nerve endings[12]. Recordings made from single fibres in cat extraocular muscle nerves show that the receptors function both as length and tension detectors[6,12]. The functional role of the receptors has been a matter of conjecture for many years and is still not understood. We have recently demonstrated that the projection to the superior colliculus from extraocular muscle afferents in the cat is very rich, and that more units may be activated by these receptors than by retinal stimulation[2]. The projection in part is concerned with head movements, for many of the cells in the

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superior colliculus excited by extraocular muscle afferents are cells of origin of the tectospinal tract[2]. The experiments now described were concerned with the nature of information conveyed in the projection to the superior colliculus from extraocular muscle receptors in the cat.

METHODS

Experiments were performed on 15 adult cats, weighing 2.5–3.8 kg, anaesthetized with chloralose (60 mg/kg, I.V.) after the induction of anaesthesia with ethyl chloride and ether. The cats were placed in a stereotaxic machine (La Precision Cinematographique, Asnieres, France), with their visual fields unobstructed. Just prior to electrical recording the animals were paralyzed with gallamine triethiodide (Flaxedil®, Poulenc). The animal was then artificially ventilated and a bilateral pneumothorax created. The temperature of the animal was maintained between 37 and 39 °C using a heated operating table.

One eye, contralateral to the superior colliculus from which unit recordings were to be made, was protected from drying with a conventional contact lens. The other eye was prepared for passive movement of extraocular muscles by the technique of Fuchs and Kornhuber[14], and occluded with an opaque black contact lens. After appropriate bone removal a ligature was looped under the insertion of the lateral rectus muscle of the occluded eye, led forward around a pulley located in front of the cat, and attached to a Ling-Altec Vibrator (model 203) so that movement led to stretch of the lateral rectus. A ramp generator controlled the eye displacement and the eye was allowed to return passively to its rest position. The ramp generator was designed to allow the magnitude and velocity of eye movement to be controlled independently. Magnitude of movement could be varied from 0 to 30 ° and velocity could be varied from 0 to 750 °/sec. It was also possible to start the movement up to 30 ° nasal to the primary position. Movement was monitored with a length transducer (Hewlett-Packard type 24 DCDT-250) attached to the moving element of the vibrator. The transducer output was displayed on a digital voltmeter and a storage oscilloscope to give a record of both initial position and induced deflection. Movements are expressed in degrees. To convert the transducer length to angular rotation, the globe diameter was assumed to be 19 mm when 1 mm = 6 ° (see ref. 9).

Unit activity within the superior colliculus was recorded with tungsten microelectrodes (Haer, type 25-10-3) introduced stereotaxically through a trephine hole over the occipital cortex. Spontaneous activity was rare in the superior colliculus, so, as the microelectrode was slowly advanced into the superior colliculus units were activated by a 2-msec flash of light to the non-occluded eye[2] or by electrical stimulation of one of the nerves to the neck muscles. It proved difficult to isolate single units in the superficial layers of the superior colliculus, and some of the recordings made there were from multi-unit preparations. Each unit or multi-unit preparation encountered was tested for its responsiveness to passive eye rotation. Upon completion of each electrode penetration the deepest position was marked by a small lesion made by passing 50–100 μA through the recording electrode for 1–2 sec. The amplification and display

techniques were conventional. In most experiments, unit spikes were converted into standardized pulses using an amplitude analyser (Haer) and successive sweeps were stored in one frame of a storage oscilloscope (Tektronix D13) with the aid of a raster generator (W-P Instruments).

At the end of each experiment, the brain was perfused *in situ* with normal saline and then with 25% formaldehyde in saline. The location of each recording site in the midbrain was established from serial frontal sections, 30 μm in thickness, stained for fibres and cells[16]. Units within the superior colliculus were assigned to either the superficial, intermediate or deep layers of the superior colliculus using the anatomical criteria established by Cajal[8], Gordon[15] and Szekely[23]. Since the colliculotegmental border is not clearly defined in the cat[19], units which were ventral to a line extending horizontally from the lateral extent of the central grey were arbitrarily assigned to the tegmentum.

Correlation coefficients were calculated by the least squares method.

RESULTS

The responses of 57 well isolated single units to passive rotation of the eye have been examined. Forty-five (79%) of the units were found in the superficial, intermediate and deep layers of the superior colliculus. The remaining 13 units were located in the tegmentum ventral to the superior colliculus. Sixteen multi-unit recordings were also made in the superficial layers of the superior colliculus. Data from a further 7 multi-unit responses in other parts of the superior colliculus have also been used.

All movements of the eye were made in a nasal direction. Constant velocity nasal rotation of the eye provoked a brief burst of a few impulses only (Fig. 1), at all collicular and tegmental recording sites. Latencies of response were long and dependent on the velocity of eye rotation. For a 30° rotation at the maximum velocity employed (750°/sec), latencies from the commencement of rotation to the recording of discharge

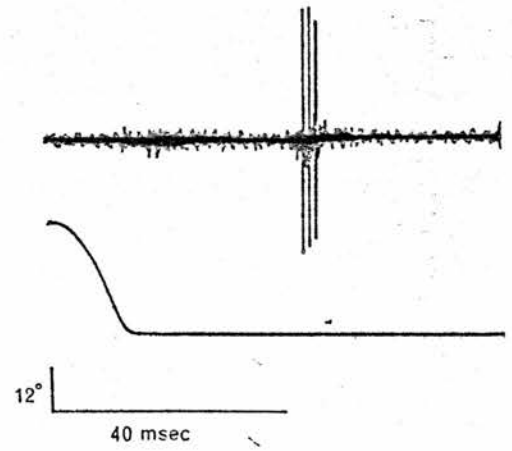

Fig. 1. Relationship of eye rotation and unit discharge in the superior colliculus. Top, unit discharge; bottom, transducer output. Rotation of 24° at 600°/sec completed in 40 msec.

ranged from 25 to 170 msec, with 61 of 80 (76%) response latencies greater than 40 msec. A 30° rotation at 750°/sec is complete within 40 msec so that unit discharge within the superior colliculus frequently occurs after eye movement is completed.

We have previously remarked on the rapid habituation of unit responses in the superior colliculus following electrical excitation of extraocular muscle nerves[2]. Habituation was equally pronounced when eye movement was used as a stimulus. A second attempt to elicit unit discharge was often unsuccessful if the interval between the two stimuli was too short. Responses could only be reliably recorded from the superior colliculus if the interval between successive rotations exceeded 10 sec.

Effect of magnitude of eye rotation on unit response

In a series of experiments the velocity of eye rotation was kept constant at 750°/sec. The velocity of 750°/sec was chosen as it is well above the upper limits of saccadic velocity for the cat eye[9,21,22], and thus should excite all movement-sensitive receptors. The eye was then rotated from the primary position to a position 30° nasal. In a series of measurements, the movement was reduced by 3° steps to a minimum rotation of 3°. Invariably, the discharge pattern was a brief burst and was unaltered as the magnitude of eye rotation decreased until rotation fell below a critical value. At this value, eye rotation no longer led to unit discharge. If the magnitude of eye rotation was now increased unit discharge to movement reappeared as soon as the critical value was exceeded. The minimum eye rotation necessary to elicit response in any given unit was a constant for that unit which we have defined as the displacement threshold. Any eye rotation at 750°/sec, whose magnitude was greater than the displacement threshold, produced the same discharge pattern. A wide range of displacement thresholds was found for units in the superior colliculus ranging from less than 6° to more than 24°. Fig. 2 shows the distribution of displacement thresholds mea-

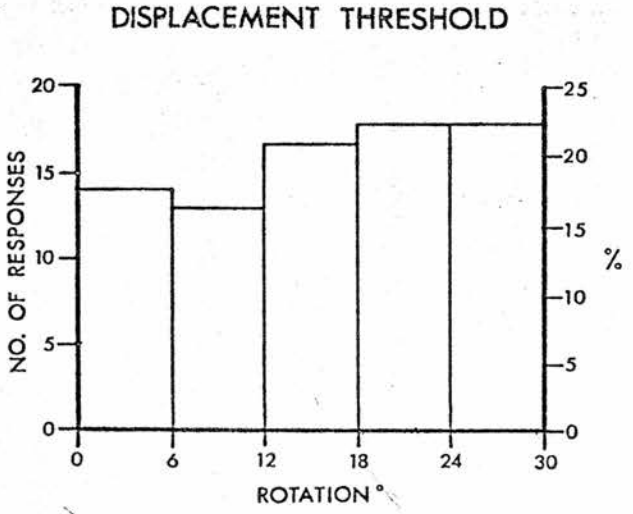

Fig. 2. Histogram illustrating the distribution of displacement thresholds of 57 isolated units and 23 multi-unit preparations recorded in the superior colliculus and adjacent tegmentum.

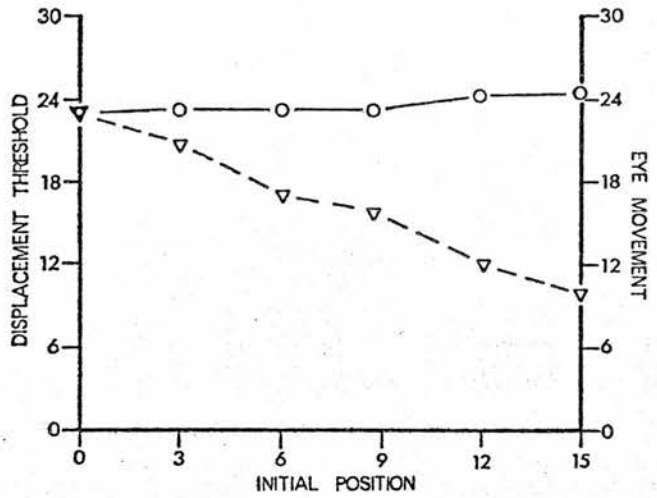

Fig. 3. The effect of displacing initial eye position in a nasal direction on the amplitude of constant velocity movement producing unit discharge in the superior colliculus. ▽ – – – ▽: actual movement necessary to produce unit discharge from the range of initial nasal displacements indicated on the abscissa. The displacement needed reduces as the initial position is moved nasally. ○ – – – ○: displacement from the primary position which initiates unit discharge. This value is obtained by adding the initial displacement to the movement, so that the value given is the actual displacement from the primary position at which unit discharge is initiated in the superior colliculus.

sured at 750°/sec. It can be seen that more than 75% of the units were excited by eye movement smaller than 24°.

Experiments were performed on 10 units with large displacement thresholds (> 20°) to examine the effect of altering initial eye position on the displacement threshold. Displacement threshold was first determined from the primary position at a velocity of 750°/sec. The eye was then subjected to a series of rotations each of which was initiated from a progressively more nasal position and the displacement necessary to elicit unit discharge established from each new initial position. These experiments established that when a unit has a large displacement threshold, unit discharge is not normally determined by the magnitude of passive rotation but occurs when the eye passes a position that is fixed with respect to the primary position (Fig. 3). The triangles in Fig. 3 show the reduction in actual movement necessary to initiate discharge as the eye is progressively moved closer to the displacement threshold. This may not be a characteristic of units with small displacement thresholds. Because of the design of the experiments just described, units with small displacement thresholds were not normally examined. However, on one occasion the relationship between initial eye position and displacement threshold was determined for a unit with a displacement threshold of only 5°. This unit did not fire at a fixed position within the orbit, but to any nasal movement from 4.5° to 3° depending on initial position. At an initial nasal deviation of 12°, a further nasal displacement of 3° was necessary for excitation. With an initial nasal displacement of 3°, a further nasal displacement of 4.5° was necessary for excitation.

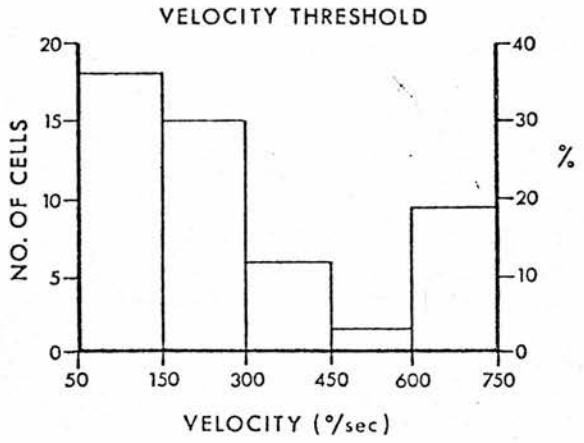

Fig. 4. Velocity thresholds of 50 units in superior colliculus and underlying tegmentum.

Effect of velocity of eye rotation on unit response

Velocity of movement itself can affect unit discharge in the superior colliculus. The nature of this effect was examined in experiments on 50 units. The eye was rotated a constant 30° at velocities ranging from 50°/sec to 750°/sec. These experiments showed that a velocity threshold exists, but discharge is independent of the velocity of rotation until velocity falls below a critical value. The lowest velocity of eye movement at which unit response could be recorded we have defined as the velocity threshold for that unit. Velocity thresholds for 50 units are plotted in Fig. 4. Most units (78%) had velocity thresholds of between 50°/sec and 450°/sec. Two units responded between 450°/sec and 600°/sec and 9 units (18%) required velocities in excess of 600°/sec for their excitation. Experiments were performed on 9 units to see whether displacement

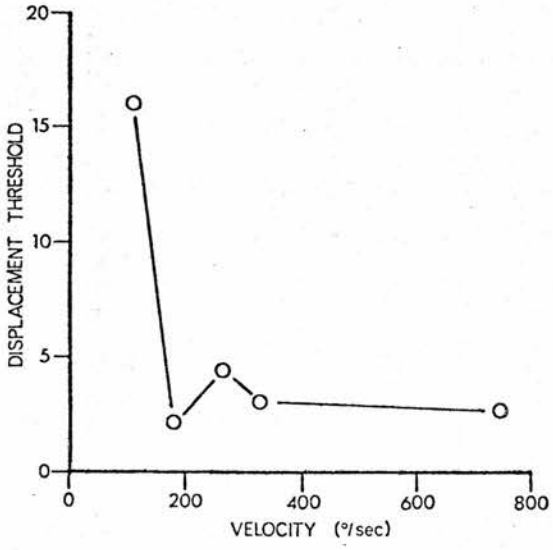

Fig. 5. The effect of displacement velocity on displacement threshold of unit in the superior colliculus.

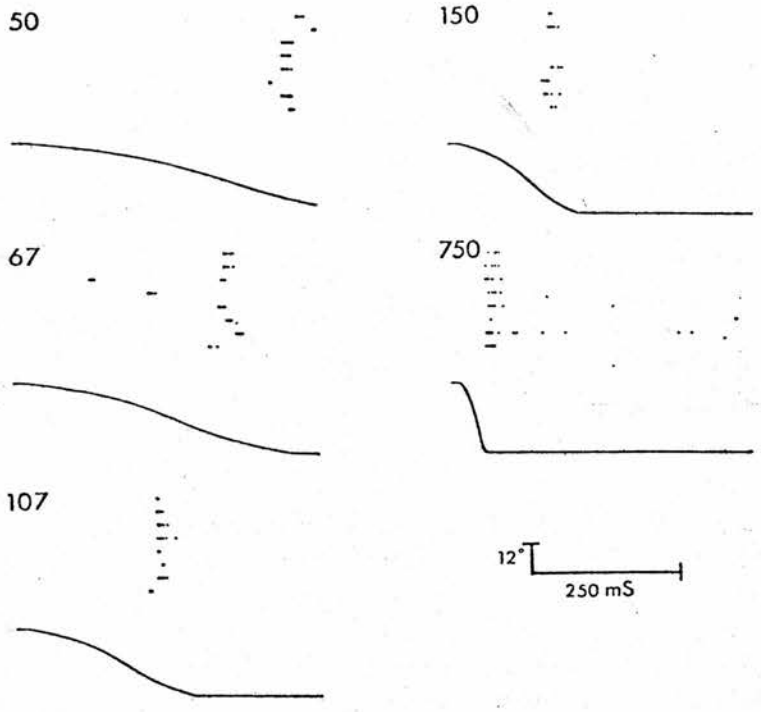

Fig. 6. Discharges from unit in the superior colliculus to 8 sequential 30° nasal rotations. Velocities indicated in degrees/sec. Interval between rotations, 10 sec.

threshold is dependent on eye velocity. As can be seen in Fig. 5, except at velocities close to threshold displacement threshold is independent of velocity. In the experiment illustrated the displacement threshold remained relatively constant as velocity decreased from 750° to 200°/sec, but as the velocity threshold of the unit of 100°/sec was approached the displacement threshold increased sharply.

The existence of a fixed displacement threshold independent of velocity was

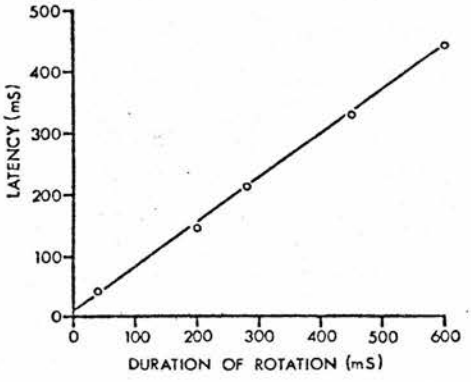

Fig. 7. Data from Fig. 6 replotted to show the relationship of response latency to duration of rotation. The ordinate intercept gives the central conduction time to the superior colliculus. $r = 0.999$, latency = 7.1 msec.

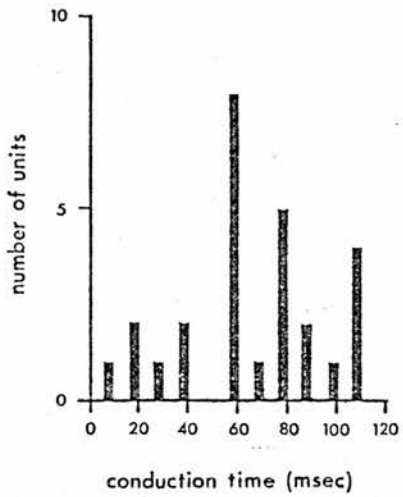

Fig. 8. Ordinate intercepts (central conduction time) of 27 units tested at a range of displacement velocities. Data from these units where correlation coefficients < 0.05.

emphasized in experiments in which the latency of collicular response was measured during fixed displacements at a range of velocities. The overall latency from the onset of movement to the onset of unit discharge determined in these experiments is dependent not only on the central conduction times, but also the displacement velocity (Fig. 6). A slow movement will take longer to arrive at the displacement threshold than a fast movement. The stability of the displacement threshold is reflected in the linearity of the slope when response latency is plotted against the duration of eye rotation (Fig. 7). In 27 of 33 units tested the linearity of the relationship was such that the correlation coefficient was significant at the 0.05% level. Lines plotted in this way have an ordinate intercept and this is the conduction time to the superior colliculus, for the value of the intercept represents the latency of response to an infinitely fast rotation. The range of central conduction times estimated in this way was from 7 to 108 msec (Fig. 8), values which are essentially the same as the conduction time to the superior colliculus following electrical excitation of extraocular muscle nerves[2].

DISCUSSION

In her original experiments on extraocular muscle stretch, Fillenz[13] described only a few responses in the superior colliculus, and those that were found were confined to the deep layers. From our experiments, it is clear that passive eye movement can lead to a discharge of cells in all layers in the superior colliculus of the cat as well as the underlying tegmentum, providing that both velocity and amplitude of movement are adequate. At no time in our experiments did we observe the sustained discharges that Fillenz[13] saw in the mesencephalic nucleus of the fifth nerve and in the deep collicular layers in barbiturate anaesthetized and decerebrate cats. The pattern of unit discharge that was invariably observed in our experiments was a brief-burst discharge similar to one pattern described by Fillenz[13].

The characteristics of passive muscle stretch that excite the receptors of the inferior oblique muscle of cat have been examined by Cooper and Fillenz[12] and by Bach-y-Rita and Ito[6]. Both groups of workers report that some receptors fire at rest, but the results of Bach-y-Rita and Ito[6] show that such units constitute a small minority, and that the majority of receptors (48 of 52) are dynamic receptors and only discharge to muscle length changes. The eye movement used in our experiments should mainly excite receptors in the lateral rectus muscle. Assuming that the receptors in the lateral rectus muscle have properties similar to those in the inferior oblique, then the dynamic nature of response in the superior colliculus is largely a reflection of receptor characteristics.

When the data contained in Fig. 9 in the article by Bach-y-Rita and Ito[6] is replotted it is apparent then that in their experiments a linear relation exists between the time of onset of receptor discharge and the duration of the pull. Thus, there is a threshold stretch for receptor activation and the displacement threshold that we have observed in the superior colliculus may be a reflection of this receptor property. The relationship between passive eye movement and unit discharge in the superior collicular cells reported are, however, not entirely a replication of the sensory properties of extraocular muscle receptors. Two major differences exist between the responses of superior collicular cells described here and the responses of extraocular muscle stretch receptors described by Bach-y-Rita and Ito[6]. Receptors in the inferior oblique muscle increase their firing frequency as the velocity of stretch increases[6], but, in the superior colliculus, once the critical velocity is exceeded, the pattern of discharge recorded in the superior colliculus is fixed and independent of the velocity of eye movement. Bach-y-Rita and Ito[6] also found that the number of impulses and frequency of discharge to a constant stretch increased as initial muscle length was increased. Thus, the firing pattern of the receptor is sensitive to initial position unlike the firing pattern of units in the superior colliculus. It is unfortunate that in our experiments only one unit with a small displacement was tested for initial position effects. Small displacement thresholds of 6° or less were found in 18% of the units. If the one small displacement threshold unit is typical, then it is possible that while the fixed displacement threshold is a characteristic of almost all collicular units, it is not an invariant pattern.

Recently, we showed that almost 60% of cells of origin of the tectospinal tract in the superior colliculus are excited by electrical stimulation of extraocular muscle afferent nerves[2]. It seems reasonable to conclude that a significant percentage of units examined in this study are cells of origin of the tectospinal tract and thus make disynaptic connection with neck muscle motoneurones[4]. Output to neck muscle motoneurones is thus likely to be influenced, in part, by movement of the eye in the orbit.

The data now obtained shows that little influence is likely to be exerted at low eye velocities, or when the movements are small. It is only when large movements are executed at saccadic velocities that the eye movement is likely to affect neck motoneurones. Then, because of the wide range of conduction times to the superior colliculus, an eye movement can set up a temporally dispersed bombardment of tectospinal cells which will greatly outlast the eye movement. In the cat eye movements from temporal to nasal extreme of 90° are possible[9] although 40% appears to be the

normal range[22]. The minimum execution time of a 90° movement at a saccadic velocity of 600°/sec is 150 msec, and it is only in these extreme movements that most of the discharge will occur in the superior colliculus during the eye movement. At more normal levels of saccadic eye velocity of 300°/sec and for more restricted movements precise information will be transmitted to the superior colliculus only after the eye movement has been completed and perhaps even after a new eye movement has commenced. It should be noted that the effects of velocity are such that while most units are activated at velocities appropriate to saccadic movements in the cat, a proportion only fire at velocities which probably never normally occur. Because extraocular muscle afferents have the most abundant projection to the superior colliculus[2], exceeding retinal and other muscle afferent projections[3], it is likely that these units are activated by such extraocular afferents. However, the technique of eye movement chosen, while it avoids the problem of limited receptor viability that occurs after enucleation[13] does mean that other structures might be contributing to responses recorded in the superior colliculus.

An extraordinary habituation has been demonstrated in the afferent system to the superior colliculus. This is not a receptor property for a similar habituation was observed when the afferent nerves were electrically excited[2]. The long latency together with the adaptation phenomena make it very difficult to propose a role for the system taking origin in the extraocular muscles and projecting to neck motoneurones. It is possible that to a degree these characteristics are imposed by the conditions of anaesthesia. However, chloralose does have some advantages as an anaesthetic in this situation, for it enables the long-latency responses of the superior colliculus to be demonstrated[2], and it is known that brain responses to extraocular muscle stretch under chloralose are similar to those observed in the decerebrate cat[13].

Few data exist on the relationships between eye movement and unit discharge in the superior colliculus of the conscious cat. Relationships between spontaneous eye movement and unit discharge have been examined in the non-anaesthetized *encéphale isolé* by Straschill and Hoffman[20] and by Arduini *et al.*[5]. Straschill and Hoffman[20] reported that 10% of the units that they observed fired in synchrony with spontaneous movement during total darkness. Discharge could occur prior to or during the movement, or 50–100 msec after the onset of movement. Neither the velocity nor the magnitude of movements was reported in their experiments. Movement was recorded in the experiments of Arduini *et al.*[5] by a modified Robinson[18] technique permitting accurate resolution of movement. They report that eye movement in the *encéphale isolé* during 'sleep' has excursions of from 4 to 5° and peak velocities of 1°30′/sec. During 'arousal' the excursions were reduced below 1°, but velocities increased to 3–20°/sec. In this situation Arduini *et al.*[5] claimed that more than 80% of units in the superior colliculus had activity correlated with the small eye movements that they observed. Usually unit discharge preceded eye movement from a few to 100 msec. It seems unlikely that the firing patterns that were observed in the superior colliculus in either of these reports could be due to activation of extraocular muscle receptors. Our data would suggest that spontaneous eye movement in the *encéphale isolé* never achieves either sufficient velocity or amplitude to activate units in the superior colli-

culus. The movements are also below threshold for extraocular muscle receptors[6]. The data so far obtained in the *encéphale isolé* must have sources other than extraocular muscle receptors and may signal events leading up to movement and connected with movement, but not information arising from the extraocular muscle receptors

ACKNOWLEDGEMENT

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Velocity and displacement characteristics of passive eye movements which initiate unit discharge in the superior colliculus

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The relationships between the magnitude and velocity of eye movement that lead to unit discharge in the superior colliculus have been examined. Experiments were performed on cats anaesthetized in chloralose. One eye was occluded and a ligature was looped around the insertion of its lateral rectus muscle. The ligature was attached to a shaker (Ling-Altec, Model 203) so that the eye could be moved in a nasal direction under the control of a function generator. Movement led to a unit discharge when the eye passed a fixed position, and this fixed position, which we have termed a displacement threshold, was characteristic for each unit. In tests conducted at a velocity of 750°/sec displacement thresholds were found to be evenly distributed in the range of 6–30°.

The displacement threshold is dependent on the velocity of eye movement. Units have a velocity threshold, and movements below this velocity do not cause discharge. The majority of velocity thresholds lay in the range of 50–450°/sec. At velocities above the velocity threshold, displacement threshold is constant. When velocity approaches the velocity threshold of that unit, displacement threshold abruptly increases.

The latency between the onset of eye movement and superior colliculus discharge ranges from 10 to 110 msec. Since both velocity and displacement thresholds are relatively high, the appearance of unit discharge in the superior colliculus will only occur at relatively high velocities, and except for exceptionally large movements will frequently occur after the eye movement is completed.

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Absence of monosynaptic reflex in dorsal neck muscles of the cat

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The monosynaptic reflex originating from receptors in muscle spindles is usually regarded as part of a fundamental servo mechanism controlling muscle length[6]. The monosynaptic reflex does not exist in the oculomotor muscles[3,7–9], and we now provide experimental evidence that a monosynaptic reflex is not present in the dorsal muscles of the neck of the cat despite the fact that monosynaptic connections exist[11]. These muscles, which serve to elevate and turn the head, have an exceptionally dense spindle content in man, rat and cat[1,5,10].

The initial observations were made in 5 cats anaesthetized with chloralose (60 mg/kg, i.v.) in which the central cut end of C2, C3 or C4 dorsal roots were stimulated. Monosynaptic reflexes recorded electrically from nerves to the muscles complexus, biventer cervicis or splenius should have a latency of approximately 2 msec. Those reflexes recorded had a latency of 16–20 msec.

In experiments on a further 11 chloralose-anaesthetized cats extracellular recordings were made with tungsten microelectrodes from motoneurones of complexus, biventer cervicis and splenius. The C2 and C3 dorsal roots were cut and their central ends placed on stimulating electrodes for orthodromic stimulation (Fig. 1). Unit antidromic potentials were elicited by stimulation of the appropriate muscle nerve and were regarded as being from motoneurones if (a) they had a short constant latency (0.3–1.4 msec), and (b) if they were all-or-none. Ninety-six motoneurones were identified in this way. Only one could be orthodromically activated by single or paired stimuli at a latency of 2 msec or less even though the strength of the orthodromic stimulus was sufficient to excite all Gp I and II fibres. In the lumbosacral cord, 100% of motoneurones can be fired by orthodromic stimulation at the peak of post-tetanic potentiation[4]. To see if a similar procedure could elicit monosynaptic reflexes in the upper cervical cord, 21 units were tested for orthodromic responsiveness following 15 sec of dorsal root stimulation at 300/sec. In no instance did this provoke a monosynaptic discharge. Orthodromic stimulus was not particularly effective in exciting neck motoneurones and the firing which was observed in 26 of the 96 units tested occurred after a 7–25 msec latency.

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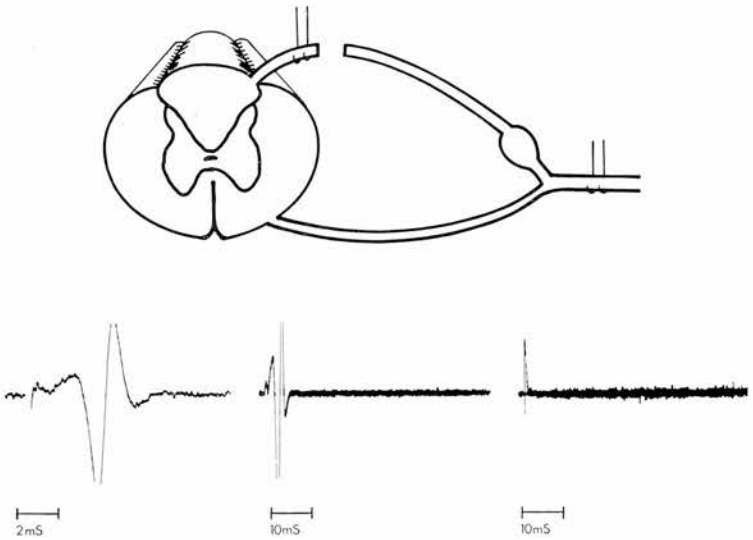

Fig. 1. Top, arrangement of stimulating electrodes for orthodromic and antidromic excitation of motoneurones. Bottom left, antidromic action potential; middle, same, slow sweep; right, orthodromic stimulation showing stimulus artefact and no orthodromic firing.

It is concluded that there is no significant monosynaptic reflex in the dorsal neck muscles of the cat. Whatever the role of the dense spindle accumulation in these muscles, there can be no simple servo loop of the kind that operates in respiratory and locomotor muscles. Presumably the role of neck muscle spindles is confined to longer control loops such as those operating through the tectospinal tract[2].

This research was supported by the Medical Research Council of Canada.

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Can. Physiol., 6, 1975, p. 50.

50

Spinal and medullar projections of afferents from dorsal neck muscles.
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A number of anatomical studies have been made which suggest that primary afferents from the neck terminate segmentally and in the cuneate nucleus, the external cuneate nucleus, the intermediate nucleus of Cajal and in the region of the spinal nucleus of the trigeminal nerve. Electrophysiological examination has now been made of the spinal and medullary course of primary afferents that originate in neck muscles. Bundles of Gp I, II and III fibres ascend in the lateral margin of the dorsal columns. Many of these fibres travel in the dorsolateral periphery of the medulla and turn ventrally at the level of the external cuneate nucleus. Primary afferents may then be traced into the spinal nucleus of the trigeminal nerve where second order cells have also been identified. These second order cells appear to be exclusively innervated from muscle afferents and do not appear to have facial fields. The only other substantial relay that has been found from neck muscle afferents is to the ventral part of the cuneate nucleus. We have been unable to trace primary muscle afferents into the grey matter of the upper cervical spinal segments, nor have we been able to elicit monosynaptic reflexes from upper cervical segments.

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48

Muscle spindle complexes in the dorsal muscles of the cat neck.
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It has long been known that muscle spindles may exist in conjunctive forms. These include relationships in which spindles overlap physically (paired units), share intrafusal fibres (tandem units), or share capsular components (parallel units). Most dorsal muscles of the cat neck are characterized by very high spindle densities, in which conjunctive complexes have proved to be so common that in some muscles, the majority of spindles exist in conjunctive forms. Spindle complexes are most commonly composed of 2 or 3 conjoined spindles but some complexes are much larger, containing up to 6 identifiable spindle units and supporting over 20 primary endings. Certain of these complexes are composed of units linked in tandem, (in one instance by the same intrafusal fibre which ran sequentially through 5 units), while others contain all 3 linkage forms in their structure. Spindles were also frequently seen in close association (dyad) with Golgi tendon organs.

These arrangements are likely to influence the sensory functions of the spindle by (a) modifying the forces exerted on individual spindle receptors and (b) by allowing individual intrafusal fibres to exert effects on more than one spindle.

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955

THE DISTRIBUTION AND MORPHOLOGY OF MUSCLE SPINDLES IN DORSAL NECK MUSCLES OF THE CAT. F. Richmond* and V.C. Abrahams. Department of Physiology, Queen's University, Kingston, Ontario, Canada. K7L 3N6.

The morphology and distribution of spindles has been examined in 5 pairs of dorsal muscles that insert into the lamboidal crest. Four muscles had very high spindle densities ranging from 45 to 110 spindles/g., with individual muscles containing as many as 254 spindles. The fifth muscle, occipitoscapularis, had comparatively low spindle densities of 13-19 spindles/g. Some of the muscles are characterized by large numbers of conjunctive spindle forms, with more than 50% of their spindles existing in paired, tandem or parallel complexes. While such complexes are most frequently composed of 2-3 spindles, complexes of 4 to 6 spindles in a variety of arrangements have been observed. Spindles were also found closely associated with Golgi tendon organs, in some cases with apparent capsular contiguity. Muscles had characteristic distributions of spindles, and certain muscles tended to have characteristic contents of conjunctive spindle forms. These morphological findings suggest that the nature, distribution and density of spindles may be characteristic to each muscle, and that the functional roles of muscle spindles may vary from one muscle to another. (Supported by M.R.C. of Canada.)

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Morphology and Enzyme Histochemistry of Dorsal Muscles of the Cat Neck

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WHILE THERE HAS BEEN a considerable body of investigation on descending systems that control neck motoneurons (2–4, 19–22), there has been virtually no investigation of the muscles which are innervated by these motoneurons and which serve primarily to move the head. A clear understanding of the physiology of these muscles is fundamental to the study of the head-movement system. A preliminary histochemical study has been made of the dorsal muscles that are most immediately responsible for head movement (1), and the histochemical profiles obtained at that time suggested that the muscles might fall into at least three functional categories. It was also found that the innervation of the three larger muscles was unusual. Each muscle received innervation from three to five cervical roots, but instead of the individual nerves fusing into a single motor nerve, each branch preserved its identity and entered the muscle at a different level.

We now give a more detailed description of these muscles and their histochemistry. It has been found that the arrangement of multiple motor nerves is associated with unusual arrangements of long and short muscle fibers, which may form the basis of unusual physiological properties of the muscle. It has also been found that the muscle fibers are composed of the same three histochemically identifiable types as those recently described in the cat hindlimb (5).

METHODS

Histochemical preparation

The neck muscles, occipitoscapularis, splenius, biventer cervicis, complexus, and rectus capitis major, were obtained from six cats weighing from 2.2 to 3.5 kg, anesthetized with a single intraperitoneal injection of diallylbarbituric acid and urethan (Dial, locally synthetized) 0.5 ml/kg, or sodium pentobarbital (Nembutal, Abbott), 35 mg/kg. One 10-mm segment was removed from the belly of each of the muscles at the level of the C_2 and C_3 vertebrae, immediately frozen onto a cryostat chuck with Freon, and transferred to liquid Freon. Transverse serial sections were then cut at 8 μm on a cryostat, mounted on slides at room temperature, and thawed. Sets of five serial sections were cut, and one of each set stained with the following five histochemical techniques to identify ATPase (acid and alkali stable) and oxidative enzyme activity. *1*) The Ca^{++} dependent method for ATPase without preincubation (13). *2*) The ATPase method preceded by formalin fixation and preincubation at pH 10.4 for 15 min (10). *3*) The ATPase method with preincubation in an acetate buffer at pH 4.35 for 10 min (10). *4*) A nitro blue tetrazolium method for reduced diphosphopyridine nucleotide (DPNH) diaphorase (14). *5*) A nitro blue tetrazolium method for succinic dehydrogenase (SDH) (12).

To determine the histochemical characteristics of individual fibers, three fiber fasciculi were located from widely separated sites in each muscle. Approximately 10 fibers in each fasciculus were then examined in sections stained with the different methods using a Leitz comparison microscope. The results were independently checked by a second person.

Of the approximately 500 fibers examined in this way, all fell into one of three categories (Table 1). In order to establish the proportions of each fiber type it was not necessary to examine each fiber for its reactions to all the histochemical procedures. In most instances, muscle fibers could be typed from their reaction for ATPase following alkali preincubation (10). Most sections subjected to this procedure have fibers that stain with one of three gradations corresponding to each of the three separate fiber types. This gradation was sufficiently reliable to form the basis of classification for most fibers.

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TABLE 1. *Types of staining profiles observed in dorsal neck muscles.*

Stain	A	B	C
Myofibrillar ATPase	Dark	Light	Dark
Alkali preincubated ATPase	Dark	Light	Dark or moderate
Acid preincubated ATPase	Light or moderate	Dark	Light
SDH and DPNH	Light	Blue, granulated appearance, rosettelike under lower powers	Purple, linearly arranged often diffuse diformazan deposits; may be prominent subsarcolemmal activity

Column A corresponds to fibers labeled type A fibers in the histochemical nomenclature of Guth and Samaha (10) and Yellin and Guth (23) or fast fatigable (FF) fibers in the functional nomenclature of Burke et al. (5). Column B corresponds to type B (10, 23) or slow (5) fibers. Column C corresponds to type C (10, 23) or fast fatigue-resistant (FR) (5) fibers.

In experiments where the shade distinctions were not sufficiently clear for classification to be made, it was necessary to examine two serial sections, one stained for SDH and the other for ATPase (with alkali preincubation or without preincubation). The procedure relies on the fact that only one fiber type has a low SDH content (Table 1), and the number of these fibers in a section can easily be counted. A second fiber type has a low ATPase activity after alkali preincubation or when no preincubation was used (Table 1). The numbers of the second fiber type could thus be obtained from the second section.

The numbers of the third fiber type present in a section could be obtained by totaling the fibers in the section under examination and subtracting from this number the total number of fibers so far classified.

The estimate of fiber types in each muscle was based on an examination of from 900 to 1,100 fibers per muscle. Fiber types are not always randomly distributed within a muscle and, in order to minimize differences that might occur from a nonrandom distribution, counts were made on a band of muscle running across the muscle from its dorsal to its ventral surface. The validity of the sampling technique was evaluated by counting several such bands from the same muscle, each separated by a few millimeters from the other. It was found that differences in the proportions of any fiber type in the bands never exceed 5% and were usually within 1–2%. The relative content of each fiber type within the muscle was expressed as a percentage.

The anatomical descriptions of the muscles and their innervation are based on dissections from the above experiments and on dissections made on a further 20 cats in the course of other experiments. In addition, sections prepared for a histological study (15) were used to establish some of the details of fiber morphology.

Single-fiber dissections

Because of the multiple innervation of neck muscles it was possible that, like avian neck muscles (7–9), individual fibers might have multiple end plates.

To investigate this possibility, about 30 single muscle fibers were dissected from two each of the five muscles. Each muscle was divided longitudinally into 5-mm strips, which were fixed for 2–4 h in a medium composed of 50 ml 8% paraformaldehyde, 50 ml 0.3 M Na cacodylate buffer, 1 ml 0.73 M KCl, 1 g sucrose. The strips were further divided into finer bundles and fixed for at least 1 additional hour. The strips were rinsed gently in maleate buffer, then placed in incubating medium for the visualization of cholinesterase activity (11) for 1–1.5 h. The strips were then returned to fixative for the dissection of single fibers.

RESULTS

Gross muscle morphology

Occipitoscapularis, the most superficial muscle of the five examined, is a long, thin, straplike muscle, originating from the coracovertebral angle of the scapula (Fig. 1*B*). It has parallel fibers, which run the length of the muscle around a core of blood vessels and nerves. The innervation of the muscle is by a single nerve bundle from the third or fourth cervical segment.

Deep to occipitoscapularis lies splenius, a broad sheetlike muscle whose long parallel fibers travel laterally from an extensive origin in the spinous processes of upper thoracic vertebrae and nuchal

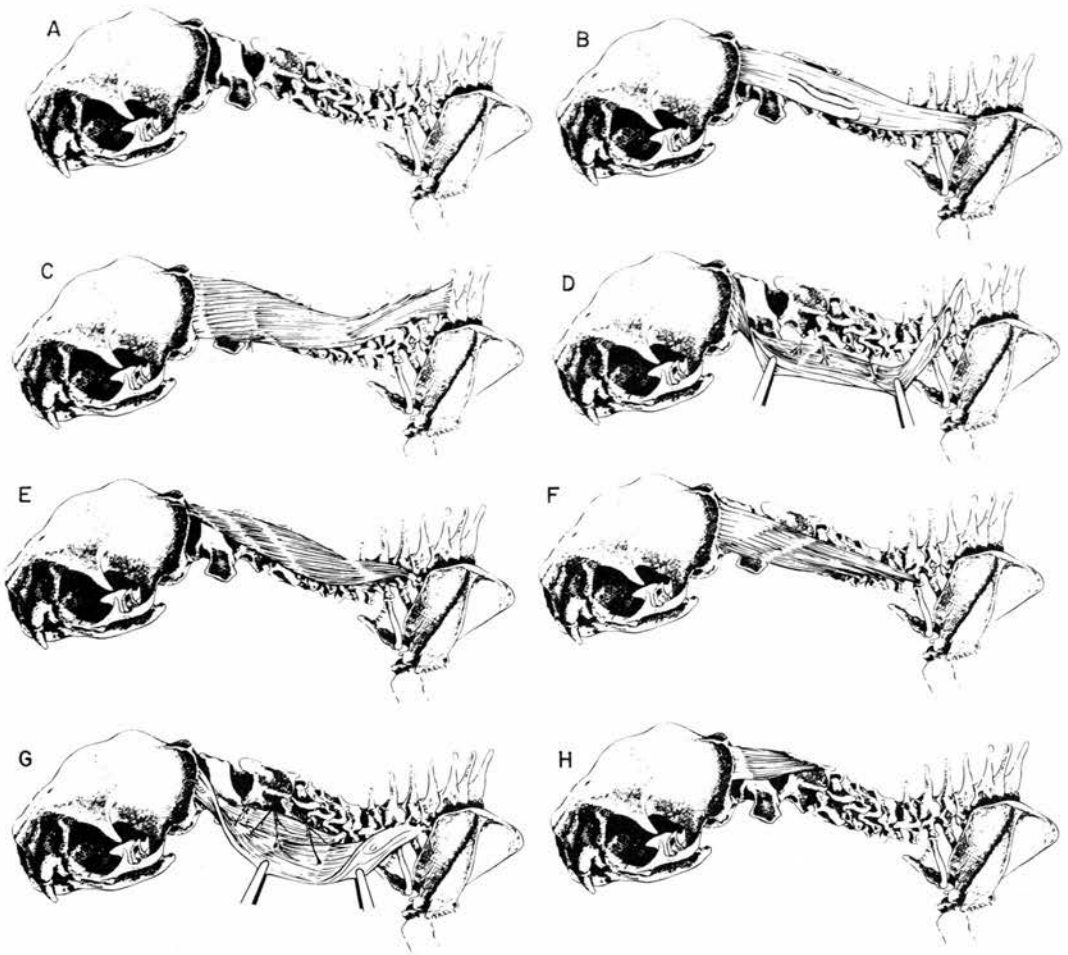

FIG. 1. Arrangement and innervation of the dorsal muscles of the cat neck. *A:* skeletal structure; *B:* occipitoscapularis; *C:* splenius to show two tendinous inscriptions; *D:* splenius, retracted to show arrangements of multiple innervation; *E:* biventer cervicis showing three tendinous inscriptions; *F:* complexus showing single inscription; *G:* biventer cervicis retracted to show innervation; *H:* rectus capitis major.

midline to insert by a short flat tendon along the whole width of the lamboidal crest (Fig. 1*C*). Traversing the muscle at an angle roughly perpendicular to its fibers are two inscriptions of tendinous tissue (Fig. 1*C*). The more rostral inscription is located about one-third of the way from the muscle insertion. This rostral inscription does not completely span the muscle, but extends from the lateral border of the muscle to terminate abruptly 1–2 cm from its medial border (Fig. 1*C*). The second inscription lies midway between the first inscription and the muscle origin. It transverses only the lateral half of the muscle, thus terminating somewhat more lateral than the first inscription. Splenius muscle fibers, which originate most caudally and lie most laterally, are traversed by both inscriptions. Fibers in the middle third of the muscle are traversed by only the rostral inscription, and the most medial fibers are not traversed by inscriptions.

Inscriptions in splenius are not attached to bone or tendon. When examined histologically, they are seen as a dense fibrous network surrounding long perforating muscle fibers. Shorter muscle fibers insert into the inscriptions, either directly or by inserting into Golgi tendon organs located in the inscriptions (Fig. 2).

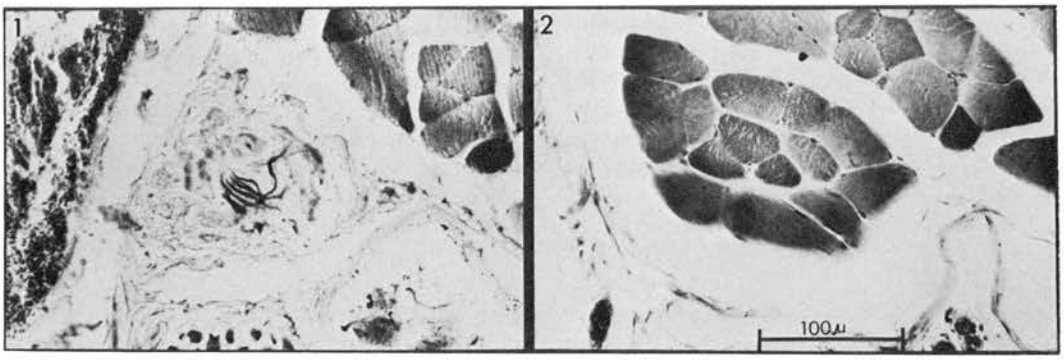

FIG. 2. Receptor and fiber fasciculi arrangements in a tendinous inscription of splenius. *1:* Golgi tendon organ (center) is associated with spindle (lower right). Note the branching arrangement of nerve fibers within the tendon organ. *2:* section through the same muscle approximately 500 μm distant. The fiber fasciculus that inserts into the tendinous inscription via the Golgi tendon organ illustrated can be seen. The spindle capsule and some of its intrafusal fibers can be seen (bottom right). Holmes silver stain.

Palpation of splenius shows that the muscle caudal to an inscription is thicker, reflecting the termination of short caudal fibers at this point.

The nerve supply to splenius enters its ventral surface and largely consists of branches from the second, third, and fourth cervical roots (Fig. 1*D*). Nerve branches from C_2 enter the muscle rostral to the inscriptions, branches from C_3 between the inscriptions, and branches from C_4 caudal to the inscriptions. In most cats, splenius is also innervated by small nerve branches from the first cervical root which enter the muscle close to its insertion. Blood vessels to splenius either accompany one or more of the main nerve branches and enter the muscle ventrally or enter splenius dorsolaterally at two or more levels, often accompanied by fine nerves.

Biventer cervicis and complexus lie beside one another deep to splenius. Biventer cervicis is the more medial and is a wedge-shaped muscle with a narrow insertion in the medial quarter of the lamboidal crest and a multiple origin in lower cervical and upper thoracic vertebrae (Fig. 1*E*). The medial edge of biventer cervicis is closely adherent to the tough connective tissue of the nuchal midline, and its lateral edge overlaps the medial edge of complexus.

Tendinous inscriptions are particularly wide and deep and, thus, are prominent in biventer cervicis. On the dorsal surface of the muscle they appear as three or four white, glossy bands running from the lateral to the medial edge (Fig. 1*E*) in a rostral direction. The inscriptions penetrate the muscle mass from its ventral to its dorsal surface and are oblique to the muscle so that the dorsal margin of the inscription is more rostral than the ventral margin. This is particularly noticeable in the most rostral inscription where strands of tendinous tissue, on reaching the dorsal surface of the muscle, run superficial and parallel to the muscle fibers to attach to the lamboidal crest. As in splenius, the inscriptions surround the long muscle fibers and serve as insertions for shorter fibers so that the muscle mass is reduced toward the muscle insertion. Successive insertions into the inscriptions by shorter muscle fibers located primarily in the lateral edge or dorsal border cause narrowing and thinning of the muscle toward the midline. Medially located fibers, in contrast, usually pass through the inscriptions and run the length of the muscle.

Nerves from the second, third, and fourth cervical segments enter the ventral surface of the biventer cervicis muscle (Fig. 2*G*). The C_2 nerve branch, which innervates the muscle between its insertion and its most rostral inscription, is accompanied by the greater auricular nerve, which penetrates through biventer cervicis without branching and runs rostrally over the lamboidal crest toward the pinna (Fig. 1*G*). Branches of C_2, which innervate

the biventer cervicis muscle, are comparatively small. The C_3 nerve branches to biventer cervicis are numerous and are consistently divided into two large bundles, the first entering the muscle at or just below the most cephalad inscription and the second entering at or just below the next inscription. The C_4 branches are also extensive and are also usually divided into two bundles. When biventer cervicis has four inscriptions, one C_4 bundle usually supplies the region between the third and fourth inscription and another the space between the fourth inscription and the muscle origin. In muscles with only three obvious inscriptions, two bundles usually enter one of the interspaces between inscriptions. Considerable variation can be seen in the relative size and in the pattern of nerve branching from one cat to another. No C_1 branches innervate biventer cervicis, but it is common for one or two small nerve branches to the muscle to take origin from segments caudal to C_4. Most of the blood supply to biventer cervicis enters via vessels accompanying the large nerve bundles.

Complexus is located laterally and slightly deep to biventer cervicis. It originates mainly from transverse processes of lower cervical vertebrae and stretches in a broad sheet to a wide tendinous insertion on the lateral two-thirds of the lamboidal crest (Fig. 1*F*). Its medial edge follows biventer cervicis closely and both muscles are bound together by a thin fascial layer, which overlies both dorsal and ventral surfaces. Laterally, the muscle follows the contour of the neck and its lateral border lies halfway down the side of the neck. Complexus usually has only one tendinous inscription, which traverses the muscle midsection from its lateral to its medial edge. The prominence of this inscription varies from one cat to another. In most cats, the inscription appears as an obvious glossy white line, but in others, the tendinous tissue is much reduced and all that can be seen by inspection is a line caused by the irregular abutment of one muscle fiber on another across the muscle.

Complexus receives the bulk of its innervation from the first three cervical segments. Nerves from C_1 enter complexus very close to its tendon of insertion. Nerve branches to complexus from C_2 and C_3 accompany the nerve branches to biventer cervicis. Blood vessels to complexus also enter ventrally with the large nerve bundles.

Rectus capitis major is a much smaller and shorter muscle lying deep to biventer cervicis and complexus. Its fibers fan out from their origin along the spinous process of the axis to insert into the medial one-third of the lamboidal crest (Fig. 1*H*). Rectus capitis major has no tendinous inscriptions. It is innervated by a large nerve branch from the first cervical segment.

Single-fiber dissection

Approximately 30 single extrafusal fibers stained for cholinesterase activity were dissected from each of the muscles examined. Fibers from occipitoscapularis and rectus capitis major muscles could be dissected with relative ease for the entire length of the muscle. Dissection of fibers in the other three muscles, splenius, biventer cervicis, and complexus, was more difficult because of fiber binding by tendinous inscriptions. Some perforating fibers could be freed of this dense tissue and traced the entire length of the muscle. Short fibers could be dissected only to the inscriptions, where they terminated. These dissections made it possible to construct a diagram of the arrangement of fibers of different lengths in splenius, biventer cervicis, and complexus, as shown in Fig. 3.

Single dissected fibers never had more than one end plate along their length. All of the muscles were traversed by several discrete bands of end plates and neighboring fibers often had end plates at quite different levels, each corresponding to a different end-plate band.

Histochemistry

Histochemical testing showed that all fibers fitted into one of three patterns (Table 1), which are the same as those described histochemically (5, 10, 23) for cat hindlimb muscle. The ATPase-poor fibers, previously demonstrated (1) by the Ca^{++}-dependent ATPase technique of

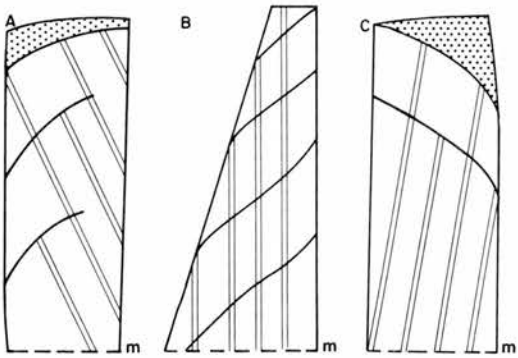

FIG. 3. Diagrammatic representation of fiber arrangements and orientation in *A*, splenius; *B*, biventer cervicis; *C*, complexus. Top of each diagram is the lamboidal insertion; m, medial aspect of muscle. Note the single tendinous inscription in complexus, the four inscriptions in biventer cervicis which completely traverse the muscle, and the two inscriptions in splenius, which incompletely traverse the muscle.

Padykula and Herman (13), form a homogeneous group. These fibers all stain poorly for ATPase after preincubation at pH 10.4, but all stain intensely for ATPase following preincubation at pH 4.35 (Fig. 4) and show high SDH and DPNH activity. After incubation for SDH and DPNH, evenly distributed blue or turquoise, beadlike diformazan deposits can be seen across a light background. The staining profile of this group is the same as that of the fiber group labeled B according to histochemical characteristics (10, 23) and identified by Burke et al. (5) as functionally slow.

Fibers which stain darkly when tested only for Ca^{++}-dependent ATPase without preincubation are not a homogeneous group but can be subdivided into a further two groups on the basis of more extensive enzyme testing. One fiber subgroup stains intensely following alkali preincubation, but shows reduced ATPase activity following acid preincubation. After acid preincubation, the intensity of coloration varied in different staining batches from an almost complete lack of coloration to more moderate coloration. When stained for SDH and DPNH activity, this fiber group demonstrates only a sparse network of diformazan deposition throughout the nonstaining cytoplasm.

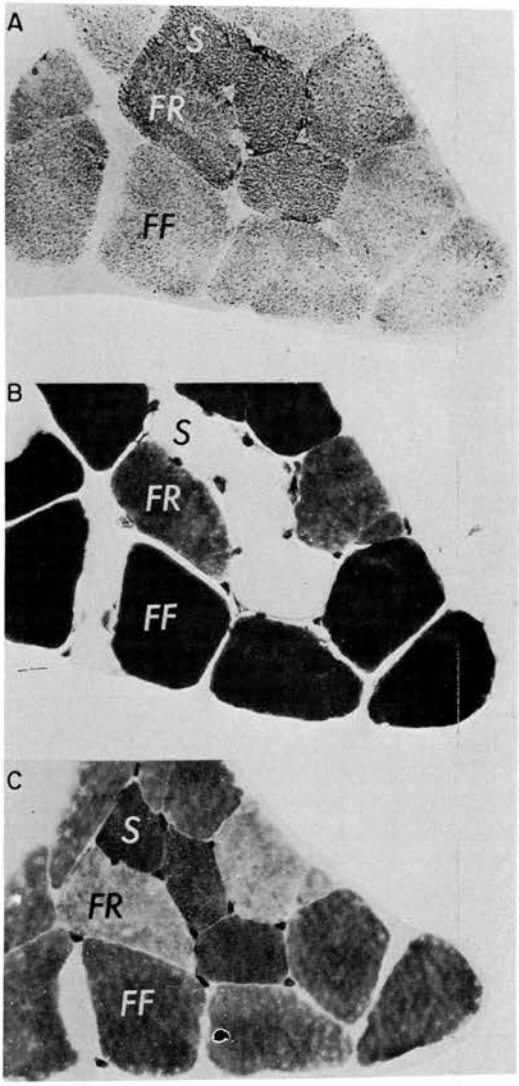

FIG. 4. Serial sections subjected to different histochemical procedures to show the three fiber types. *A:* SDH stain; *B:* ATPase, alkaline preincubation; *C:* ATPase, acid preincubation. S, small fiber which shows the staining pattern typical of *B* (slow) fibers. FR, a large fiber which shows the staining pattern of *C* (fast, fatigue resistant) fibers. FF, a large fiber with staining characteristics of type A (fast, readily fatigued) fibers. Vacuolation present in *C* is due to freezing artifact. Note the predominance of S fibers in the core and the predominance of FF fibers in the periphery and FR fibers both in core and periphery of fasciculus.

Following alkali preincubation, the second fiber subgroup stains with an intermediate coloration distinctive from both other fiber groups. Following acid prein-

cubation, these fibers consistently stain very lightly. Like slow fibers, they demonstrate prominent SDH and DPNH activity. However, the pattern of diformazan distribution observed under high magnification is more diffuse than that observed in slow fibers and is arranged in a pattern of intersecting lines rather than discrete granules. These deposits are also a deeper blue-purple color than deposits in slow fibers. Some fibers in this second group showed heavy diformazan deposition beneath the fiber sarcolemma, as has been observed by Stein and Padykula (16). The regions of deeper coloration did not always ring the periphery of the fibers evenly, but rather appeared discontinuously at one or more edges. These two subgroups correspond to the groups categorized histochemically as A and C, respectively (10, 23), and functionally as fast, readily fatigued (FF) and fast, fatigue resistant (FR) (5).

Previous investigations (1) have shown that the five dorsal neck muscles could be divided into three functional categories based on their relative contents of fast and slow fibers. When the proportions of the three fiber types were examined in the present experiments, differences between the muscles, particularly in their proportions of FF and slow fibers, again became obvious (Table 2). Splenius and rectus capitis major had about 50% FF fibers and about 25% slow fibers (Table 2). In contrast, biventer cervicis and occipitoscapularis had only about 27% FF fibers but about 50% slow fibers. Complexus again occupied an intermediate position with approximately equal numbers of FF and slow fibers. FR fibers constituted from 14 to 36% of the fiber populations in the neck muscles examined (Table 2). This variation in content of FR fibers was as great between the same muscles from different animals as between muscles of different types. For example, the FR proportions in three rectus capitis major muscles were 16, 17, and 35%. Part of the data were taken from one pair of rectus capitis major muscles and one pair of complexus muscles from the same animal. As shown in Table 2, the relative contents of each fiber type are virtually identical from one side of the cat to the other.

TABLE 2. *Percentage of three histochemically identified fiber types in dorsal neck muscles*

	FF, %	FR, %	Slow, %
Biventer cervicis			
A (R)	30	24	46
E (L)	32	14	54
D (R)	21	31	48
Occipitoscapularis			
A (R)	27	16	57
C (L)	29	16	55
B (R)	21	23	56
Splenius			
A (R)	50	28	22
E (L)	57	17	26
C (L)	54	16	30
Rectus capitis major			
A (R)	41	35	24
A (L)	42	36	22
C (R)	56	17	27
Complexus			
D (R)	31	27	42
A (R)	32	30	39
A (L)	32	29	38

The cats from which the tissue was taken are labeled *A–E;* R, right; L, left

The distribution of the three fiber types followed a pattern. Slow fibers were most commonly found at the core of muscle fasciculi, while FF fibers tended to occupy peripheral positions (Fig. 4). This may be of some consequence in sensory as well as motor mechanisms. This arrangement means that the fasciculus that enters a tendon organ is not functionally homogeneous and, in consequence, the receptor can be differentially affected by activity in fast and slow motoneurons. FR fibers are more randomly distributed in both core and peripheral positions. In all muscles save occipitoscapularis, slow fibers also predominate in the core of the muscle as a whole, while FF fibers are concentrated peripherally. FF fibers are particularly populous close to the muscle's dorsal surface. They are also concentrated in the lateral edge of splenius and in circumscribed regions near tendinous inscriptions in biventer cervicis and complexus muscles.

Occipitoscapularis was the only muscle

in which the proportion of each fiber type appeared uniform across the whole cross section, although the same intrafascicular organization observed in other muscles was present, with slow fibers in the core of fasciculi and FF fibers in their periphery.

DISCUSSION

The anatomical characteristics suggest that of the major neck muscles, biventer cervicis supports and elevates the head, whereas splenius is of more importance in turning movements. The fibers of splenius are directed laterally and insert along the entire length of the lamboidal crest, while those of biventer cervicis run parallel to the vertebral column and are organized to direct their force onto a very restricted insertion at the midline of the crest. The role of complexus is less clear, but its intermediate characteristics both mechanically and functionally may imply a synergist action to both biventer cervicis and splenius. Rectus capitis major must also contribute to head movement because of its origin on the axis, but the nature of this contribution, whether it be to extend the head or lock the upper cervical vertebrae, is not obvious. Occipitoscapularis originates from the scapula rather than the vertebral column and, as Elliott (6) described, probably has its major role in rotation of the scapula.

Splenius, biventer cervicis, and complexus all have a very complex internal architecture, which is based on the presence of tendinous inscriptions. Tendinous inscriptions are a well-known characteristic of rectus abdominis, but in these muscles have been dismissed as evolutionary vestiges (18). While the presence of tendinous inscriptions has been noted in biventer cervicis by Elliott (6), the relationship of tendinous inscriptions to the fiber organization of these muscles does not seem to have been appreciated previously. At their simplest, tendinous inscriptions allow long muscles to taper to a reduced insertion while maintaining a parallel fiber orientation. The inscriptions also permit a mixture of long and short fibers to coexist in the same muscle in a parallel orientation. Long fibers that are bound by inscriptions transfer tension developed by short fibers inserting into these inscriptions. Presumably, all the tension developed by the muscle must ultimately be transmitted through the relatively few fibers inserting on the tendon.

The mixture of short and long fibers could have interesting functional implications. By irregular contraction of short and long fibers it is possible that at any initial muscle length, a significant proportion of individual fibers could be at a length optimum for peak tension development. This property could lead to a flattening of the normal length-tension relationship and allow the muscles to develop relatively high tension over a wide range of initial muscle lengths. The precise arrangements must be different for each of the three muscles for there are significant differences of fiber orientation and organization of short and long fibers between muscles.

There is no evidence to suggest that the multiple innervation of dorsal neck muscles is associated with multiple innervation of single fibers, as has been found in avian dorsal neck muscles (7–9), for only one end plate has been found on each single fiber. Multiple innervation appears related more to the unusual arrangements of short and long fibers within each muscle. Nerve branches from the more anterior cervical roots, for the most part, innervate only those long muscle fibers that penetrate the inscriptions to terminate close to or at the bony insertion, while branches from posterior roots for the most part innervate the short fibers which terminate at the inscriptions. Such a differential innervation of short and long muscle fibers could mean some differences in functional roles between fibers taking origin in upper roots and fibers having their origin in lower roots. This question can only be resolved when the organization of motor units is fully understood.

Dorsal neck muscles of the cat are very rich in receptors (2, 15), and it may be the complex internal arrangements of these muscles which provide some of the logic for this characteristic. The unusual fiber mix and the arrangement of inscriptions

suggest that the muscles may be composed of functional subunits, which can be differentially activated in different movements. For the adequate motor control of such muscles it is not enough for the information to be available about muscle performance as a whole, but separate information is needed from each of the subunits. The high concentration of Golgi tendon organs in the inscriptions and the very rich accumulation of spindle arrays that are present in these muscles (15) suggest a sensory system well able to provide information concerning the performance of localized muscle regions.

Previous studies on the content of Ca^{++}-dependent ATPase in neck muscle fibers (1) led to the conclusion that biventer cervicis is predominately a slow muscle, splenius predominately a fast muscle, and that complexus occupies an intermediate role. The extended histochemical testing now undertaken supports and extends this previous finding. It is also now apparent that, like hindleg muscle, neck muscle is histochemically, and thus functionally, composed of three fiber types. No fibers were seen which did not clearly fit one of the three staining profiles, but the possibility that further subgroups might be demonstrable with the use of a more extended range of testing cannot be excluded. The proportion of fast and slow fibers in any given muscle have proved to be remarkably consistent in both this and the previous study (1). The consistency of fiber types within neck muscles may relate to the relative fixity of their task. Unlike locomotor muscles, neck muscles are rarely called on to work against widely varying loads or to meet great variation in their patterns of activity.

When the distribution of the three fiber types is examined, it can be seen that the FR fiber proportions are relatively consistent from one muscle group to another and these fibers have never been found to exceed 36% of the muscle mass. On the other hand, both FF and slow fiber proportions vary rather more from one muscle group to the next and can form more than half the fibers in a muscle. Thus, it may be the content of FF or slow fibers, which is important in dictating the overall functional character of any muscle. The histochemical examination supports the idea that biventer cervicis is a tonic muscle that supports the head and splenius, a phasic muscle which is primarily responsible for quick turning movements. It is noteworthy in this regard that Anderson, Yoshida, and Wilson (4) showed that unilateral electrical stimulation of the superior colliculus (which will lead to contralateral turning of the head) gives rise mainly to EPSPs in splenius motoneurons but IPSPs in ipsilateral motoneurons innervating the complexus/biventer cervicis complex. In a further study of vestibulospinal effects, Wilson and Maeda (19) found that stimulation of the lateral vestibular canal nerve selectively produced EPSPs in splenius motoneurons. Suzuki and Cohen (17) had previously shown the same stimulation in the conscious cat to cause fixed patterns of head turning. These experimental findings are all consistent with splenius being the major neck muscle involved in head turning.

The head-movement system has much in common with the oculomotor system in the range of movements that can be executed and the fact that most movements are executed against constant inertia and loading. Despite the relative simplicity of the motor task, it is clear that the muscles that are most immediately responsible for the support and movements of the cat head have an intrinsic complexity, which may be unmatched by any other of the muscle systems of the body.

SUMMARY

An examination has been made of the five dorsal muscles of the cat neck which insert into the lamboidal crest. The three larger muscles, splenius, biventer cervicis, and complexus, are characterized by the presence of tendinous inscriptions which serve as the insertion points of shorter muscle fibers which do not run the length of the muscle. Longer fibers are bound by the inscriptions and, thus, can transmit tension developed by shorter fibers. These three muscles are multiply innervated by nerves emerging from upper cervical spinal roots. Multiple innervation is not associated with multiple end plates

but with an arrangement so that lower roots innervate a high proportion of short fibers and higher roots, a high proportion of long fibers. Occipitoscapularis and rectus capitis major are, by comparison, simple muscles, each with a single motor nerve. Of the muscles examined, occipitoscapularis alone does not have its origin on the vertebral column but on the scapula, and is unlikely to have any major function in head movement.

Histochemical examination of the five muscles has revealed the presence of three fiber types only. On the usual association of histochemical profile and functional characteristics, splenius and rectus capitis major are predominantly fast muscles and biventer cervicis and occipitoscapularis, slow muscles. Complexus occupies an intermediate position between fast and slow.

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Morphology and Distribution of Muscle Spindles in Dorsal Muscles of the Cat Neck

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IN THE PREVIOUS PAPER (29) we have shown that dorsal muscles of the cat neck, which serve primarily to move the head, have an unusual fiber organization and pattern of innervation that set them apart from other muscle systems. We now report that the sensory apparatus of these same muscles also has a number of unusual characteristics. Some of these characteristics may be of importance in the motor physiology of head movement, but there is also evidence that neck muscle receptors play a role in postural regulation that extends beyond the immediate problems of motor control of neck muscles (for reviews, see ref 2, 12). As in man (18, 35) and rat (34), it has been found that there is a very high spindle index (spindles per gram) in cat dorsal neck muscles and, because of the relatively large size of the muscles, a very high spindle content.

Many of the spindles in neck muscles are in complex forms so that elements are shared between spindles and, in some muscles, complex forms connect with one another to form elaborate spindle arrays. The arrangement of spindles in dorsal neck muscles not only has implications for the sensory role of the spindles in dorsal neck muscles, but also for their fusimotor control.

METHODS

Neck muscles were obtained from cats weighing from 2.8 to 3.5 kg anesthetized with intraperitoneal sodium pentobarbital (Nembutal, Abbott), 35 mg/kg, or intraperitoneal diallylbarbituric acid and urethan (Dial, formulated locally), 0.5 ml/kg. Four muscles, occipitoscapularis, splenius, biventer cervicis, and complexus, were separated rostrally from the lamboidal crest and caudally below their innervation by C_4 root branches. This particular caudal boundary is above the point where the latter three muscles divide into their multiple origins in lower cervical and upper thoracic vertebrae (21, 29). The fifth muscle, rectus capitis major, has its origin on the axis and was removed in toto. All muscles were weighed immediately after removal and then fixed by immersion in formal sublimate (19) for 2–24 h. After fixation, the muscles were rinsed in 70% alcohol, placed in 70% alcohol with 0.5% iodine for 16–24 h, and dehydrated through graded alcohols to toluene. The muscles were impregnated and embedded in a mixture of paraffin and plastic polymers (Paraplast, Sherwood).

Sections of 15 μm were cut transversely throughout the entire length of each muscle. Muscle is brittle tissue to cut and the surface of the block was routinely soaked for 16–24 h in a tissue-softening agent (Mollifex, BDH) prior to cutting; but even so, only a restricted number of sections could be cut at any one time. Sections were mounted in order and a note made of any that were damaged and discarded in cutting so that accuracy could be maintained in serial reconstruction. Sections were stained with Holme's silver method (19).

Spindle reconstructions were made from sketches or photographs of at least every 20th section. Whenever necessary to define a point of anatomy clearly, serial sections were examined. Spindle distribution within a muscle was plotted on profile tracings of transverse muscle sections 100 μm apart, made with the aid of a photographic enlarger.

To examine fully the morphological conjunctions that occur between two or more spindles, it was necessary to examine every serial section in a muscle. This could be done with relative ease in all specimens of the two smaller muscles, occipitoscapularis and rectus capitis major. However, because of the large spindle population, it was not practical to do this on all specimens of the three larger muscles, splenius, biventer cervicis, and com-

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plexus. Therefore, one of each large muscle was subjected to a detailed examination of every section, in the course of which every intrafusal fiber of every spindle was, in turn, traced beyond its capsular extremities to its points of termination. Where measurements of length are given, these are the actual measurements that were made, with no correction for shrinkage.

RESULTS

The first part of RESULTS details the general characteristics of neck muscle spindles. In the second part, a detailed survey of the spindles of each muscle is given.

General spindle morphology

A total of 1,578 spindles were examined in 15 muscles, 3 each of occipitoscapularis, biventer cervicis, and splenius, 2 of complexus, and 4 of rectus capitis major. The basic structure of spindles in cat neck muscles is essentially the same as that of spindles in the large hindlimb muscles (13, 31). Capsule lengths, from the beginning to the end of the multilayered lamellar sheath, are similar in all neck muscles and measurements from 312 spindles ranged from 1,000 to 3,400 μm (mean, 2,200; SE, 40). Spindles contained both nuclear bag and nuclear chain intrafusal fibers. Nuclear bag fibers typically extend beyond the capsule extremities for distances up to and occasionally exceeding 2,000 μm. Nuclear chain fibers seldom extended more than 100 μm beyond the spindle capsule. Nuclear bag fiber diameters ranged from 12 to 18 μm at equatorial regions and from 12 to 25 μm at polar regions. Nuclear chain fibers had diameters of 5–10 μm at their equatorial regions and 5–15 μm at their polar regions. In a previous histochemical study (4), Ca^{++}-dependent ATPase in nuclear bag fibers of neck muscle spindles was found to be present either in high or low concentrations. In that study, the two types of nuclear bag fibers were found to be present in approximately equal numbers. Because the classification was based on histochemical differences and not on morphological differences, no attempt could be made to classify nuclear bag fibers in the present study.

Intrafusal fiber content, accurately determined in 1,382 spindles, ranged from 1 to 10 fibers. The spindle content of intrafusal fibers was similar in all the muscles examined except occipitoscapularis (Table 1). Spindles in occipitoscapularis tended to have more bag fibers and fewer chain fibers than the other neck muscles. Spindles with only one bag fiber were never observed in occipitoscapularis, but were numerous in the other four muscles and constituted 25–35% of spindles in splenius and complexus. Spindles most commonly had two nuclear bag fibers, and spindles with three bag fibers were relatively uncommon, never exceeding 10% of the spindle population in any one muscle (Table 2). No spindle was seen with more than three bag fibers. Intrafusal fiber content was thus lower in neck muscle spindles than in hindleg, with the exception of occipitoscapularis. Occipitoscapularis muscles, like hindlimb muscles (20), had average bag fiber contents equaling or exceeding 2.0 (Table 1).

Nuclear chain fibers were found in all spindles except three. In most spindles, one to eight chain fibers were present and chain fibers usually outnumbered bag fibers. As Table 2 shows, spindles with only one bag fiber tended to have fewer chain fibers (mean = 3.36) than spindles with two or three bag fibers (mean = 4.72 and 5.13, respectively).

TABLE 1. *Chain and bag fiber content of neck muscle spindles*

	Bag Fibers	n	Chain Fibers	n
Occipitoscapularis	2.03 ±.03	29	4.03 ±.14	29
Splenius	1.71 ±.02	466	4.12 ±.06	466
Biventer cervicis	1.83 ±.02	369	4.58 ±.05	369
Complexus	1.66 ±.02	392	4.42 ±.06	392
Rectus capitis major	1.87 ±.04	126	4.45 ±.10	126

Values are means ± SE.

TABLE 2. *Relationship between chain and bag fiber content of neck muscle spindles*

No. of Bag Fibers	No. of Chain Fibers									
	0	1	2	3	4	5	6	7	8	Total
1	2	10	60	150	98	37	12	3	0	372
2	1	0	3	52	354	405	155	14	2	986
3	0	0	0	2	7	6	4	5	0	24
Total	3	10	63	204	459	448	171	22	2	1,382

Conjunctive forms

We have introduced the term "conjunctive form" as a blanket term to include all spindles which either share elements or which exist in close physical contact with other spindles. In occipitoscapularis, only a minority of spindles (Table 3) were in conjunctive forms. However, in all the other muscles a high proportion of spindles occurred in one of three forms of conjunction. These spindle conjunctions have been previously described and are called tandem or series linkages (11, 14, 18, 32, 34), parallel or compound linkages (10, 18, 30, 34), and paired linkages (34). The morphology of the tandem linkage in cat neck muscles is as varied as in hindlimb muscle (11). Sometimes only one intrafusal fiber projects beyond one spindle capsule into a second spindle capsule, to link them in series. In other spindles, all the intrafusal fibers may enter a second capsule, rarely to form an identical spindle pair, or more commonly to be joined by additional intrafusal fibers. The distance between the capsules of tandem spindles varied considerably but only rarely exceeded 1,000 μm. Well-separated tandem spindles generally shared only one intrafusal fiber, while spindles closely approximated at their poles were often linked by more than one intrafusal fiber. Closely approximated tandem spindles had complex junctional arrangements so that there is no obvious discontinuity between capsules (Fig. 1).

As in cat hindlimb muscles (11), tandem linkages can exist between more than two spindles, and arrays involving up to five spindles were found in all of the three larger muscles. Arrays were either linked by a single fiber running through all capsules in succession or by a series of linkages formed by a few fibers, each linking in succession two or three capsules of the complex. Such an array is illustrated in Fig. 2. Some of the morphology of the array is shown in Figs. 3 and 4.

Paired linkages

Spindles which have some form of mechanical contact with one another without sharing intrafusal fibers have been regularly described in human (18) and cat muscle (10, 11), and have been called paired complexes (34). In cat neck muscles, paired complexes are abundant. The degree of contact between spindles varies from one extreme where only the ends of the capsules abut, to the other extreme where two capsules lie side by side throughout their entire length, with their

TABLE 3. *Distribution of single and complex spindle forms in dorsal neck muscles*

Muscle	Cat	Total	Single	Tandem	Paired	Parallel
Occipitoscapularis	*A*	6	6	0	0	0
	D (R)	15	13	0	0	2
	D (L)	11	7	0	0	4
Splenius	*E*	189	97	60	38	2
Biventer cervicis	*C*	173	55	57	54	4
Complexus	*F*	190	62	57	84	2
Rectus capitis major	*A*	30	12	2	16	0
	B (L)	44	19	2	23	0
	B (R)	38	15	9	14	0
	C	57	21	10	29	2

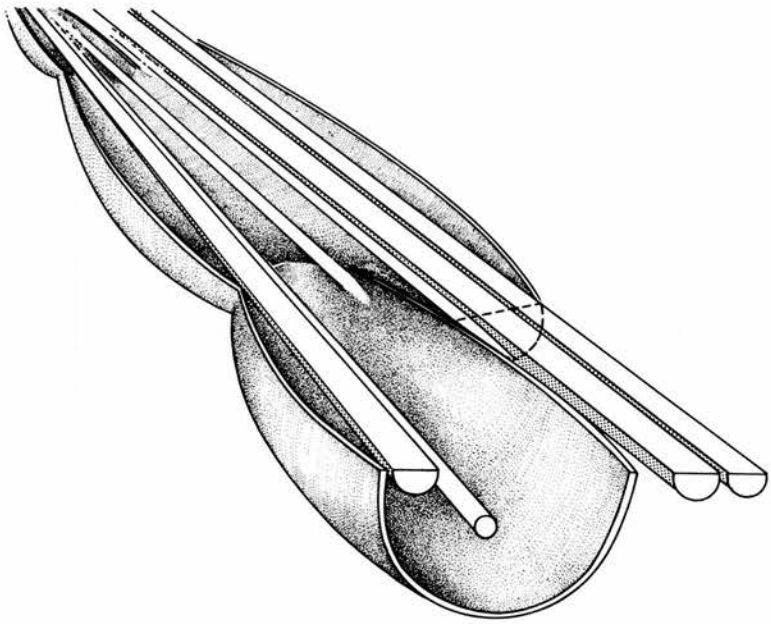


FIG. 1. Diagram to show the junctional arrangements of tandem spindles. It can be seen that the two spindles have a continuous capsule. The second capsule forms a lamellar partition in the first, closely investing the shared fiber and acting as a termination point for shorter unshared fibers. Long unshared intrafusal fibers lie to one side of this wall and extend for up to 1 mm beyond their capsule to terminate in perimysial connective tissue or tendon. Nuclear bag fibers shown as thick rods; chain fibers, as thin rods.

equatorial enlargements staggered. In contrast to spindles in tandem, paired spindles are usually of similar size, each containing two bag and four to six chain fibers.

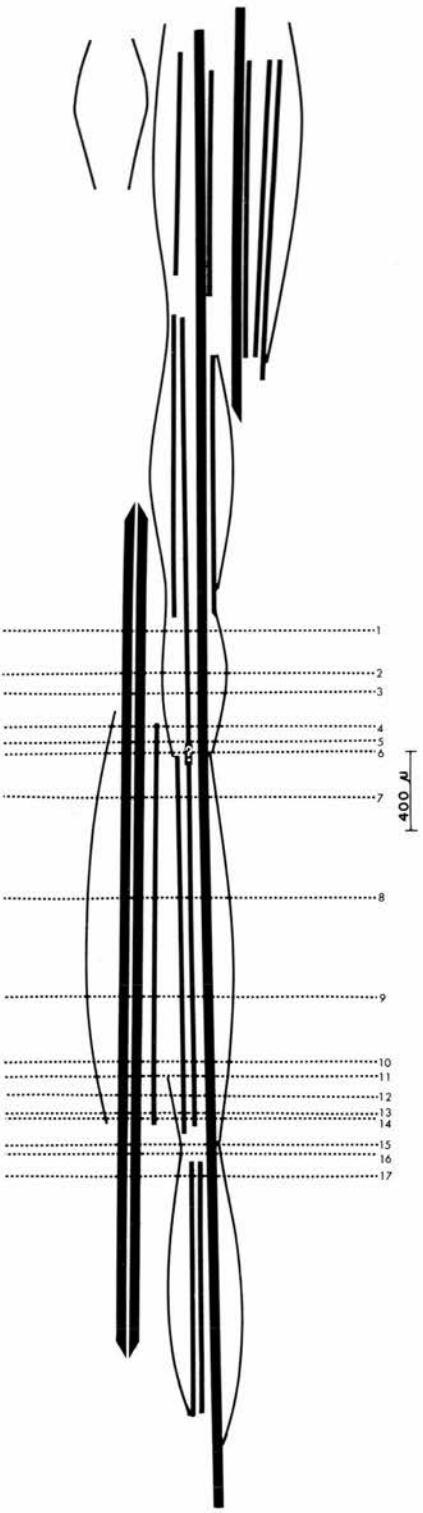
Each spindle in a pair appears as an autonomous unit, but the two spindles are surrounded by perimysial connective tissue threads which hold the spindles together. Occasionally, the investing membranes are thickened to give the appearance of an outer capsule investing the two spindles.

Parallel spindles

A third type of complex that was seen is called a compound complex (10, 18, 30) or a parallel complex (34). In this arrangement, spindles are separated at their polar regions into distinct capsules, which are bound closely together by contiguous outer lamellae. However, the lamellae attenuate toward the equatorial region and terminate so that there is no separation of intracapsular spaces at the equatorial region. Both sets of intrafusal fibers lie in a common capsule for a distance of 100–500 μm (Fig. 5), with the equatorial aggregations of nuclei and primary innervation of each set of intrafusal fibers staggered and about 100–500 μm apart. The myelinated branches of primary afferent endings to one set of intrafusal fibers remained well separated within the capsule from those of the other set, but it was not obvious whether these two sets of afferent branches merge outside the capsule.

Spindle complexes

Elaborate spindle complexes formed by several spindles are common in amphibia (23) and have occasionally been described in cat hindlimb (11). They have now been found in the three large muscles, splenius, biventer cervicis, and complexus. These complexes may be formed by one kind of linkage only, e.g., tandem or parallel (Fig. 6), but more usually incorporate more than one linkage type in their structure. Complexes may also be in close association with a Golgi tendon organ (Fig. 2). Spindles in such complexes frequently participate in more than one variety of



linkage, and two spindles were found which participated in all three varieties of linkage. They were parallel to each other, paired with a third unit, and in tandem with a series of three further units.

Spindle content of individual neck muscles

Table 4 summarizes the numbers and densities of spindles in the 15 cat neck muscles examined in this study. Spindles which form part of a spindle complex are listed individually. Splenius, biventer cervicis, complexus, and rectus capitis major all have very high spindle indexes (46–107 per gram), but occipitoscapularis has a much lower spindle index, in the range of 13–19 per gram.

Although spindle indexes for the same muscle may vary between animals, in cat neck muscle, they are, as in rabbit (9), much more consistent for the same muscle in the same animals. Two muscle pairs, one occipitoscapularis and the other rectus capitis major, were examined, each pair from a different animal. The spindle indexes for the two occipitoscapularis muscles were 18.7 and 16.1; for rectus capitis major, 58.7 and 61.3.

The incidence of complex forms in the muscles examined is shown in Table 3. It should also be noted that since one spindle may participate in a variety of conjunctive forms, that spindle will be counted in more than one category. For this reason, the total number of spindles in the subcategories in Table 4 usually exceeds the actual spindle count.

OCCIPITOSCAPULARIS. This muscle had the lowest spindle contents of any of those examined, with no more than 15 spindles in any one muscle and spindle densities ranging from 13 to 19 spindles per gram (Table 4). Spindles were well separated

FIG. 2. Diagram of spindle array in which five spindles exist in tandem, sharing one common nuclear bag fiber. Spindle capsules are shown as curved light lines; nuclear chain fibers, as thin straight lines; nuclear bag fibers, as thick straight lines. The outline at the top of the diagram to one side of the spindle is of a Golgi tendon organ that is in dyad with the upper spindle. The transverse lines indicate the plane of section illustrated in Figs. 3*A* and *B* and 4*A* and *B*.

but located solely in the central core of the muscle (Fig. 7*A*). The large majority of spindles in occipitoscapularis muscles were single, and no spindles were found in tandem or paired linkages. In one muscle (Table 3), 4 of 11 spindles were in parallel; 2 other parallel spindles were found in a second muscle.

SPLENIUS. A total of 509 spindles were examined in three splenius muscles with spindle densities ranging from 47 to 66 spindles per gram. Spindles were confined to the core of each muscle, evenly spaced in a plane roughly parallel to the dorsal surface (Fig. 8). We have shown in histochemical experiments (29) that core regions of splenius contain most of the slow fibers.

A considerable number of splenius spindles were arranged serially in chain-like tandem or paired complexes of up to six units. In the one splenius muscle carefully scrutinized for the presence of conjunctive linkages, 60 of 189 spindles (31%) formed tandem linkages and 38 (20%) formed paired linkages; 51% of spindles did not form part of a complex.

BIVENTER CERVICIS. A total of 421 spindles were found in three biventer cervicis muscles. Spindle density ranged from 74 to 96 spindles per gram. The distribution of spindles within this muscle was scattered and uneven, with some circumscribed regions of the muscle containing large numbers of spindles, while other regions were almost devoid of spindles. Unlike both occipitoscapularis and splenius, spindles were not confined to the muscle core but were also located close to most muscle edges (Fig. 9*A*) and were particularly common in the region of tendinous inscriptions.

Conjunctive spindles were particularly difficult to trace in biventer cervicis because they were frequently associated with the tendinous inscriptions that distorted and obscured spindle fine structure. Nonetheless, in the one muscle carefully examined for conjunctive forms, 60 of 173 spindles (35%) were found to be in tandem linkages and 54 spindles (31%) were paired. Only four spindles (2%) were observed in parallel. The distribution of the conjunctive spindle population within this muscle is shown in Fig. 10.

COMPLEXUS. The two complexus muscles examined contained the largest population of spindles of the muscles surveyed (Table 4). One complexus contained 254 spindles and had a spindle density of 107 spindles per gram, while the second had 190 spindles with a somewhat lower density of 71 spindles per gram.

The distribution pattern of spindles in complexus resembles that of splenius, with a large majority of spindles aligned in the muscle core from the lateral to the medial edge. Complexus is a somewhat thicker muscle than splenius and the spindles were scattered across a band of about half the total muscle thickness, midway between dorsal and ventral surfaces (Fig. 9*B*). Only a few spindles were located very close to dorsal and ventral surfaces. These peripherally placed spindles usually ran parallel to groups of fibers which inserted into tendinous inscriptions (29) close to the muscle surface.

As in splenius and biventer cervicis, several spindle complexes involving paired and tandem linkages were observed in complexus muscles. The one muscle in which spindles were categorized had 44% of its spindle population in paired arrangements and 30% in tandem arrangements; 37% of the spindles were found to be single and not part of a spindle complex.

RECTUS CAPITIS MAJOR. The four rectus capitis major muscles had variable spindle densities, ranging from 48 to 84 spindles per gram with an average density of 63 spindles per gram (Table 4). Rectus capitis major muscle is a small muscle, weighing less than 1 g (4), so that total spindle content (ranging from 30 to 57) is relatively small. Spindles were unevenly distributed, with most spindles in the muscle core and very few in the periphery (Fig. 7*B*). Rectus capitis major, like splenius, is a fast muscle with a high proportion of slow fibers in its core (5, 28) so that most of its spindles, too, are in the slow fiber region.

Rectus capitis major muscles had the highest proportion of paired spindles

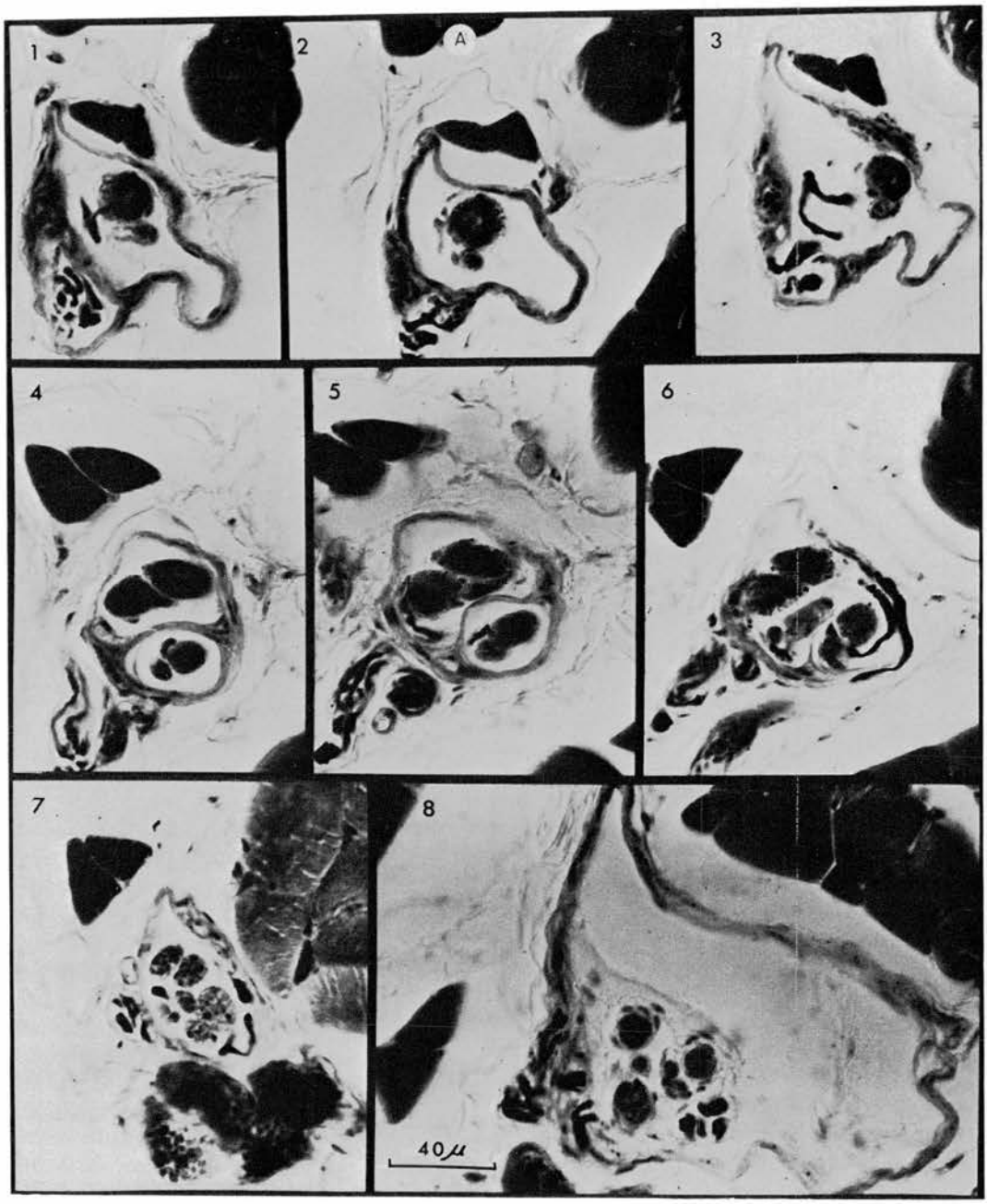


FIG. 3. *A:* photomicrographs of the spindle complex illustrated in Fig. 2 to show transitional zones in a spindle complex. The two large fibers in the upper left in each section are extrafusal fibers. Numbers refer to the level of section in Fig. 2. *B:* line drawings of the intrafusal fibers and capsules shown in *A*.

seen in neck muscles. Three of the four muscles had at least 50% of their spindles in paired arrangements (Table 3). Tandem arrangements were less common and ranged in frequency from 5 to 24%. The proportion of single spindles in all four muscles examined was about 40% (range 37–43%).

Golgi tendon organs

In the three large muscles, Golgi tendon organs were commonly found in as-

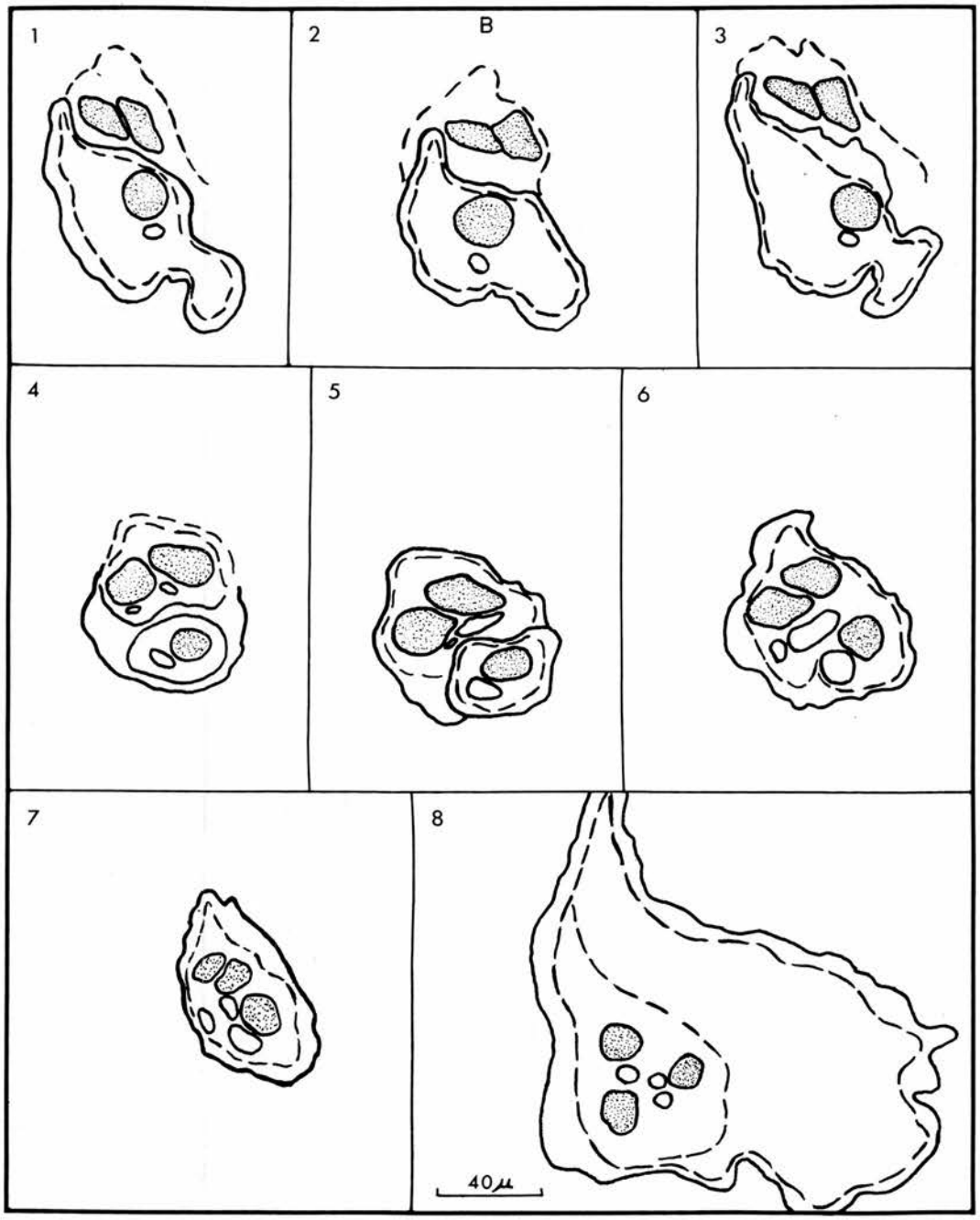


FIGURE 3*B*.

sociation with tendinous inscriptions. Although no special attempts had been made to preserve the tendon at the origin and insertion of the muscles, tendon organs were also seen in these regions. In one biventer cervicis muscle, 51 tendon organs were found in the tendinous inscriptions alone. Short fibers insert into these tendon organs in the same manner that fibers insert into tendon organs at the origin and insertion of muscles. No tendon organs were found in the main mus-

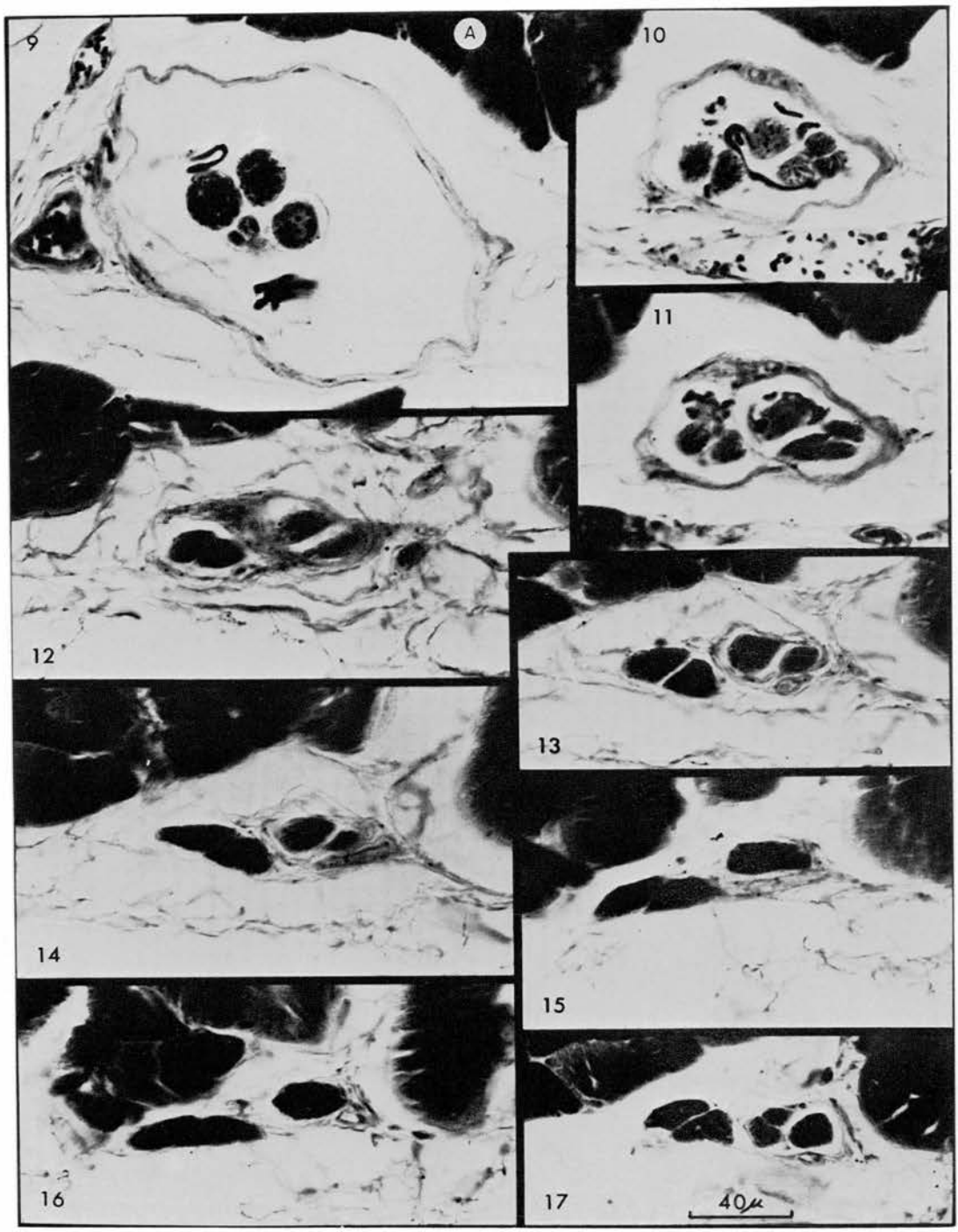


FIG. 4. *A:* photomicrographs of the spindle complex illustrated in Fig. 2 to show transitional zones in a spindle complex. Numbers refer to the level of section in Fig. 2. *B:* line drawings of the intrafusal fibers and capsules shown in *A*.

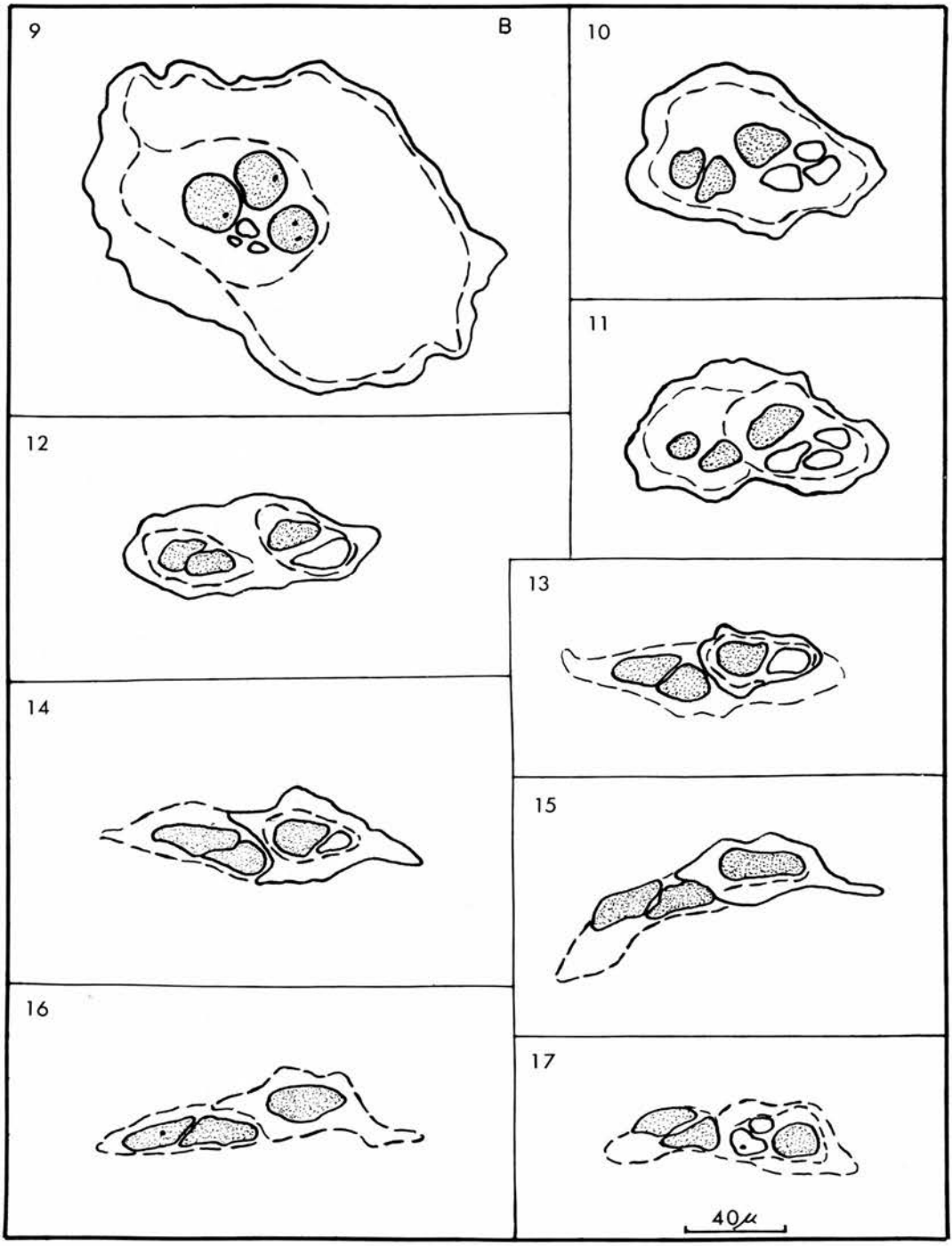


FIGURE 4*B*.

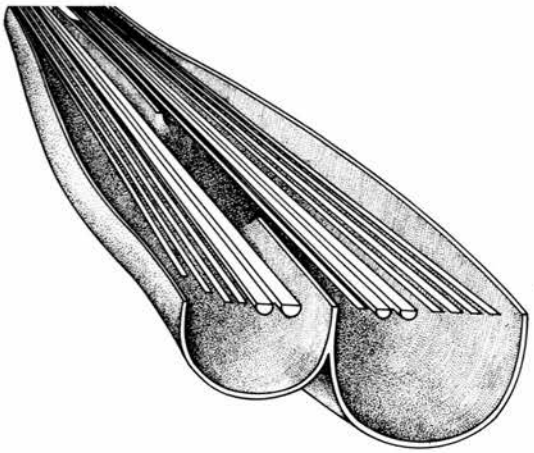


FIG. 5. Diagram to show typical arrangement of parallel spindles. Note the capsule discontinuity at the equatorial region of spindles.

cle mass of either occipitoscapularis or rectus capitis major, neither of which possess tendinous inscriptions.

As has been reported in many other muscles (25–27), a large number of tendon organs exist in close contact with muscle spindles. In some of these spindle-tendon organ dyads (25), the two receptors were structurally quite independent of one another and merely lay side by side, but other dyads were so closely associated that spindles and tendon organ capsules were contiguous. Most commonly, spindles participating in close dyad associations were small and contained a single nuclear bag fiber and only a few nuclear chain fibers.

DISCUSSION

The most obvious characteristic of neck muscle receptors is their sheer abundance in those muscles which serve to move the head. Indeed, this small group of muscles contains more spindles than any equivalent muscle mass in the cat. The common factor associated with high spindle density does not appear to be just that the muscle is a neck muscle or that it is inserted into the lamboidal crest, but that the muscle has head movement as its prime action. Occipitoscapularis probably plays little

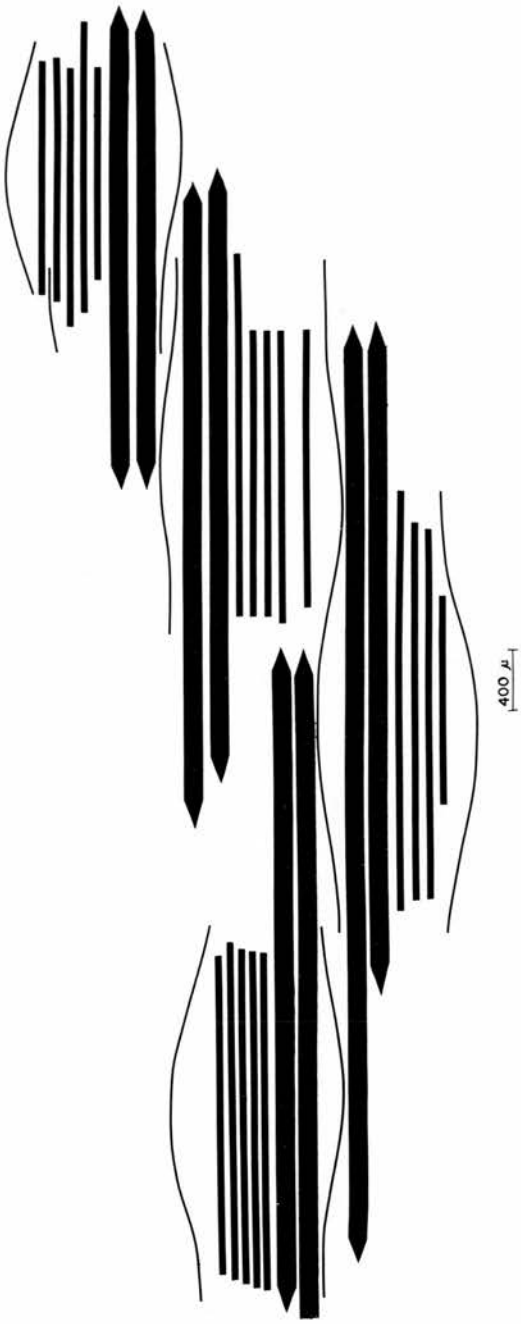


FIG. 6. Diagrammatic representation of spindle complex composed of four paired spindles. Nuclear bag fibers, thick lines; nuclear chain fibers, thin lines; capsules shown as curved outlines. Note that no intrafusal fibers are shared.

role in head movement, but has its origin in the scapula and has as its primary function, rotation of the scapula (21, 29). It

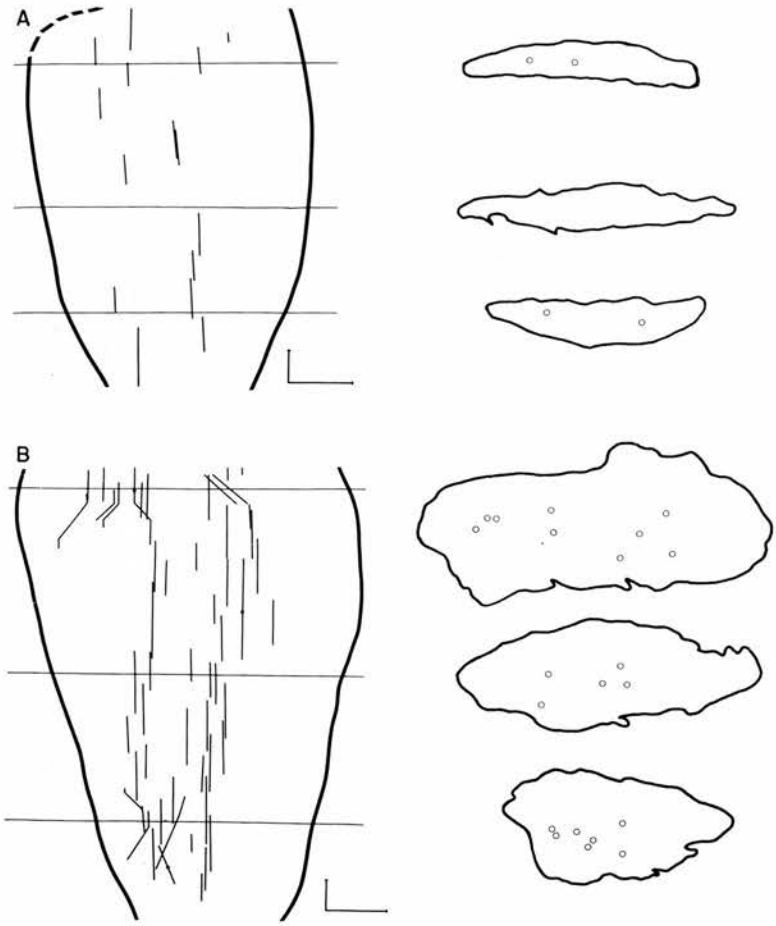


FIG. 7. Diagram to show spindle distribution in a representative *A*, occipitoscapularis and *B*, rectus capitis major muscle. Diagrams are foreshortened and vertical scale = 2 mm, horizontal scale, 3.7 mm (*A*) and 2.2 mm (*B*). Lines indicate spindle capsules. The transverse sections indicate spindle distribution within the muscle substance and were made at levels shown by horizontal lines.

TABLE 4. *Spindle contents and spindle index in dorsal neck muscles*

Muscle	Cat	Muscle Weight, g	No. of Spindles	Spindle Index
Occipitoscapularis	*A*	0.46	6	13.0
	D (R)	0.93	15	16.1
	D (L)	0.59	11	18.7
Splenius	*B*	3.18	148	46.5
	G	2.59	172	66.4
	E	2.99	189	63.2
Biventer cervicis	*B*	2.42	180	74.4
	A	0.91	68	74.7
	C	1.80	173	96.1
Complexus	*F*	2.66	190	71.4
	G	2.38	254	106.7
Rectus capitis major	*A*	0.62	30	48.4
	B (L)	0.75	44	58.7
	B (R)	0.62	38	61.3
	C	0.68	57	83.8

Spindle index measured as spindles per gram.

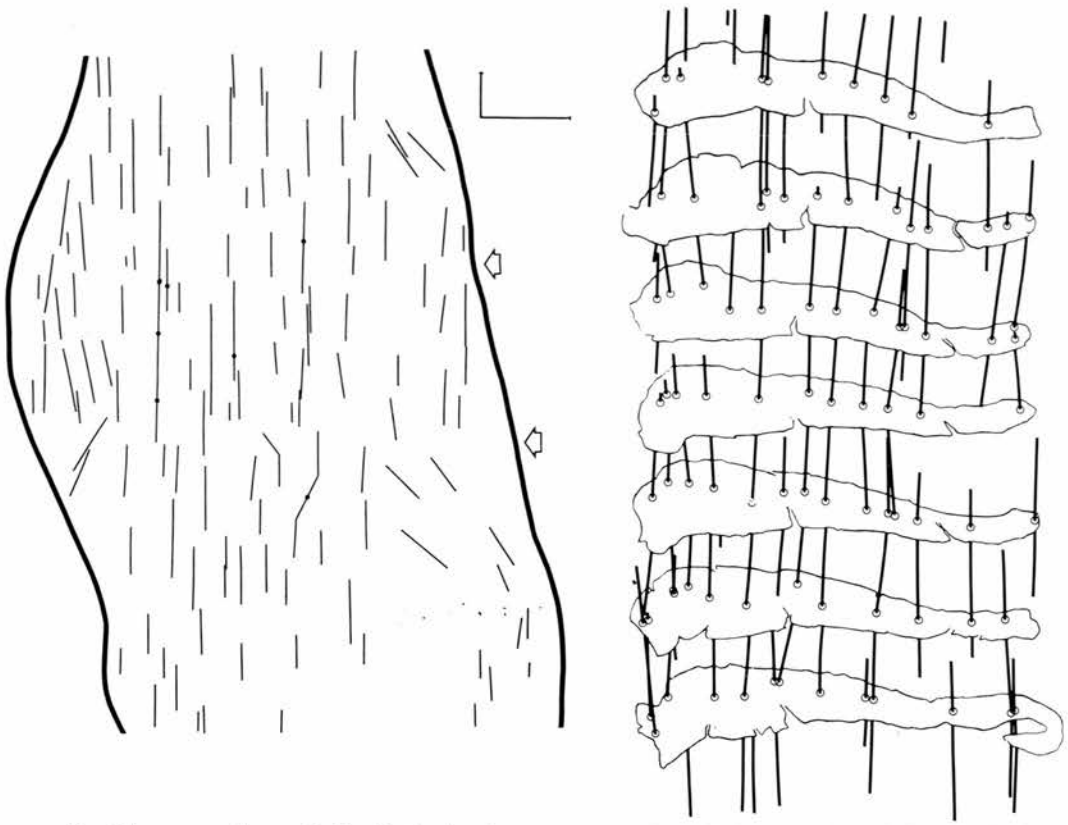


FIG. 8. Diagram to show spindle distribution in a representative splenius muscle. Left, longitudinal reconstruction with spindle capsules indicated as straight lines. Well-defined tandem junctions indicated by dots. Right, series of transverse sections at 1-mm intervals in the tissue segment between the two arrows in the left-hand diagram. The distribution of spindles in muscle substance is indicated in this diagram. Vertical scale, 2 mm; horizontal scale, 4.3 mm.

may, therefore, be of some significance that this muscle has a spindle density similar to that of locomotor muscles (22) and far lower than the other neck muscles. The presence of high spindle densities in neck muscles concerned with head movement has now been reported in such disparate species as man (18, 35), rat (34), and cat, and must relate in some way to the motor control of these muscles.

In general, high spindle densities have been associated with small muscles subserving fine motor tasks (27). Only rectus capitis major, of the neck muscles examined, can be regarded as small, and splenius, biventer cervicis, and complexus, which have equally high spindle densities, are all relatively large muscles. These large muscles, however, have a most unusual internal structure based on the presence of tendinous inscriptions, so that the muscles are formed of elaborate complexes of short and long muscle fibers (29). This elaborate muscle fiber organization is paralleled by an equally complex innervation. Individual nerves to each muscle arise from several cervical segments and terminate in relatively fixed positions determined by the particular organization of tendinous inscriptions (29). Thus, each of these three large muscles forms an anatomical entity but is composed of subunits which may be functionally dissimilar. The density of receptors in these muscles might, in part at least, derive from the role of spindles and Golgi tendon organs within the individual subunits that go to make up the muscle.

It has long been known that spindles are not randomly distributed within mus-

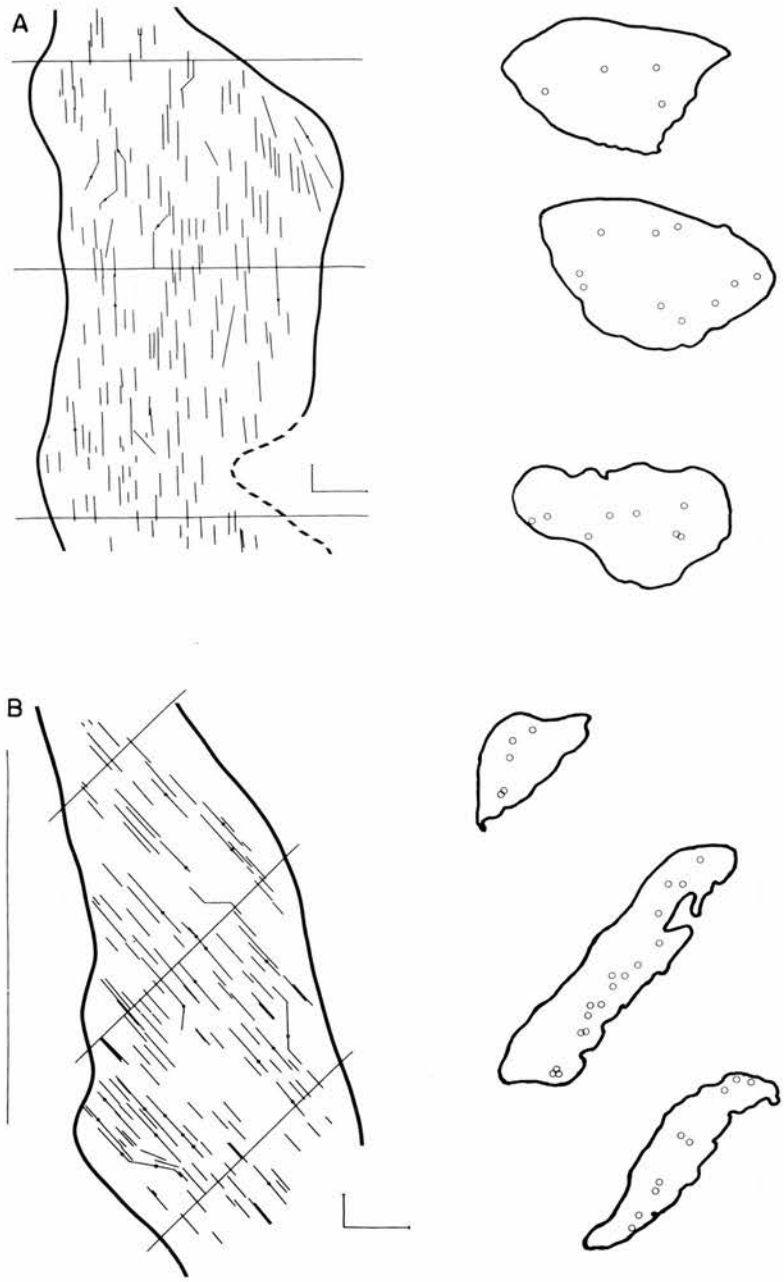


FIG. 9. Diagrammatic reconstruction to show spindle distribution in *A*, biventer cervicis, and *B*, complexus. Black dots indicate well-defined tandem spindle junctions. Cross sections are from levels indicated and show the intramuscular spindle distribution. Line alongside complexus represents midline of animal. Vertical scale, 2.0 mm; horizontal scale, top, 2.0 mm, bottom, 4.0 mm.

cles, but tend to occupy fixed positions (13, 15, 24, 30, 32). Indeed, as a historical note it should be remembered that Sherrington (30) first directly implicated the spindle in the stretch reflex by taking advantage of the location of spindles in the aponeurosis of the rectus femoris muscle. In those experiments, Sherrington (30) was able to show that removal of the aponeurosis (and thus the spindles)

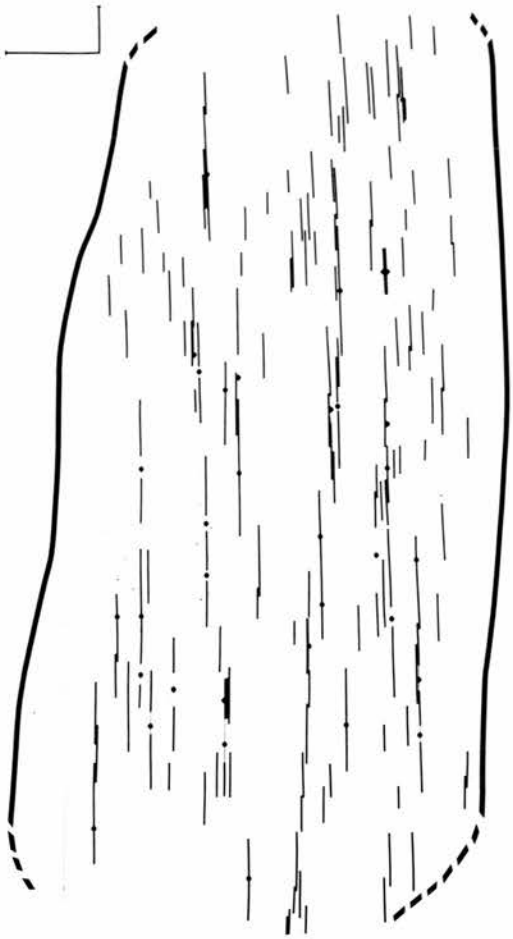


FIG. 10. Spindle content and distribution of complex forms in one biventer cervicis muscle. Paired spindles lie side by side and are indicated by line thickening. Tandem spindles marked by dots; widely separated tandem spindles joined by thin lines. Two parallel spindles marked by diamonds. Calibration: horizontal, 1.7 mm; vertical, 2 mm.

abolished the stretch reflex. In general, there has been little regard paid to the significance of spindle positioning, but recent experiments have shown that, in the cat extensor digitorum longus, the position of a spindle in the muscle is important in determining its static response (28). In splenius, biventer cervicis, and complexus, the fairly fixed patterns of spindle distribution may reflect functional differences between individual spindles in a muscle population, which are essential to the motor control of that muscle.

Another striking aspect of spindle location in neck muscles is the relationship of spindles to muscle fibers of different enzyme profiles. Individual fibers of neck muscle have histochemical profiles consistent with their classification as fast, fatigue resistant; fast, readily fatigued; or slow (29). On the basis of the predominant fiber content, the muscles themselves can be categorized as fast, slow, or intermediate (4, 29). As in the muscle of the cat hindleg (31), the spindle index of the slower neck muscles is higher than the spindle index of the fast muscles. Furthermore, even in predominantly fast muscles such as splenius, the spindles are restricted to those parts of the muscle which contain mainly slow fibers (28). Yellin (37) has described a similar association of spindles with slow fibers in a number of rat muscles, and the rich accumulation of spindles in extraocular muscles lie in those peripheral parts of the muscle (17) where small and functionally slow fibers predominate (8). This association of spindles with slow muscle fibers appears now to be so common that it must be regarded as subserving a functional role.

That muscle spindles exist in conjunctive forms with one another has long been known (18, 30). The incidence of muscle spindle complexes varies from muscle to muscle. In cat rectus femoris and soleus, 16–20% of spindles are in tandem (11, 32), and one gastrocnemius muscle has been described with up to 44% of its spindles in tandem (32). Of the muscles we have examined, splenius, complexus, and biventer cervicis all have about 30% of their spindles in tandem. Biventer cervicis and complexus additionally contain large numbers of paired spindles, so that the majority of spindles in both these muscles exist in complex forms. Swett and Eldred (32) proposed that the tandem linkages, which they observed to stretch from aponeurosis to aponeurosis in cat gastrocnemius, may serve to identify length change anywhere in the muscle. However, there is no experimental evidence to suggest that complex spindles have unique or specific sensory properties.

In the rat tail, parallel spindles constitute up to 40% of the population, but an examination of the physiological charac-

teristics of rat tail receptors failed to provide any evidence that parallel spindles had different sensory characteristics from simple spindles (7, 34). It is possible that the importance of spindle complexes lies not in their sensory role, but rather in the manner in which fusimotor control is exercised. A fusimotor fiber shared by two or three spindles will exert effects on many more receptors than a single unshared fiber. It would seem that to the existing complexities of fusimotor control systems must be added the complexities created by the existence of a hierarchy of fusimotor fibers. The rank of a fusimotor neuron in the system will be determined by the number of receptors that it influences.

Individual spindles in neck muscles differ only marginally in their morphology from hindleg spindles but, as a general rule, neck muscle spindles have fewer intrafusal fibers. Unlike hindleg spindles (20), neck muscle spindles often have only a single bag fiber and never have more than three bag fibers. The reduced number of intrafusal fibers in neck muscle spindles may mean that the number of receptors in these spindles is fewer than in hindlimb spindles.

While no comprehensive study of Golgi tendon organs was possible in the present work, the presence of numerous tendon organs in tendinous inscriptions was striking. As in many other studies (25, 26), these tendon organs were frequently found associated with spindles in dyads. The anatomical contiguity of spindles and tendon organs should enable different information concerning the same muscle site to be collected. This might be of particular importance in muscles organized in subunits like splenius, complexus, and biventer cervicis. The occurrence of dyads can no longer be considered a random event, with the association of spindles and tendon organs "by chance" (27), but the dyad should be recognized as a common muscle receptor entity which must serve a functional role.

The muscles that serve most immediately to move the head have a number of unusual specializations, as do their receptors. The central projections from these afferents also have some unusual features. While monosynaptic connections to neck motoneurons have been reported from cervical afferents (36), the bouton density on upper cervical motoneurons is low (33) and, despite the large numbers of spindles that are present, no significant monosynaptic reflex exists in the upper cervical cord (5). Afferent input from neck muscles is clearly of importance in the control of neck motoneurons, but this input operates through long loop pathways. For example, neck muscle afferent information is combined with inputs from the retina and extraocular muscles in the superior colliculus to influence output to neck muscles through the tectospinal tract (6). Neck muscle afferents also influence reticulospinal output through connections to the tectoreticular system (unpublished observations) and can influence lumbosacral excitability through mechanisms involving the cerebral cortex (1). The neck muscle spindle system, while it has no simple segmentally based role, is an integral part of the complex descending systems that control head movement and enable head movements to be integrated with the requirements of the visual, auditory, and olfactory systems. It is perhaps the close and necessary association of the neck muscle afferent system with these major sensory systems that explains the extreme postural consequences of interference or damage to neck muscle afferents, which have so often been reported (2, 3, 12, 16).

SUMMARY

Silver-stained sections have been examined from the five dorsal neck muscles, splenius, biventer cervicis, complexus, rectus capitis major, and occipitoscapularis. Every serial section was examined for at least one muscle of each type so that a complete description of the spindle distribution and morphology could be made. With the exception of occipitoscapularis (whose prime function is probably in scapula rotation and not in head movement), neck muscles have a remarkably high spindle density. Occipitoscapularis has a spindle density similar to that of hindleg locomotor muscles

(13–19 per gram), but splenius has a density of 46–66 per gram, biventer cervicis 74–96 per gram, complexus 71–107 per gram, and rectus capitis major 48–84 per gram. Such high densities have only previously been seen in small muscles whose total spindle population is not large. Because of the relatively large size of some neck muscles, individual muscles with a spindle content of up to 254 spindles have been found.

In general, those muscles, which because of their histochemistry have been classified as slow, have higher spindle indexes than those muscles which are classified as fast. Even in fast muscles, spindles are usually present in regions rich in slow extrafusal fibers. The spindle distribution for each muscle is similarly organized from animal to animal.

We have introduced the term conjunctive form to describe arrangements in which spindles either share elements or have some degree of physical contact. The proportion of conjunctive forms in some muscles is so great that the majority of spindles are in such arrangements. The content of each type of conjunctive form tends to be characteristic for each muscle. Thus, the number, distribution, and type of spindle complement is a relatively fixed characteristic for each neck muscle. Large numbers of Golgi tendon organs were present in the tendinous inscriptions, commonly in dyad with spindles.

ACKNOWLEDGMENTS

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587

P.K. ROSE*and V.C. ABRAHAMS. Department of Physiology, Queen's University, Kingston, Ontario. Unit response in the superior colliculus and its relationship to activation of receptors in extraocular muscle.

We have recently demonstrated (Abrahams and Rose, J. Neurophysiol. 38 (1975)p.10-18), that the extraocular muscle receptors constitute the most abundant source of afferent information to the superior colliculus in the cat. The nature of the information conveyed from extraocular muscles to the superior colliculus has now been examined. Extraocular muscles were passively stretched by moving an occluded eye with a shaker controlled by a function generator. It was found that the unit response in the superior colliculus to eye movement was a brief burst discharge. This occurred as the eye passed a fixed point in the orbit (displacement threshold). Such a discharge only occurred provided a minimum velocity was exceeded (velocity threshold). Displacement thresholds ranged from 6-30°. Velocity thresholds lay mainly between 50 and 450°/sec, with a second group responding at velocities ranging from 600-750°/sec. Receptors in extraocular muscles can thus signal precise information concerning eye position to the superior colliculus, not when the eye executes slow movements or movements of less than 6°, but only when the eye moves at saccadic velocities for excursions in excess of 6°.

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Proprioceptive Projections to the Spinal Nucleus of the 5th Nerve in the Cat.

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The physiology of the caudal part of the spinal nucleus of the trigeminal nerve (CspV) has still failed to resolve the clinical evidence for a role of this region in pain perception, for the bulk of its trigeminal input is from low threshold receptors. A better insight into the role of the CspV may be obtained if it is regarded, not in terms of sensory physiology, but motor physiology. The upper cervical segments in which CspV terminates contain motoneurones that serve the primary function of head movement. These are also the segments where the sensory axons from neck muscle receptors enter the nervous system. The segmental motor organisation of the upper cervical cord differs in a number of important ways from that in the lumbo-sacral cord. There is no monosynaptic reflex, segmental reflexes are weak, and there appears to be a greater dependence on long looping pathways which enable input from many sensory modalities to influence motor activity. It seems likely that trigeminal input is an important element in this motor system. A microelectrode analysis of input to the upper cervical cord shows that trigeminal afferents and neck muscle afferents excite units particularly in lamina V. These units can give rise to ascending pathways or participate in segmental reflexes (or both). Fibres which ascend in the dorsal columns from neck muscle afferents are associated with CspV. Many terminate in a restricted part of CspV close to the obex on cells that have their activity influenced by light facial stimulation. The association of cutaneous and proprioceptive afferents is characteristic of many kinds of motor organisation and it seems appropriate to revive the older idea that CspV has a motor role. An obvious role is in the organisation of head movement following any facial stimulus. If that stimulus is damaging or potentially damaging then the head movement will be aversive. The association of a pain perception with such an aversive head motor act finds an obvious parallel in the well known association of a pain perception with limb flexor reflexes.

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THE ORGANISATION OF MUSCLE AFFERENT AND CUTANEOUS INPUT TO THE UPPER CERVICAL CORD IN THE CAT.

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The motor functions of the upper cervical cord are mainly confined to head movement. The segmental organisation of this part of the spinal cord would therefore be expected to reflect the intrinsic organisation and specialisation of the head movement system. Certain unique specialisations have already been found in these segments, for example, the absence of a monosynaptic reflex (Abrahams, Richmond and Rose, Brain Res. 92, 130, 1975). We have now made a microelectrode study of the distribution of units in C1 to C3 activated by skin and neck muscle afferent stimulation. In general the distribution of such units is similar to that found elsewhere in the spinal cord in that cutaneous units predominate in the more dorsal region of n. proprius and both muscle and cutaneous afferents project to the deeper regions of the nucleus. At these deep levels convergence and interactions between cutaneous and neck muscle afferents can be demonstrated. As expected the most effective cutaneous stimuli are those applied to areas of the face supplied by the trigeminal nerve, and only a minority of cutaneously activated units could be excited by stimulation of neck skin. It may be concluded that the laminar organisation of afferent projections to the upper cervical cord is similar to that of the lumbosacral cord with one important exception. Segmental relations are displaced and trigeminal input substitutes for segmental cutaneous inflow at least in C1 and C2. Such an arrangement would seem appropriate for a segmental organisation concerned with head movement, for facial stimuli can be prepotent in leading to head movement. It also explains the projection of facial afferents into upper cervical segments, and the fact that at this level there is no morphologically distinct spinal nucleus of the trigeminal nerve. Supported by MRC and Queen's University.

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PHYSIOLOGICAL PROPERTIES OF DORSAL MUSCLES OF THE CAT NECK.

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In previous histochemical studies, we have shown that each large dorsal muscle of the cat neck has a characteristic composition of fibres. We have also shown that these muscles are composed of a mixture of short and long muscle fibres. The physiological properties of these muscles have now been examined. Length-tension curves for passive stretch, twitch and tetanic contractions were constructed. The twitch time characteristics and tetanus fusion frequencies were examined at the muscle length giving peak active tension. These experiments show that splenius is functionally a relatively fast muscle and biventer a slow muscle. Complexus has fast contraction characteristics approaching those of splenius, probably reflecting functional domination by the fast fibre component. Dorsal neck muscles are multiply innervated and previous experiments suggested that each of the nerves might innervate different populations of long and short muscle fibres. It was found that each nerve innervates a relatively fixed region of muscle close to the nerve entry and neighbouring muscle fibre populations innervated from different motor nerves may have quite different length-tension responses and speed of contraction. This could arise from the excitation by different nerves of populations of different fibre length, or it might relate to the internal order of elastic structure within the muscles, or to a combination of both these factors.

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TECTOSPINAL AND TECTORETICULAR CELLS; THEIR DISTRIBUTION AND AFFERENT CONNECTIONS.

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Stimulation of the medial longitudinal fasciculus in the region of the contralateral pontine reticular formation leads to the antidromic excitation of cells in the superior colliculus. About half of these cells can also be antidromically activated by stimulation in the contralateral upper cervical spinal cord and are therefore tectospinal tract cells. The remaining cells are tectoreticular cells. The two cell populations have been found to be unevenly distributed within the superior colliculus, with tectospinal tract cells predominating in mid-regions and tectoreticular cells predominating in anterior regions. Both cell types have similar afferent inputs and are activated by neck and extraocular muscle afferents as well as by visual stimulation. However, prolonged inhibition characteristically follows excitation of tectospinal cells. Statistical analysis did not reveal any other difference in the input to the two cell populations. Measurements of conduction velocities of tectospinal cells antidromically activated by both reticular and spinal stimulation revealed discrepancies best explained by the existence of tectospinal axon collaterals to reticular nuclei. Examination of these discrepancies in conduction velocity suggest that about 80% of the tectospinal cell population have such collaterals. Thus part of the tectoreticular system consists of collaterals of the tectospinal tract. (Supported by M.R.C. of Canada)

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Further studies on the organisation of the upper cervical cord.

The grey matter of the upper cervical cord contains a number of specialised structures which do not appear at other levels of the spinal cord. These include the descending tract of the trigeminal nerve and a precerebellar nucleus, the central cervical nucleus. In the present experiments two aspects of the physiology of these structures have been examined. The first is whether spinal connections of the trigeminal nerve have a motor role in head movement. The second is the nature of the afferent input to the central cervical nucleus. A motor role of the trigeminal system seems certain. Not only do interneurons in C2 and C3 ventral horns respond to trigeminal stimulation after a latency of 1 to 2 msec but it has also been demonstrated that neck motoneurons in C2 and C3 are activated by trigeminal stimulation with latencies as short as 7 msec. The examination of the medial cervical nucleus has shown that its cells receive input from splenius, biventer cervicis and complexus muscle afferents as well as from the trigeminal nerve. It seems likely that one role of the central cervical nucleus may be in the cerebellar organisation of head movements initiated by stimulation of the vibrissae or facial skin.

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PHYSIOLOGICAL PROPERTIES OF DORSAL MUSCLES OF THE CAT NECK. F. J. R. Richmond and V. C. Abrahams, Department of Physiology, Abramsky Hall, Queen's University, Kingston, Ontario K7L 3N6.

In previous histochemical studies, we have shown that each large dorsal muscle of the cat neck has a characteristic composition of fibres which suggests that one muscle (splenius) is functionally fast, one (complexus) has intermediate characteristics, and one (biventer cervicis) is slow. We have also shown that all these muscles have an unusual internal architecture and are composed of a mixture of short and long muscle fibres arranged around collagenous bands called tendinous inscriptions. The physiological properties of these muscles have now been examined.

Muscles were dissected free of their neighbours. The section of lamboidal crest serving as insertion for the muscle under examination was also freed and attached to a tension transducer rigidly mounted on a massive motor-driven base. The muscle origin was fixed by means of clamps on both lower cervical vertebrae and the scapula. Length-tension curves for passive stretch, twitch and tetanic contractions were constructed. The twitch time characteristics and tetanus fusion frequencies were examined at the muscle length giving peak active tension. Results of these experiments confirm the supposition based on histochemical findings that splenius is functionally a relatively fast muscle and biventer a slow muscle. Complexus, which on histochemical grounds appears to be intermediate in its properties, has fast contraction characteristics approaching those of splenius, probably reflecting functional domination by the fast fibre component.

Dorsal neck muscles appear functionally more complex than the more commonly studied cat muscles. The muscles are multiply innervated and previous experiments suggested that each of the multiple nerves to neck muscles might innervate different populations of long and short muscle fibres. In the present experiments it was found that each nerve innervates a relatively fixed region of muscle close to the nerve entry. Neighbouring muscle fibre populations innervated from different motor nerves may have quite different length-tension responses and speed of contraction. The origin and significance of this is not yet clear. It could arise from the excitation by different nerves of populations of different fibre length, or it might relate to the internal order of elastic structure within the muscles, or to a combination of both these factors.

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SENSORY AND MOTOR FIBRE SPECTRA OF NECK MUSCLE NERVES IN THE CAT. G. C. B. Anstee*, F. J. R. Richmond and V. C. Abrahams, Department of Physiology, Abramsky Hall, Queen's University, Kingston, Ontario K7L 3N6.

Descriptions of the fibre composition of muscle nerves have largely been based on a study of nerves which supply muscles of the cat hindlimb. These fibre spectra vary from muscle to muscle and are presumably related both to the content of sensory receptors in any given muscle and to its motor organization. The large dorsal neck muscles responsible for head movement differ considerably in both their motor characteristics and sensory organ content from hindlimb muscles. Dorsal neck muscles of the cat neck have the highest spindle contents of any cat muscle and each muscle is innervated by several well-separated nerves taking origin from different cervical segments. Fibre spectra of nerves to these muscles have now been constructed so that the motor and sensory innervation to these muscles could be examined. Nerve fibres were counted and measured in muscle nerves from cats chronically deafferented on one side. The fibres surviving ganglionectomy were considered to be motor, and sensory fibre content was estimated by subtracting the number of fibres surviving ganglionectomy from the fibre content of the equivalent intact nerve.

Sensory fibre spectra in neck muscle nerves depart substantially from the usual bimodal distribution and have instead a skewed distribution with a single very large peak of fibres 3 to 6 μ in diameter (Gp II and III). Motor fibres constitute only a small proportion of neck muscle nerves, usually less than 20% and fibres in the gamma size range constitute from 64 to 99% of these motor fibres. Some nerve branches were found which were purely sensory, others that were purely motor, and some motor nerves were found containing only gamma fibres. Clearly the fibre spectra of neck muscle nerves are very different from those of typical hindlimb nerves. The relative paucity of Group I fibres is unexpected considering the high content of Golgi tendon organs and muscle spindles in the nerves. The possibility may exist that the usual classification of muscle afferent function based on fibre diameter may not apply for neck muscle nerves. However, there can be no doubt that these nerves have a major sensory role.

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Motor and sensory fibres of neck muscle nerves in the cat

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Motor and sensory fibres of neck muscle nerves in the cat

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The numbers and sizes of nerve fibres to the dorsal neck muscles, splenius, complexus and biventer cervicis have been examined in the cat. The total number of fibres is unusually high as is the content of sensory fibres (estimated as the loss of fibres after ganglionectomy). The fibre spectra of these sensory nerves has an unusually large number of fibres in the group II and III range (3–7 μm) and differs markedly in this way from other muscle nerves. The motor fibres contain a high proportion (64–99%) in the gamma fibre size range. Large motor fibres are absent in the nerves to biventer cervicis (a slow muscle). The ratio of unmyelinated to myelinated fibres in neck muscle nerves is similar to that in hind legs at about 2.5:1.

RICHMOND, F. J. R., ANSTEE, G. C. B., SHERWIN, E. A. et ABRAHAMS, V. C. 1976. Motor and sensory fibres of neck muscle nerves in the cat. Can. J. Physiol. Pharmacol. **54**, 294–304.

Le nombre et la taille des fibres nerveuses se rendant aux muscles dorsaux du cou, splénius, complexe et digastrique sont explorés chez le chat. Le nombre total de fibres est élevé, tout comme le contenu en fibres sensorielles (estimé d'après la diminution du nombre de fibres après ablation ganglionnaire). Ces fibres sensorielles comprennent un nombre inhabituellement élevé de fibres du groupe II et du group III (3 – 7 μm) et diffèrent en cela des fibres innervant les autres muscles. Les fibres motrices contiennent une proportion élevée (64–99%) de fibres ayant la taille des fibres gamma. Les grosses fibres motrices sont absentes dans les nerfs innervant le digastrique (muscle lent). Le rapport (fibres non myélinisées – fibres myélinisées) dans les nerfs innervant les muscles du cou est semblable à celui observé dans les pattes arrières (à peu près 2.5:1).

[Traduit par le journal]

Introduction

The examination of sensory and motor fibres in muscle nerves has provided data essential for the analysis of peripheral mechanisms concerned with motor function. Hitherto attention has largely been directed to muscle nerves of the hind leg (Sherrington 1894; Eccles and Sherrington 1930; Rexed and Therman 1948; Lloyd and Chang 1948; Boyd and Davey 1966; Stacey 1969). The data which are now presented concern a group of three dorsal neck muscles in the cat. These muscles, splenius, complexus and biventer cervicis, have as their primary function, the support and movement of the head (Elliott 1935; Richmond and Abrahams 1975*a*). Because of its relationships with the vestibular system and the major sensory systems of vision and audition, the head motor control system poses a unique problem in motor physiology. The three muscles under examination have already been shown to possess a number of intrinsic specializations that set them apart from other muscle groups. These specializations are seen both in their sensory apparatus, for they possess an unusually high density of both spindles and Golgi tendon organs (Richmond and Abrahams 1975*b*), and also in the unusual organization of their contractile elements, for the muscles are composed of arrays of short and long fibres arranged in a matrix of tendinous inscriptions (Richmond and Abrahams 1975*a*).

In most muscles, nerve fibres from a number of spinal roots fuse to form a common motor nerve. However, in these neck muscles, the motor nerves take origin from three to four separate cervical roots and do not fuse. Instead, each nerve from each spinal root preserves its separate identity for its entire length before branching and entering the muscle (Abrahams and Rancier 1973; Richmond and Abrahams 1975*a*). An examination has now been made

[1]Studentship of the MRC.

of the content and size of individual nerve fibres in the nerve branches from C2 to C4 segments to biventer cervicis, splenius and complexus in cats after a unilateral dorsal root ganglionectomy. Because quantitative data concerning the unmyelinated fibre content of muscle nerves are scanty (*cf.* Stacey 1969), the opportunity was also taken to subject the unmyelinated fibres of selected nerve bundles to electron microscopic examination.

Methods

Surgical Preparation

Seven female cats weighing between 2.4 and 3.5 kg were anaesthetized either (*a*) with a single intraperitoneal injection of pentobarbitone sodium (Pentobarbital Sodium Injection, Haver–Lockhart) (30 mg/kg) or, (*b*) with an intravenous injection of pentobarbital sodium (4.5–5.5 mg/kg) following induction of anaesthesia with a single intramuscular injection of ketamine hydrochloride (Ketalar, Parke–Davis) (30 mg/kg). Acepromazine maleate (Atravet, Ayerst) (0.08 mg/kg, im) and atropine sulphate (0.4 mg/kg, im) were given in conjunction with ketamine anaesthesia. Under aseptic conditions and with the aid of an operating microscope, dorsal root ganglia were removed from the left side of the cats. In two cats (A and B) only the dorsal root ganglion of the second cervical root was excised. This ganglion lies several millimeters distal to the intravertebral foramen, and is easily separated from the ventral root bundle without damage to the ventral root. The dorsal root ganglion of the third cervical root lies within the intravertebral foramen between the axis and the third cervical vertebra.

In two cats (C and D) removal of the left C3 ganglion was attempted without bone removal. This procedure proved unsatisfactory as the ganglion could not be consistently removed without ventral root damage. Three further cats (E, F and G) were therefore subjected to a more extensive operative procedure in which bone overlying the lateral foramina between the axis and C3, and between C3 and C4, were removed. This approach allowed a clear view of the C3 and C4 dorsal root ganglia which could then be excised without damage to the ventral roots or the spinal blood vessels that travel with these roots. The C2 dorsal root ganglion was also removed. The ganglia were fixed in 25% buffered formal saline for histological examination. The area from which bone was removed was packed with gelatin sponge (Gelfoam, Upjohn). A mixture of polymyxin B, bacitracin and neomycin (V-sporin, Wellcome) was sprayed on the wound before closure and a streptomycin and penicillin mixture (Derapen–C, Ayerst), 0.3 cc, was administered intramuscularly following surgery. Motor behavior of all cats was observed in the immediate post-operative period.

Between 28 and 35 days after ganglionectomy, the animals were reanaesthetized with pentobarbital sodium, 30 mg/kg, ip. Segments at least 5 mm long of all muscle nerve branches to splenius, biventer cervicis and complexus were removed on both the operated and nonoperated sides approximately 5 mm distal to the point of nerve entry into the spinal cord. When branching occurred before the level of sampling, the branches were separately removed. The C2 muscle nerve branches innervating biventer cervicis are accompanied by the greater auricular nerve, a cutaneous nerve to the pinna. This nerve was usually stripped from the muscle nerve bundle before histological preparation. In two of the five animals where the presence of scar tissue prevented this dissection, the greater auricular nerve was removed and processed together with the muscle nerves. By also removing a distal segment of the isolated greater auricular nerve, the cutaneous nerve could be identified in the large fibre bundle and excluded from the count.

Histology

Ganglia

Following fixation for 7 days in 25% buffered formal-saline, ganglia were cleared and embedded in a mixture of paraffin and plastic polymers (Paraplast, Sherwood) and sections were cut serially at 15 μm. The sections were stained with Holme's silver method (Drury and Wallington 1967).

Nerve Bundles

Nerve branches were rinsed immediately upon removal from the animal in sodium cacodylate buffer (0.1 *M*, pH 7.4 $\pm$ 0.2), cut into 1–2 mm lengths, and fixed in 3% gluteraldehyde for 30–180 min. The tissue was then rinsed in Millonig's phosphate buffer at pH 7.4 and further fixed with 1% osmic acid for 30–120 min. After washing with several changes of phosphate buffer, the nerves were dehydrated and cleared in graded alcohols and propyleneoxide, and embedded in Epon 812 resin.

Transverse sections of all nerves were cut at 0.5–1.0 μm using an LKB ultramicrotome and stained with toluidine blue.

Electron Microscopy

Blocks of all normal nerves taken from the first four cats (A, B, C and D) were further cut at 60–90 nm and mounted on copper grids (mesh 300). These sections were stained with uranyl acetate (7% uranyl acetate in 30% methanol) and lead citrate and dried. A Hitachi type HS7S electron microscope was used for examination and photography.

Light Microscopy

A montage method was employed for the construction of fibre spectra in cats A, B and C. Using the light microscope, photographs of sections of the entire nerve were enlarged to an overall magnification of 1000 times, trimmed and montaged. To count and measure fibres the montage was then rephotographed and the slide projected on to a screen at a magnification of 2000 times. Myelinated fibre diameter was measured with the aid of a Plexiglas sheet with circular cut-outs of known diameter. Photomicrographs of whole nerve branches were made at 40 times magnification in cats E, F and G. This slide was similarly projected at a

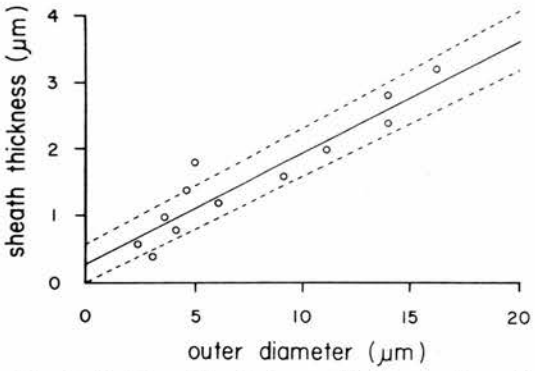

FIG. 1. Relationships between thickness of myelin sheath and overall fibre diameter. Measurements made on 625 axons from a single normal splenius nerve. Open circles, means of original data. Solid line, regression line computed from the means. Dotted lines, 95% confidence limit of the regression line.

2000 times magnification onto a screen for fibre diameter measurement.

Two procedures have been used previously to indicate the size of myelinated nerves. In the first the data has been expressed as the mean of the inside and outside diameter of the myelin sheath (Lloyd and Chang 1948). More recently, the outside diameter alone has been used for measurement (Sima 1974) and this latter procedure has been adopted in the present work. So that the data presented here may be compared with earlier measurements from which the myelin sheath diameter has been partly subtracted, the thickness of more than 600 myelin sheaths was measured in one normal splenius nerve. The relationship between overall diameter and myelin thickness is shown in Fig. 1. The regression line equation was computed for this data and showed the relationship to be $x = 0.17\ y + 0.29$ (correlation coefficient 0.94), where x is the thickness of the myelin sheath and y the overall fibre diameter.

As Fig. 2 shows, not all myelin sheaths are completely circular and some criteria must be adopted to standardize measurements. When a fibre had an irregular outline the largest diameter was measured, obliquely cut fibres were measured across their narrowest diameter (Sima 1974). Measurements were rounded off to the nearest micron. To estimate the effect of ganglionectomy the assumption was made that the nerve fibres would be approximately symmetrical in their distribution on both sides of the cat so that the unoperated side could provide control values. While this has been a common practice, in two instances the ganglionectomized side had more fibres than the unoperated side. This data implies that nerve fibre content is not always bilaterally symmetrical and measurement based on this assumption will always be open to some error.

No shrinkage correction has been applied to the measurements. It has been shown that paraformaldehyde–gluteraldehyde fixation followed by osmium

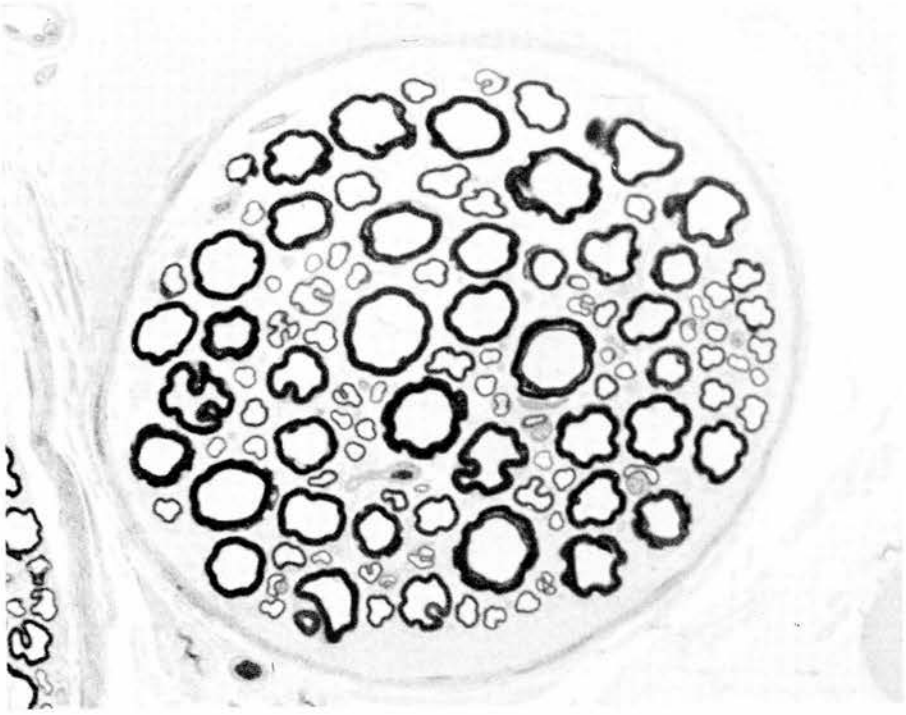

FIG. 2. Transverse section of branch of normal biventer cervicis nerve. Magnification ×125.

tetroxide post-fixation and Epon 812 embedding, a procedure similar to that used here, causes less than 5% shrinkage in nerves (Sima 1974). Since the fibre sizes measured in the present work were the same as or larger than those reported where correction for shrinkage was made (Stacey 1969), it seemed likely that little shrinkage had occurred. When classifying motor nerves as alpha or gamma, 7.5 μm has arbitrarily been used as the dividing line between alpha and gamma fibres (Matthews 1972). No functional significance can be attached to this arbitrary division as yet. However, as Fig. 4 shows, the distribution of neck muscle motor nerves is often bimodal with a natural boundary lying between 7 and 10 μm.

The counts of unmyelinated fibres were made from EM photomicrographs taken at magnifications of from 2000 to 5000 times. Six to fifteen random sample fields were taken for each nerve. The numbers of unmyelinated and myelinated axons were then counted in each field. Sample sizes per field ranged up to 35 for myelinated fibres and 200 for unmyelinated fibres. The ratio of unmyelinated to myelinated fibres for each nerve was established by pooling the data from all samples.

Results

Effects of Operative Procedures on Posture and Locomotion

Dorsal root ganglionectomy of one or more left upper cervical roots may lead to defects of posture and locomotion. The degree of deficit is variable and appears related to the extent of the deafferentation. Of the two cats which had C2 ganglionectomy, one showed few postural or locomotor alterations. The second cat had a transient ataxia from which it recovered within 7 days, reminiscent of the mild ataxia that can follow section of neck muscle motor nerves (Abrahams and Falchetto 1969). However, the three cats in which C2–C4 ganglia were removed exhibited prolonged and profound defects. These cats were unable to walk or stand for a period of up to 3 days following operation. Any attempt by the animal to rise was unsuccessful and the animal would collapse, usually to its left side. After 3 days, the animals regained some ability to move about, but lateropulsion was marked and the animals continued to fall to their left side, particularly when attempting to turn. Recovery was considerable within 1 week and although some locomotor ataxia was still obvious, the animals could progress in an adequate fashion. After 4 weeks, one cat continued to walk on a broad base and to collapse when attempting to turn. Both the other cats had recovered completely. At no time in the post-operative period was nystagmus seen in any animal.

Myelinated Fibre Content

The results to be presented include data taken from only six of the seven cats subjected to ganglionectomy as one cat (D) subjected to C3 ganglionectomy obviously suffered ventral root damage, and these nerves showed almost total degeneration. Data for the second cat (C) subjected to this same operative procedure appear consistent with those seen in subsequent C3 ganglionectomies, although the ganglionectomy was imperfect and the excised ganglion was seen to have an interrupted outer capsule and a shredded appearance. Ganglia taken from the other five animals (A, B, E, F and G) had been completely and cleanly removed. The capsule surrounding each ganglion was intact and dorsal root entry and exit zones could be identified (Fig. 3).

Compared to hind limb muscles of similar size (Boyd and Davey 1966; Abrahams and Rancier 1973), the numbers of fibres innervating neck muscles are very high. Data taken from the unoperated side of cats E, F and G show that of the three muscles examined, biventer cervicis receives the richest innervation, with an average myelinated nerve fibre count of 3282 in the C2–C4 nerves (Table 1). Splenius and complexus have an average fibre count from these same segments of 1522 fibres, and 956 fibres, respectively. These counts will not show the total innervation, for nerve fibres innervate splenius and complexus from the C1 segment and biventer cervicis from segments below C4.

The fibre spectra of intact nerves to neck muscles show some departures from the spectra observed in nerves to hindlimb muscles. In general (Fig. 4), the myelinated fibre diameters have a skewed distribution from 1.5 to 24 μm with a single obvious peak between 3 and 5 μm. The bimodal distribution which is normal for hind leg nerves (Eccles and Sherrington 1930; Rexed and Therman 1948; Boyd and Davey 1966) was only occasionally seen with a second peak at 12–17 μm (*cf.* splenius C4, complexus C2, and C3, Fig. 4). Even when such a second peak was obvious it was always reduced in comparison with the small fibre peak.

Following ganglionectomy, the majority of

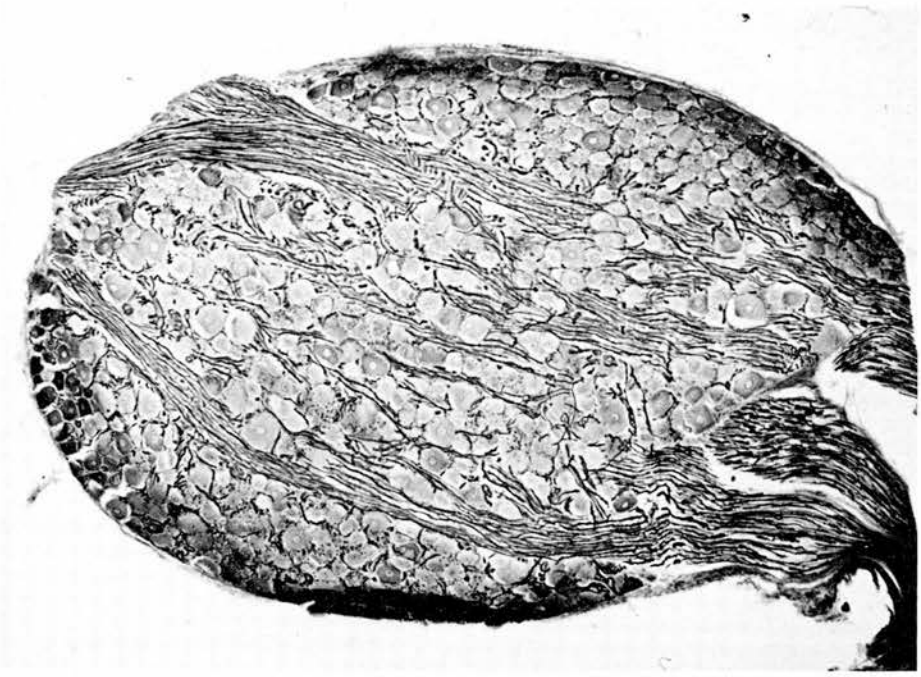

FIG. 3. Transverse section of excised dorsal root ganglion. Magnification ×50.

TABLE 1. Motor and sensory content of nerves supplying splenius, biventer cervicis and complexus. Data from three cats subjected to C2–C4 ganglionectomy. Alpha fibres are defined as those with diameters above 7.5 μm. Sensory content computed from fibre loss on operated side following ganglionectomy

Nerve	Cat	Total	Motor	Sensory	Total alpha	Total gamma	% gamma of motor component
Splenius	E	1096	298 (27%)	798 (73%)	134	164	55
	F	1520	707 (47%)	813 (53%)	221	486	69
	G	1951	398 (20%)	1553 (80%)	144	254	64
Biventer cervicis	E	4196	194 (5%)	4002 (95%)	58	136	70
	F	3131	793 (25%)	2338 (75%)	201	592	75
	G	2520	308 (12%)	2212 (88%)	23	285	93
Complexus	E	1230	163 (13%)	1067 (87%)	29	134	82
	F	945	442 (47%)	503 (53%)	152	290	66
	G	785	183 (23%)	602 (77%)	34	149	81

myelinated fibres to all three neck muscles were lost. There is no reason to suppose that this was due to ventral root damage and thus represents a deafferentation. An average of only 24% of myelinated fibres innervating complexus and splenius and 18% of fibres innervating biventer cervicis did not degenerate following ganglionectomy. All but 5% of myelinated fibres to one biventer cervicis muscle and 13% of the fibres to one complexus muscle were lost (Table 1).

Of the fibres remaining after ganglionectomy, most were of small diameter. Only 36% of splenius, 30% of complexus and 22% of biventer cervicis fibres had diameters exceeding 7.5 μm. In the classification of motor fibres, those with diameters exceeding 7.5 μm are regarded as alpha motor fibres, those with diameters below 7.5 μm as gamma fibres (Matthews 1972). Biventer cervicis, which has the histochemical characteristics of a slow twitch muscle (Abrahams and Rancier 1973; Richmond and Abrahams 1975*a*), rarely had any ventral root fibres with diameters exceeding

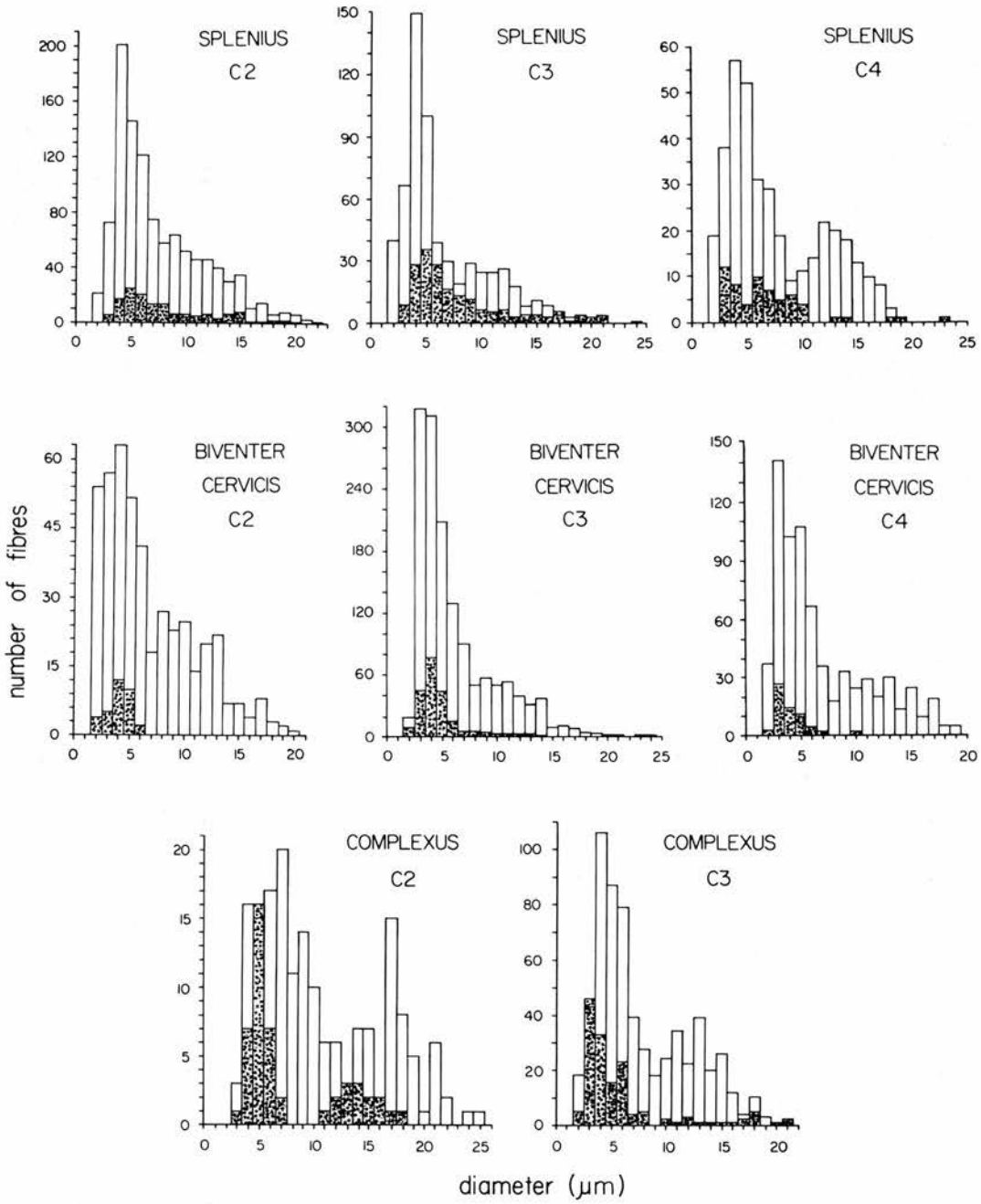

FIG. 4. Histogram of fibre diameters in cat G in individual branches to neck muscles. Open columns, total fibre count on non-operated side. Shaded columns, fibre count following C2–C4 ganglionectomy.

10 μm (Fig. 4). In contrast, splenius, and complexus, which histochemically have a substantial component of fast muscle fibres (Abrahams and Rancier 1973; Richmond and Abrahams 1975*a*) commonly have ventral root nerve fibres with diameters between 10 μm and 20 μm and some fibres between 20 and 25 μm in diameter.

Alpha fibres were not evenly distributed in different nerve branches. Of the 84 deafferented nerve branches examined, 21 contained no alpha fibres and consisted exclusively of fibres with diameters below 7.5 μm. By definition these branches are entirely composed of gamma fibres and thus could be exclusively fusimotor. Of the 21 branches which contained only gamma fibres, 15 innervated biventer cervicis, four innervated complexus and two innervated splenius. No deafferented branches were found which contained only alpha fibres.

A few nerve branches were found which may have been exclusively sensory. In cat F, 8 of the 34 deafferented branches showed degeneration of all myelinated fibres. These presumed sensory nerves were present at all three cervical levels and innervated all three muscles. The presence of normal myelinated fibres in the remaining 26 nerve branches from the same segments argues against the degeneration being due to damage to the ventral root or its blood

TABLE 2. Motor fibre content of nerves to neck muscles splenius, biventer cervicis and complexus, emerging at different segmental levels. Alpha fibres are those with diameters above 7.5 μm. Gamma fibres are those below 7.5 μm. Data from cats subjected to C2–C4 ganglionectomy

Cat	Segment	Total motor	Alpha	Gamma	% gamma
E	C2	258	85	173	67
	C3	302	110	192	64
	C4	95	26	69	73
F	C2	439	108	331	75
	C3	1289	464	825	64
	C4	214	2	212	99
G	C2	232	82	150	65
	C3	534	98	436	82
	C4	123	21	102	83

TABLE 3. Fibre content of normal and deafferented nerves to splenius, biventer cervicis and complexus from different cervical segments

Cat	Cervical segment	Muscle nerve	Total fibre count	Fibre count after deafferentation	% motor	% gamma (of total motor count)
A	C2	Splenius	680	197	29	83
		Biventer	860	550	64	99
B	C2	Splenius	320	750		87
		Biventer	1280	460	36	96
E	C2	Splenius	379	92	24	63
		Biventer	493	43	9	49
		Complexus	594	123	21	76
	C3	Splenius	473	131	28	38
		Biventer	2415	171	7	60
		Complexus	636	40	6	100
	C4	Splenius	244	75	31	75
		Biventer	1288	20	2	65
F	C2	Splenius	944	173	18	76
		Biventer	910	20	2	65
		Complexus	399	246	62	76
	C3	Splenius	262	450		61
		Biventer	1108	643	58	70
		Complexus	546	196	36	53
	C4	Splenius	314	84	27	98
		Biventer	1113	130	12	100
G	C2	Splenius	970	144	15	57
		Biventer	443	40	9	88
		Complexus	170	48	28	69
	C3	Splenius	617	194	31	67
		Biventer	1459	205	14	93
		Complexus	615	135	22	86
	C4	Splenius	364	60	17	70
		Biventer	618	63	10	95

TABLE 4. Content of unmyelinated fibres in electron micrographs of neck muscle nerves expressed as a ratio of myelinated nerve fibres

Muscle	Cat	Non-operated nerve	Deafferented nerve
C2 splenius	A	1.5	3.3
C2 splenius	B	2.6	2.7
C2 biventer	A	2.4	11.2
C2 biventer	B	6.1	5.3
C2 complexus	B	1.6	4.2
C3 splenius	C	1.5	
C3 biventer	C	3.9	
C3 biventer	C	1.6	
C3 complexus	C	1.4	
Mean		2.5	

supply. Such completely denervated branches were also found in cat C innervating biventer cervicis and splenius, but not complexus.

In the three cats subjected to C2–C4 ganglionectomy, most motor fibres emerge from the C3 segment and fewest from C4 (Tables 2 and 3). An average of 309 nerve fibres originate from the C2 segment, 708 from the C3 segment and 144 from the C4 segment. About $\frac{1}{3}$ of the total number of motor fibres in C2 and C3 branches and 11% of fibres in the C4 branch were alpha fibres. It can be seen from Table 2 that on the average, gamma fibres outnumber alpha fibres by a ratio of 2:1, but this ratio approaches 4:1 in some nerve branches, and in one instance, almost all fibres taking origin in C4 were gamma fibres.

Figure 5 shows the distribution of sensory fibres to splenius, biventer cervicis and complexus in cat G, but is typical of all those examined. It can be seen that because most of the fibres are sensory the histograms are very similar to those of whole nerves. It has been noted in some muscle nerves that afferent nerve spectra exhibit a trimodal distribution corresponding to group I, II and III fibres (Lloyd and Chang 1948; Stacey 1969). Such clear trimodality does not appear in neck muscle fibre spectra. The dominant peak is still at 4–6 μm, and although there are substantial numbers of large fibres present, they are evenly distributed, forming a continuum from 7 to 15 μm and then gradually decreased in number with the largest fibres being 23–25 μm in diameter.

In two instances, as referred to in Methods

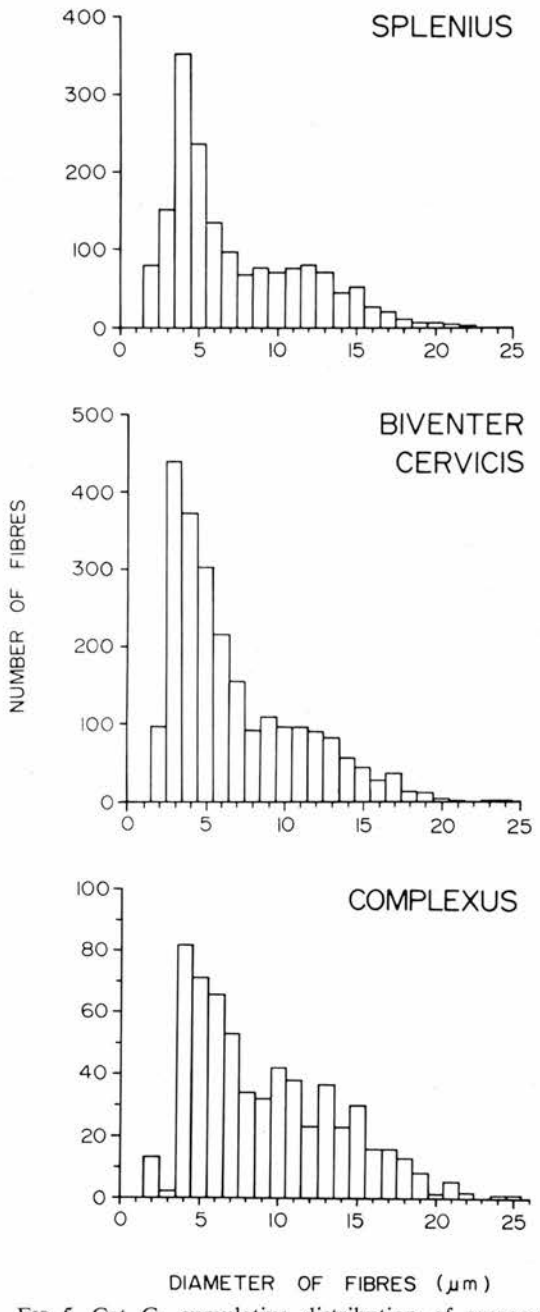

FIG. 5. Cat G, cumulative distribution of sensory fibres to neck muscles from C2 to C4 dorsal roots.

(cat F, C3 splenius, and cat B, C2 splenius; Table 3) the number of fibres found on the deafferented side was greather than that on the nonoperated side. In both these nerve roots, the fibre count after deafferentation was unusually

high suggesting some lack of symmetry between equivalent nerves on the two sides of the animal.

The ratio of unmyelinated to myelinated fibres was examined in samples taken from a total of nine normal muscles nerves (Table 4). These ratios ranged from 1.5:1 to 6.1:1 (mean 2.5:1). A segment of greater auricular nerve was also examined and showed a ratio of 12:1, closely resembling that normally found in cutaneous sensory nerves. Unmyelinated fibres were also examined in deafferented nerves. In these nerves it was common to see Schwann cells with highly irregular outlines suggesting either the loss of unmyelinated fibres or Schwann cell fractionation (Fig. 6). Schwann cells of normal nerves were usually found to contain from 4 to 20 unmyelinated fibres. After ganglionectomy Schwann cells usually contained only one to three unmyelinated fibres and never more than eight.

Discussion

The present experiments reinforce the fact that the composition of muscle nerves varies considerably from one skeletal muscle to another. In neck muscle nerves, three characteristics are outstanding, the very high number of nerve fibres, the very high proportion of sensory fibres and the very high proportion of sensory fibres in the group II and III size range. The number of nerve fibres innervating each of the larger hind limb muscles in the cat range from 400 to 1000 of which approximately 35–60% are sensory (Boyd and Davey 1966). The innervation of neck muscles is obviously quite different from large hindlimb muscles, but resembles instead the composition of the plantar nerves which supply the small muscles of the cat paw. Plantar nerves have a total of from 1700 to 3000 nerve fibres of which from 40 to 90% are sensory (Boyd and Davey 1966).

Most hind limb muscle nerves have a clear bimodal fibre distribution with one peak between 3 and 8 μm, and a second equal peak between 10 and 16 μm (Eccles and Sherrington 1930; Rexed and Therman 1948; Boyd and Davey 1966; Stacey 1969). Neck muscle nerves exhibit one very large peak in the 3–8 μm (groups II and III) range, and only in a few branches is this accompanied by an equal peak in the 10 to 16 μm (group I) range. The plantar nerves, despite their superficial similarity to neck muscle nerves, differ in this respect and have more group I fibres than group II fibres (Boyd and Davey 1966).

Most of the group II fibres in neck muscle are sensory and might be expected to come from the dense accumulation of spindles. Group II and III fibres are normally considered to serve spindle secondary endings and free nerve endings respectively (Stacey 1969). The numbers of primary and secondary spindle endings in a muscle are approximately the same (Matthews 1972) and thus the groups I and II fibre populations should be similar. The great preponderance of smaller sensory fibres in neck muscle could be due to an unusually high number of spindle secondary endings or to little peripheral branching in fibres serving them. Alternatively, the large number of group II and III fibres could be related in some way to the high proportion of conjunctive spindle forms in muscle (Richmond and Abrahams 1975*b*). Other possibilities are that many small afferent fibres may originate from as yet undiscovered receptors, or the relationship between an afferent fibre's size and the receptor that it serves may not hold in neck muscle afferents.

The gamma fibre content in hind limb muscle motor fibres is usually less than 55%, and in soleus and medial gastrocnemius falls below 40% (Eccles and Sherrington 1930; Boyd and Davey 1966). Neck muscle nerves have a gamma fibre content in individual nerve branches from 55 to 93%. This presumably relates to the high content of spindles in neck muscles which have spindle indices four to six times greater than hind leg muscles (Richmond and Abrahams 1975*b*).

Splenius and complexus motor nerves contain alpha fibres up to 24 μm in diameter but biventer motor fibres were never found to exceed 19 μm and generally were less than 15 μm in diameter. Histochemical (Abrahams and Rancier 1973; Richmond and Abrahams 1975*a*) and functional testing (Abrahams, Lywood, Milligan, and Richmond, unpublished observations) indicate that biventer cervicis is predominantly a slow twitch muscle, while splenius and complexus are predominantly fast muscles. Thus neck muscle nerves follow the general rule that fast motor units are supplied

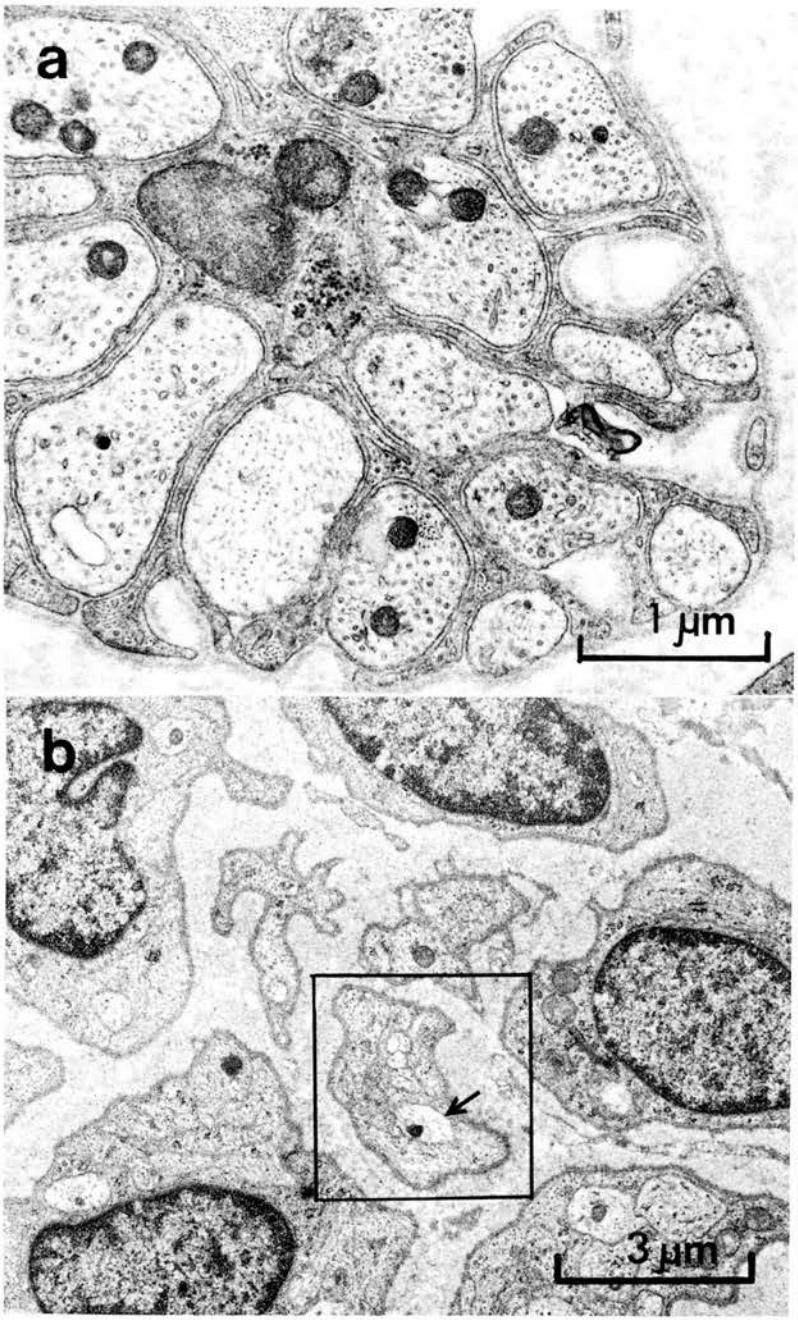

FIG. 6. Electron micrographs of Schwann cells in normal and ganglionectomized muscle nerves. (*a*) Schwann cell from normal C2 splenius nerve. Note the regular outline and the presence of large numbers of unmyelinated axons. (*b*) Schwann cells from ganglionectomized C3 biventer cervicis nerve. The Schwann cells have proliferated so that the section shows irregular protoplasmic fragments each containing but a few axons. The arrow indicates a single axon in an irregular Schwann cell fragment.

by large fibres and slow motor units by small fibres (Henneman and Olson 1965).

Motor nerves to the same muscle taking origin in different cervical segments have been found to have some characteristic differences in both their motor and sensory fibre distribution. For example, there is a preponderance of larger motor fibres in the C2 branch to complexus. These differences assume some division of functional responsibilities between roots. What was not anticipated was the presence of nerve branches which may be purely fusimotor or purely sensory.

The examination of unmyelinated fibres has shown that the ratio of unmyelinated to myelinated fibres is very similar to that found in hind leg muscle nerve (Stacey 1969). The role that might be played by unmyelinated nerve in muscle is uncertain. The fact that they consistently outnumber myelinated fibres attests to an important functional role. It would be of interest to know whether myelinated and unmyelinated afferent fibres carry out separate and unrelated functions, or whether, as in cutaneous nerve, some act together to influence the ascending transmission of proprioceptive impulses at spinal levels.

In an incidental way these experiments confirm the unusual and widespread nature of postural and locomotor deficits that follow damage to neck muscles or their nerves (Cohen 1961; Biemond and DeJongh 1969; Abrahams 1972). There seems little doubt that the information that is derived from the receptors in neck muscle plays a critical role in the organization of posture and locomotion.

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SPINAL AND MEDULLARY PROJECTIONS FROM NECK MUSCLE AFFERENTS IN THE CAT

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SUMMARY

Microelectrode recording in the upper cervical cord and lower medulla of chloralose anaesthetized cats has been used to trace the pathways followed by neck muscle afferents. The major ascending pathway contains group I, II, and III fibres and travels in the lateral margin of the dorsal column and ascends into the medulla dorsal and medial to the spinal nucleus of the trigeminal nerve. Second and later order units are activated by neck muscle afferents in the spinal trigeminal nucleus and in the deep layers of the cuneate nucleus. Complex interactions occur in the trigeminal nucleus between neck muscle projections and those from facial afferents. In the spinal cord many neck muscle afferents terminate on lamina IV and lamina V interneurons in regions served by facial afferents. Complex interaction occurs between the two sets of afferents at this level. No evidence could be obtained for the existence of a monosynaptic reflex in the upper cervical cord.

KEY WORDS

Neck muscle afferents; spinal cord; medulla; trigeminal system.

RUNNING TITLE: NECK MUSCLE AFFERENT PROJECTIONS

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INTRODUCTION

The dorsal neck muscles that serve to move the head of the cat are characterised by an unusually high content of muscle spindles and Golgi tendon organs (Richmond and Abrahams, 1975b). Section of neck muscle nerves (Abrahams and Falchetto, 1969), local anaesthesia of upper cervical dorsal roots which serve neck muscles (Cohen 1961), local anaesthesia of neck muscles (Biemond & DeJongh, 1969; Abrahams & Falchetto, 1969) or upper cervical dorsal root gaglionectomy (Richmond, Anstee, Sherwin and Abrahams, 1975) all give rise to generalized defects of posture and locomotion. Little is known of the precise nature of mechanisms that could underly such widespread postural defects, but it is known that neck muscle afferents project to cortex (Landgren & Silvenius, 1968), cerebellum (Berthoz & Llinas, 1974), and superior colliculus (Abrahams & Rose, 1975). It is also known that neck muscle afferents give rise to powerful spino-spinal effects (Abrahams & Falchetto, 1969; Abrahams, 1970, 1971) and influence neural activity in Deiter's nucleus (Mori & Mikami, 1973).

The spindles of neck muscles are distinctive, not only because of their abundance, but also because of their consistent and orderly pattern within neck muscles and because of the high incidence of conjunctive spindle forms. In the present work, we have examined the immediate destination of afferent fibres leaving neck muscles.

Extensive anatomical studies have previously been made of spinal and medullary degeneration following section of whole dorsal roots (Ranson, Davenport and Doles, 1932; Corbin and Hinser, 1935; Escolar, 1948, Liu, 1956; Imai and Kusama, 1969; and Kerr, 1972) but the anatomical method does not differentiate between cutaneous and muscle afferent projections. An electrophysiological examination has therefore been made of the projections to the upper cervical cord and lower medulla

from neck muscle afferents in the cat. The afferents examined take origin in 3 large dorsal muscles, splenius, complexus and biventer cervicis that support, elevate and turn the head (Elliott, 1935; Fichmond & Abrahams, 1975a). Convergence between these muscle afferent and cutaneous afferent pathways has also been examined. Some of the data has previously been reported in brief (Abrahams and Rose, 1974; Abrahams, Richmond and Rose, 1975).

METHODS

Experiments were performed on cats weighing between 2.8 and 3.3 Kg, anaesthetized with a single i.v. dose of 60 mg/Kg chloralose following induction with ethyl chloride and ether. The animals were then placed either in a conventional stereotaxic head holder (Type "A":, La Precision Cinematographique, Asniere, France) or one for use in visual experiments (Type "U", La Precision Cinematographique, Asniere, France), so that the face was readily accessible. Laminectomy was performed to expose the lower medulla and upper cervical spinal segments. For more extensive medullary explorations, the posterior fossa was opened and the cerebellum removed by sub-pial aspiration, but in later experiments the cerebellum was left intact. The C2 or C3 branches to the neck muscles, biventer cervicis and complexus run together and the joint nerves were placed on small paired platinum stimulating electrodes and secured in place with low melting point (39°) paraffin wax. When C2 nerves were employed, great care was taken to separate the greater auricular nerve (which travels with biventer cervicis nerve) so that it was not stimulated. In a number of experiments, splenius nerve was also

prepared for stimulation. Accurate placement of stimulating electrodes was checked by monitoring the threshold stimulus strength necessary to elicit muscle contraction. With the stimulator used (Devices type 2533) this normally ranged from 0.05 to 0.2 V.

In some experiments, the presence of a monosynaptic reflex was investigated. In the earliest experiments this was done by recording from whole muscle nerves while stimulating the central cut end of dorsal roots. In later experiments biventer cervicis, splenius and complexus motoneurones were identified by antidromic excitation and then tested for their responsiveness to orthodromic stimuli. In these experiments, the upper cervical dorsal roots were cut close to their point of emergence from the dura. Upper cervical rootlets are short and each segmental input is distributed over 1 cm or more of cord. To stimulate the rootlets, long (about 15mm) paired platinum stimulating electrodes separated by 1 mm were laid close to and parallel to the dorsal columns and all the rootlets from the segment placed across the electrodes.

Cutaneous receptive fields were mapped by light touch with a fine camel hair brush. When necessary, timed discrete cutaneous stimuli could be produced by the movement of a fine bristle or fine tungsten wire mounted on a Vibrator (Ling-Altec, Model 203 or Bruel and Kjaer, Type 4810). Pulses formed by a function generator and triggered by a Digital Programmer (Devices No. 4030) controlled the timing and excursion of the stimulus. Gentle movement of limb joints was also examined for evidence of limb proprioreceptive projection.

To minimize tissue movement during unit recording, a bilateral pneumothorax was performed, and the animal was artificially ventilated following paralysis with gallamine triethiodide (Flaxedil, Poulenc). The stereotaxic machine was raised from the table so that the animal's body was partially suspended. Body temperature was maintained at or close to 38°C by a heated blanket controlled by a rectal thermistor. All exposed nervous and muscle tissue was protected by a coating of low melting point paraffin wax.

The recording and display equipment used was conventional and incorporated spike filtering and raster generating circuits. Commercially available tungsten microelectrodes (Haer, Type 25-10-3) were used for recording. The criteria for a primary afferent unit potential was an all or none (usually monophasic) response of short latency which reliably followed stimuli delivered at 300-500/sec. Similar potentials with short latencies and the ability to follow very high frequencies, but which increased their amplitude with increasing stimulus strengths were regarded as field potentials. Units with short latencies which followed stimuli up to about 10/sec were classified as 2nd order units. Stimulation voltages are expressed as a multiple of threshold (T) which was the voltage necessary to produce a just detectable compound action potential at the point of dorsal root entry, or the shortest latency component of a primary afferent field potential. At the termination of each micro-electrode penetration, a current of approximately 50 micro amps was passed through the recording microelectrode for 1 to 4 seconds to produce a lesion (microelectrode cathode). At the termination of the experiment, the brain was perfused <u>in situ</u> with 25% formalin in saline through one carotid artery. The spinal cord was sometimes

fixed by whole body perfusion with formalin or more commonly, removed unfixed and fixed by immersion in 25% formalin in saline. The brain or spinal cord was then serially sectioned at 30 μ on a freezing microtome, and sections were stained with Luxol Fast Blue and Cresyl Violet (Klüver and Barrera, 1953). All sections were retained and examined so that the point of maximal electrode penetration could be determined, and the electrode track then reconstructed.

In later experiments, the electrode was advanced only a few hundred microns beyond the recording site before marking (in order to localise recording sites with the maximum certainty).

RESULTS

Primary afferent projections - upper cervical cord

The potentials associated with conduction by primary afferents in the spinal segments C1 to C3 were examined in 9 experiments. In all experiments, field potentials were observed in the dorsal columns from an ipsilateral region close to the dorsal root entry zone. The initial low threshold waveform had a latency of 0.4 msec, but as the stimulus strength was increased, later waves appeared with latencies of up to 4.5 msec (Figure 1a). The amplitude of the field potential increased as the electrode was advanced, reaching a peak in the lateral regions of the dorsal columns close to the junction with the grey matter of the dorsal horn. It was then common to record short latency all or none unit potentials (Figure 9 b). Occasional all-or-none spikes with latencies of about 0.4 msec were also recorded as the electrode was advanced into the dorsal horn.

In lamina IV of the dorsal horn, the most common response to muscle afferent nerve stimulation was a burst of activity with a latency of from 1.5 to 6.0 msec exhibiting the characteristics of second order units. Unit discharge with latencies in excess of 6 msec was present in lamina IV and as the electrode entered lamina V this long latency response became the dominant response. The distribution of primary afferent fibres and second order units within the spinal cord is shown in Figure 2.

Primary afferent projections, Medulla

The medullary course of primary afferents was examined in 24 experiments. In 7 of these experiments, explorations were confined to planes 2 - 8 mm anterior to the obex, mostly in the region of the external cuneate nucleus. In all experiments except one, biventer cervicis/complexus nerves only were stimulated. Primary afferents were only rarely encountered in these anterior penetrations, and then were found in a narrow band just dorsal to the spinal nucleus of V. In the remaining 17 experiments in which penetrations were made at levels close to or posterior to the obex, primary afferent field and unit potentials with minimum latencies from 0.6 to 0.8 msec were consistently found to follow stimulation of ipsilateral muscle afferent nerves. Both unit and field potentials were encountered in a narrow fibre-rich band ventral to the external cuneate nucleus and dorsal to the nucleus of the spinal tract of V (Figure 3). Physiologically this region was found to be deep to that from which forepaw joint movement readily elicited unit discharge and above that from which facial stimuli readily elicited unit discharge. Similar responses could also be recorded from a band

which extended ventromedially along the lateral margin of the cuneate nucleus where the cuneate and trigeminal nuclei abut. Primary afferent potentials were also consistently found along the ventral border of the spinal nucleus of V. Unlike the other primary afferent projections at medullary levels the primary afferent input here was bilateral.

Increasing the strength of stimulation of neck muscle nerves invariably led to the appearance of additional primary afferent waves with latencies of up to 6 msec at all medullary sites. The calculated conduction velocities to the medulla from neck muscle ranged from 12 to 120 msec (Figure 3).

In 5 of the experiments in which the medulla was explored, the splenius nerve was stimulated as well as the complexus/biventer cervicis nerve. Evidence was obtained in these experiments that some separation occurs between afferent systems from the different muscles. Primary afferent potentials in and close to the spinal nucleus of 5, were recorded following stimulation of either nerve. The splenius nerve projection appears confined to this region and primary afferent potentials could only be recorded from the vicinity of the cuneate nucleus following biventer cervicis/complexus stimulation.

Second order units with latencies of 1.5 to 4.0 m/sec were common in the dorsal border of the spinal nucleus of V. Units in this region could be activated by Group I afferents, but their frequency and rate of discharge increased as the stimulus strength was increased to about 10T suggesting that Group II afferents activated the same units. Second order units activated by Group I and II inputs were also found in the deep layers of the cuneate nucleus. Careful examination of brain regions in the interstitial nucleus of Cajal and the external cuneate nucleus

failed to show the presence of neck muscle primary afferents or second order units in either of these nuclei.

Long latency responses in spinal cord and medulla following neck muscle afferent stimulation

It was common when recording in both medulla and upper cervical cord to find units responding to neck muscle afferent stimuli at latencies from 15-60 msec. In general such units responded to a single stimulus with a train of potentials lasting 20 msec or more, but were often best activated by stimulus trains of at least 3 - 5 impulses at 100/sec. While such long latency units could be excited at threshold stimulus levels, they responded most consistently when the stimulus was at least 3T and usually increased their response as the stimulus was increased to 10T. Such unit discharge would not follow stimulation that was too rapidly presented, but became more consistent when stimuli were presented at intervals of 4 seconds or longer.

In the spinal cord, second order units in lamina IV were exclusively innervated from ipsilateral muscle nerves, but long latency units in lamina V to VII and in the medulla were often bilaterally innervated and also received input from muscle nerves entering the cord in segments distant to the recording site. Commonly a unit received ipsilateral exictation from one muscle and contralateral excitation from another muscle. Long latency units were not confined to the spinal grey matter, but were also present in the ventral funiculus in the regions occupied by the tectospinal and vestibulospinal tracts (Figure 4a).

In the medulla, long latency units responding to trains of neck muscle stimuli were particularly common within the spinal

nucleus of V, in the ventromedial core (which corresponds to the subnucleus magnocellurlaris). In the cuneate nucleus, long latency units were also largely restricted to deep layers. Long latency units were also found in a number of other medullary structures including the lateral reticular nucleus and medial reticular areas within the lateral tegmental field (Figure 3).

Convergence of neck muscle primary afferents in the spinal nucleus of V and the cuneate nucleus

The dorsal horn of the upper cervical segments in which many muscle activated units were found is known to receive trigeminal afferents (Kerr, 1961; Kerr and Olafson, 1961; Gordon, Landgren and Seed, 1961; Wall and Taub, 1962; Kerr, 1963; Kerr, 1972; Mosso and Kruger, 1973) and indeed merges into the spinal nuclear complex of V (Olzewski, 1950). Previous experiments (Kerr and Olafson, 1961) suggest that there is little convergence between trigeminal and segmental inputs at these spinal levels. In 7 experiments the distribution of both facial and neck muscle afferents in these regions was carefully examined.

Medial to the dorsal root entry zone in C1 and C2, neck muscle primary afferent potentials were recorded as soon as the microelectrode reached the spinal cord. A few hundred microns beyond this region very small amplitude unit potentials were found responding to light touch of the face, but not to neck muscle afferent stimulation. When the electrode was advanced a further few hundred microns, unit potentials of greatly increased amplitude were encountered which responded to both facial and neck muscle afferent stimulation. Further into the cord, facial stimulation ceased to be effective and only neck muscle afferent stimulation elicited unit discharge. In lateral penetrations through the dorsal root entry zone, not only were primary

muscle afferent potentials recorded superficially, but second-order unit and wave potentials could be recorded from the same sites. In these more lateral penetrations, facial stimuli did not elicit unit activity until the electrode was advanced a further few hundred microns.

In dorsal horn regions where both facial and neck muscle afferent stimuli elicit unit activity, it is not easy to establish whether the same units are responsive to both stimuli. Very light touch to the face and threshold muscle nerve stimulation both normally elicit bursts of multi-unit discharge and only occasionally have well isolated units been observed. The few single units that could be isolated were activated by both stimuli (Figure 5 a). That some form of convergence is present can readily be demonstrated in multi-unit preparations, as activity initiated by one stimulus modality may be influenced by prior stimulation of the other modality (Figure 6). Considerable and complex interactive convergence occurs between neck muscle and trigeminal afferents in upper cervical segments.

Overlap and convergence also occurs at medullary levels. Units activated by trigeminal and neck muscle afferent stimulation were found in the subnucleus gelatinosa of the spinal nucleus of V. Many of the units were of low amplitude and only just discernible above noise, but even the most trivial facial stimuli induced by a small (100-300μ) displacement of facial hair or of a single vibrissa, activated many units. Small amplitude units in these same regions could also be activated by neck muscle afferent stimulation. The impression was gained in these experiments that recordings were obtained from micro-bundles or cell groups which were homogeneous in their afferent input. At any recording site in the subnucleus gelatinosa, it

was commonly found that unit activity in response to one stimulus modality was usually of greater amplitude than unit activity from the other modality.

In the subnucleus magnocellularis of the spinal nucleus of V, the amplitude of muscle and trigeminal unit discharge was considerably higher than in subnucleus gelatinosa, but the intensity of discharge still made it difficult to isolate single units. However, interactions were observed in multi-unit preparations between cutaneous and muscle afferent stimulation which leave no doubt that convergence occurs between trigeminal and muscle afferent pathways in this region too (Figure 6). On the rare occasions when well isolated units were obtained in the trigeminal nuclei, no difficulty was found in establishing that both trigeminal and neck muscle afferent stimuli could activate the same unit. Such units had latencies exceeding 15 msec. As the electrode was advanced ventrally beyond the border of the spinal nucleus of V, facially activated units were lost, but neck muscle afferent stimulation frequently elicited long latency unit discharge.

Unfortunately, no systematic attempt was made to examine convergence on cuneate units activated by neck muscle afferents, but in one instance it was noted that a unit bilaterally activated by biventer cervicis stimulation also had a large cutaneous receptive field on the ipsilateral shoulder (Figure 5 b).

Afferent connections to motoneurons in upper cervical cord

One well known segmental connection from Group Ia afferents is that which gives rise to the monosynaptic reflex (Eccles, Eccles and Lundberg, 1957; Lloyd, 1943). No evidence could be found in

the present experiment for the existence of any appreciable monosynaptic reflix in upper cervical segments.

In 6 experiments the central cut ends of the dorsal roots of C2 and C3 were stimulated and attempts were made to record reflexes eletrically from electrodes placed on biventer cervicis and splenius muscle nerves. While reflex activity could be consistently recorded to strong stimulation after latencies of 16-20 msec, no activity was recorded at latencies appropriate to a monosynaptic reflex. To examine this question further, 96 motoneurons or axons (34 from splenius and 62 from either biventer cervicis or complexus) were identified by antidromic excitation in C2 and C3 segments. Attempts were then made to orthodromically drive these units by single or paired stimuli to the dorsal roots. Of the 96 units examined, only 26 could be regularly activated by orthodromic stimuli and then at latencies usually exceeding 9 msec (Figure 7). Only one unit was activated at a latency sufficiently short to be regarded as monosynaptic. The lack of orthodromic responsiveness even at relatively long latencies could not be attributed to an inadequate stimulus strength, for the same stimulus was sufficient to secure the discharge of many interneurons in both dorsal and ventral horns.

In the lumbosacral cord, 100% of a motoneuron pool can be orthodromically monosynaptically activated at the peak of post-tetanic hyperpolarisation (Jefferson and Benson, 1953; Clamann, Gillies, Skinner and Henneman, 1974). Twenty-one antidromically identified motoneurons were tested for their orthodromic responsiveness at 2 sec intervals after 15 seconds of dorsal root stimulation at 300 sec. In no instance could any of these cells be activated in the post-tetanic period at monosynaptic latencies. Recordings made following stimulation of dorsal roots from adjacent segments

also failed to demonstrate the presence of monosynaptic reflexes.

The description of the histology of the upper cervical cord suggest that ventral horn motoneurons in this region are in two columns, the nucleus ventromedialis and the nucleus spinalis accessorii, (Rexed, 1954). It has also been suggested that cells of the medial cervical nucleus may also be motoneurons, possibly of the spinal accessory nerve (Rexed, 1954). Figure 8 shows two composite diagrams of the cervical spinal cord on which have been plotted the points at which a maximal antidromic unit potentials could be recorded. The points in white matter are presumable from axons and those in gray matter are from both axons and cell bodies. In both C2 and C3 segments, splenius and biventer cervicis/complexus potentials were found to be widely distributed in the gray matter of cord in laminae VI to IX. The spread of potentials in C2 was considerable and some of the potentials were found in laminae VI and VII, some were distributed along the lateral border of the grey matter, while others were scattered close to and below the medial cervical nucleus (Figure 8). In C3 most potentials were close to the anatomically defined motor nuclei (Figure 8). There appears to be some anatomical separation of potentials recorded from different muscles. Antidromic potentials following splenius stimulation usually occupied a more superficial position than those from biventer cervicis/complexus stimulation. No splenius unit potentials were found in nucleus ventromedialis in the ventral horn of the second cervical segment, although beventer cervicis/complexus potentials were found in this region. However, it was common while recording in the deep parts of Lamina VIII to find antidromic potentials which were not all-or-none, but which were graded and increased in amplitude as the stimulus strength to both

splenius and biventer cervicis/complexus was increased. These potentials may have been due to groups of closely approximated motoneurones, but because they did not meet the all-or-none criteria for a single unit, they were not included in the experimental sample.

In the course of recording in the spinal grey matter following muscle nerve stimulation, trains of unit discharges were occasionally observed (Figure 9). The records are essentially the same as those reported by Renshaw (1946) and are presumed to be due to activation of Renshaw cells via recurrent collaterals. A further population of cells were also seen which could be antidromically activated, but which had latencies of 4 m/sec or more indicating conduction velocities in the range of 10 - 20 m/sec. These units were assumed to be small fusimotor neurones.

DISCUSSION

Correlation of Anatomical and Physiological Experiments

Older anatomical experiments (Ranson, Davenport and Doles, 1932; Corbin and Hinsey, 1935; Escolar, 1948) utilizing the Marchi technique firmly established the major characteristics of ascending projections from the upper cervical cord in the cat. Degeneration was found in the cord in the lateral parts of the cuneate fasciculus, in the dorsal horns, in the intermediate nucleus of Cajal and a few degenerating fibres were traced into the ventral horn. Fibres were also found which projected to the medulla and degeneration was present in the cuneate nucleus, and dorsomedial to the spinal tract of V, occupying the triangle between the substantia gelatinosa and the cuneate nucleus. Degeneration was also found in the intermediate nucleus of Cajal,

in the medial part of the restiform body and in the descending vestibular nucleus. Later studies in the cat utilizing the Nauta-Gygax and Fink-Heimer technique (Liu, 1956; Kerr, 1961; Imai and Kusama, 1969; Kerr, 1972) substantiated the previous accounts and also reported the presence of degeneration in the boundary between the spinal tract and spinal nucleus of V and in a small region ventral to the spinal nucleus of V (the convergent area "C" of Kerr, 1972). Although it has been reported (cf Kerr, 1972), that degeneration occurs in the external cuneate nucleus, this is not substantiated (at least is lower medullary regions) by the available maps which show degeneration not in the nucleus itself but in the fibre-rich zone ventral to it (Imai and Kusama, 1969; Kerr, 1972). However, in the rat, electrophysiological evidence has been found for a projection from neck muscles to more anterior regions of the external cuneate nucleus (Campbell, Parker & Welker, 1974). In the experiments reported here, only lower medullary explorations were carried out, but within these limits it is apparent that the anatomically described projections from upper cervical segments to cord and medulla all carry afferents from neck muscle, with the exception of that to the intermediate nucleus of Cajal. Presumably that projection is exclusively cutaneous.

The termination of primary afferents within spinal segments as observed electro-physiologically bears out the known anatomy, with most fibres terminating in laminae IV and V, and few penetrating the ventral horn (Ranson et al, 1932; Corbin and Hinsey, 1936; Szentagothai, 1948; Escolar, 1948; Kerr, 1961). Indeed, the failure to demonstrate a monosynaptic reflex might have been predicted from the observations of Szentagothai (1948)

who showed on the basis of bouton degeneration following rhizotomy, that few primary afferent fibres terminate on motoneurones in the upper cervical cord. Presumably those that do are sufficient to generate synaptic potentials (Wilson and Maeda, 1974), but insufficient to initiate action potentials.

It is paradoxical that neck muscles, which contain the largest accumulation of spindles anywhere in the body (Richmond and Abrahams, 1975a) should not have a monosynaptic reflex.

Physiology of spinal and medullary projections from neck muscle afferents

The operation of the monosynaptic reflex plays an important role in most theories of feedback control of muscle (cf Houk, 1974), but clearly the importance of this particular role will vary from muscle to muscle, and even with respect to the particular task being performed by that muscle. Melvill Jones and Watt (1971) have demonstrated that in the muscular control of landing from a fall a "functional stretch reflex" is elicited at a latency considerably in excess of that expected from a monosynaptic reflex. A similar situation has been reported for the human biceps (Hammond, Merton and Sutton, 1956).

The muscles of the neck, in common with the muscles of the eye (McCouch and Adler, 1932; Sasaki, 1963; Keller and Robinson, 1971; Bach-y-Rita, 1972) clearly do not possess a conventional excitatory monosynaptic reflex and such reflexes as are set up by neck muscle afferents will be of some greater complexity. Pathways from neck muscle project to the cerebral complex and participate in reflex interactions from neck muscle afferents (Landgren & Silfvenius, 1968; Abrahams, 1970, 1972). Long loop pathways have also been demonstrated from neck muscles with the superior colliculus as an important relay station (Abrahams and Rose, 1975).

Undoubtedly some reflexes of neck muscles are likely to be subcortical for Sherrington (1898) noted in the decerebrate cat that "on excitation of the central end of the 2nd cervical nerve, or of a branch, even a small twig of that nerve, the high-held retracted head drops almost as if knocked down by a blow from above. The muscles causing the retraction can be seen and felt to relax at once under the excitation; the completeness and suddeness of the relaxation is surprising."

The projection of neck muscle afferents to the deep layers of the cuneate nucleus and their convergence at this level with cutaneous afferents from the shoulder reinforces the previous evidence (Rosen, 1967, 1969 a & b; Rosen and Sjolund, 1973) that the cuneate nucleus is not purely a cutaneous relay centre, but is also an important region of relay for Gp I and II muscle afferents. The neck projection to the cuneate nucleus is confined to receptors from the slow muscle, biventer cervicis and this raises some interesting functional questions. The slow and fast muscles of the neck are not simply synergistic and the faster muscles splenius and complexus probably play and major role in turning movements while the slow muscle biventer cervicis is probably primarily responsible for head elevation (Elliot, 1935; Richmond and Abrahams, 1975a). The separation of afferent pathways at medullary levels may have some relation to the particular functional tasks subserved by individual neck muscles.

Neck Muscle afferents and the spinal trigeminal complex

The close association between the trigeminal and neck muscle afferent systems could have been predicted from the known anatomy of cervical projections (Ranson et al, 1932; Corbin and Hinsey,

1936; Escolar, 1948; Liu, 1956; Kerr, 1961; Imai and Kusama, 1969; Kerr, 1972) and also from such electrophysiological examination as had been previously undertaken (Kerr and Olafson, 1961). Kerr and Olafson (1961) confined their examination to C1 and C2 and reported that "What proportion of neurons in this region (sic, C1 and C2 dorsal horns) receives afferents from both systems it is impossible to state, but it can be said that they constitute a small percentage". While simple convergence may be difficult to demonstrate in the upper cervical cord, interactions between neck muscle and facial afferents clearly indicate that the two afferent systems have a strong functional connection at this level.

In some respects, the organization of the lower C1, C2 and C3 segments resembles that of the lumbosacral cord. In both regions of the cord cutaneous projections are prominent in the more dorsal laminae and proprioceptive projections in the more ventral laminae (Wall, 1967). The major difference is that cutaneous fields in the upper cervical cord are segmentally displaced and must enter the cord by way of a cranial nerve. Wall and Taub (1962) pointed out that the trigeminal system must develop rapid pathways to the neck musculature not only for aversive reflexes of the head, but also for reflexes such as orientation and suckling orientation. Such connections must ultimately impinge on neck motoneurones. The medullary projections may be concerned with ascending connections but the possibility cannot be ruled out that they play a substantial reflex role. It should be noted that the neck muscle afferent projection to Kerr's (1972) area "C" is to a region, which, in the monkey appears activated by noxious stimuli to the face Nord and Ross, 1973).

There can be no doubt that the trigeminal system plays a major role in the motor control of neck muscles, a fact perhaps first experimentally verified by Sherrington (1898) who reported ".....after removal of the cerebral hemispheres when it is easy to apply electrodes to the division of the trigeminus on the floor of the middle fossa of the cranium, a touch with the electrodes is enough to cause the relaxation of the rigid neck muscles.....excitation of even small twigs of distribution of the 5th effects the same .....". The association of the spinal tract of V with pain perception is also a long established fact. The present experiments reinforce the association of the trigeminal system with a muscle afferent system whose primary function must be in the task of head motor control. The immediate reaction to pain is movement and the association of the spinal nucleus of V with head motor control is entirely appropriate to a system concerned both with pain perception and the immediate motor response to abate or remove the source of that pain.

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Figure 1 A. Group I and II primary afferent waves recorded from dorsal column of C2 following biventer cervicis nerve stimulation at 3T (100 superimposed at 300/sec).

B. Primary afferent unit potential recorded from ventro-lateral margin of C2 dorsal columns following C3 biventer cervicis stimulation at threshold.
S, stimulus artefact.

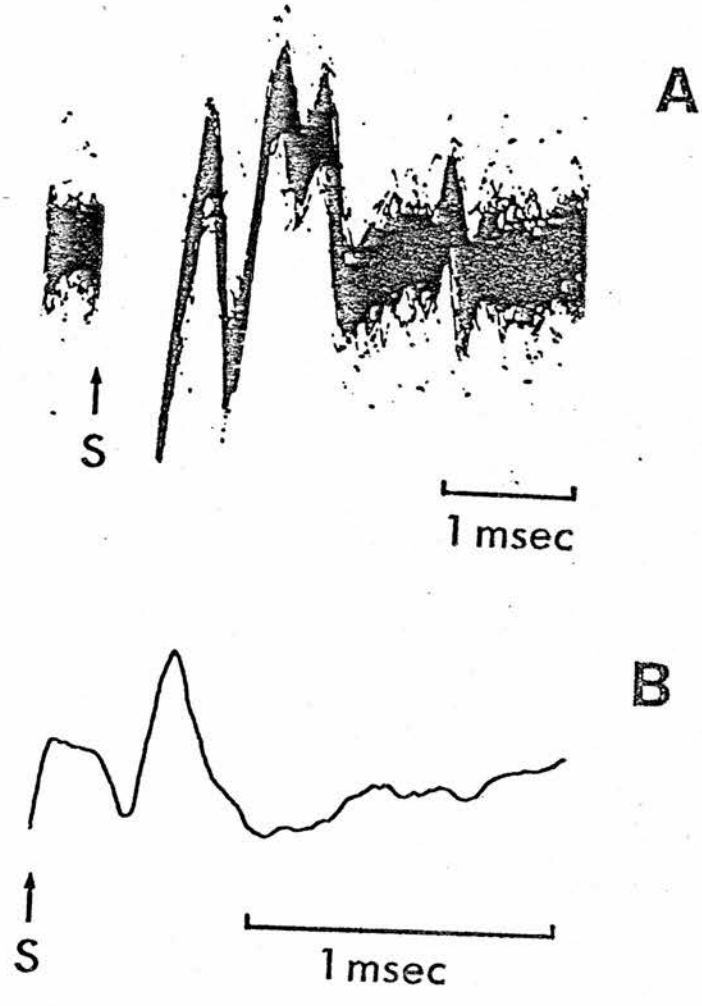
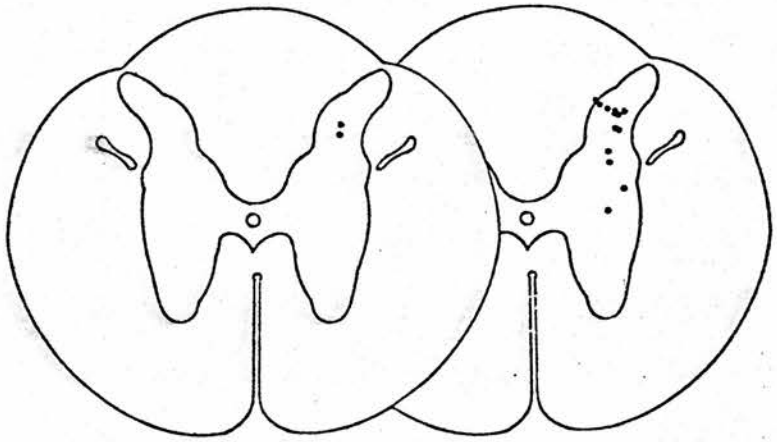
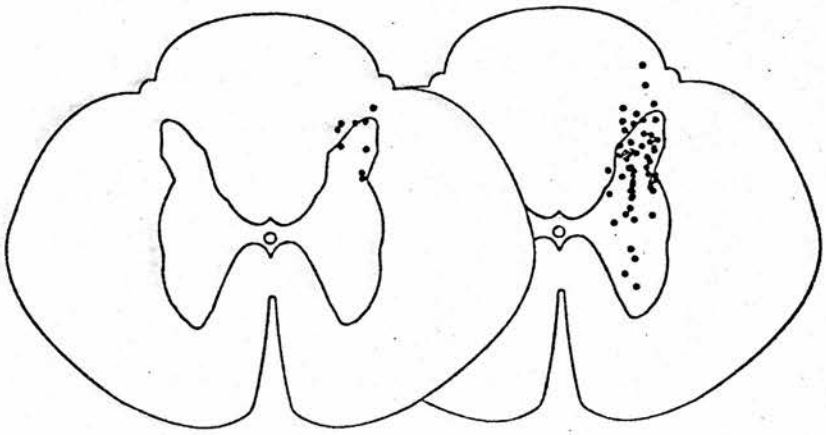

A
S
1 msec
B
S
1 msec

Figure 2. Maps of the location of primary afferents and second order units in the C2 and C3 segments. Left, primary afferents; right, second order units.



C 2



C 3

Figure 3. Distribution of primary afferent potentials and second order units in the lower medulla. Left, primary afferent potential at T (top); 3T (bottom). Time marker, 1 msec. Right, maps of medulla showing distribution of regions from which primary afferent potentials were recorded (vertical shading) and from which second order units were recorded (horizontal shading). Dots indicate locations of recording sites from which second order units were recorded in single instances.

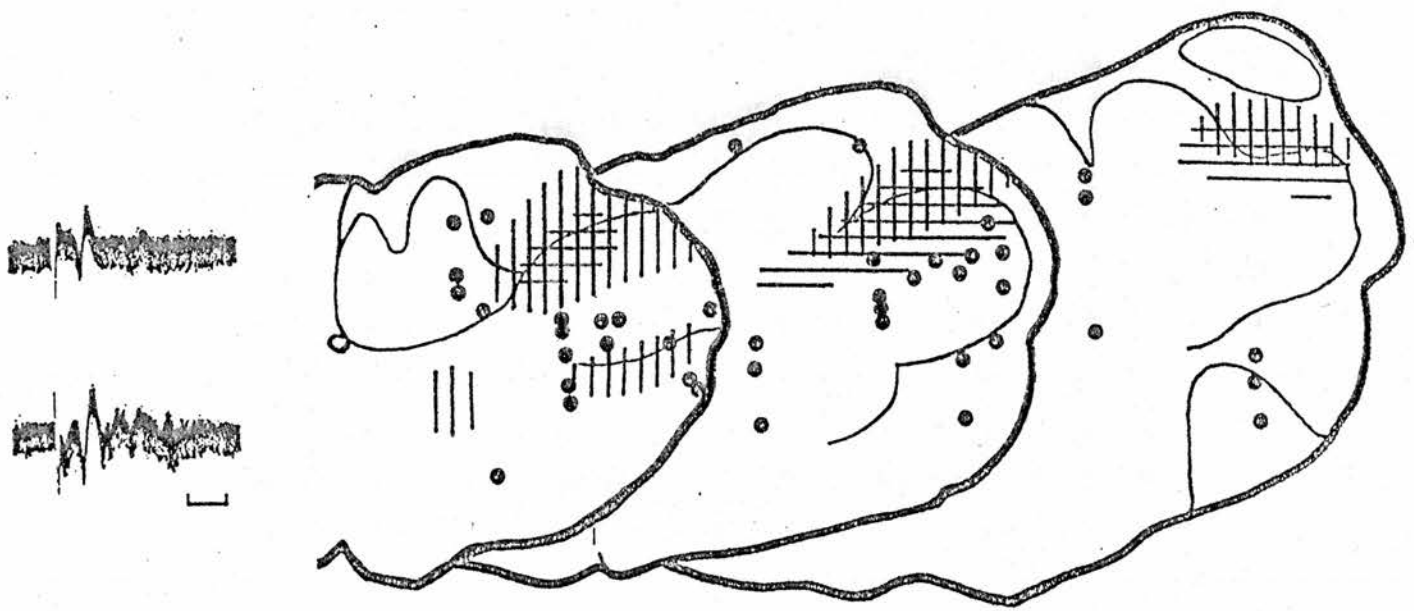
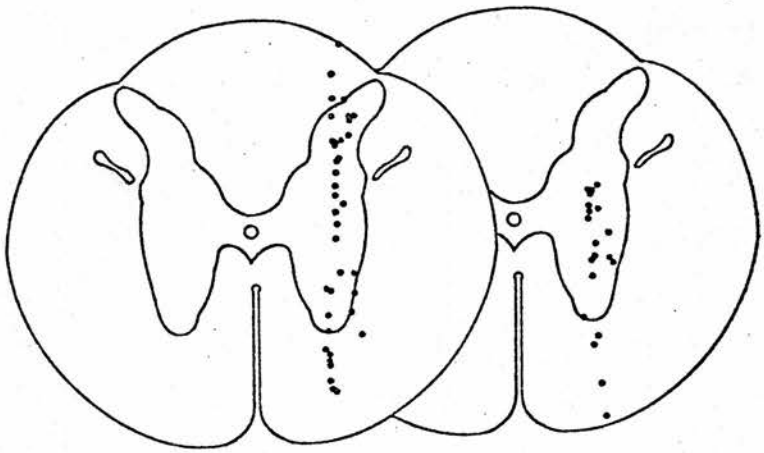
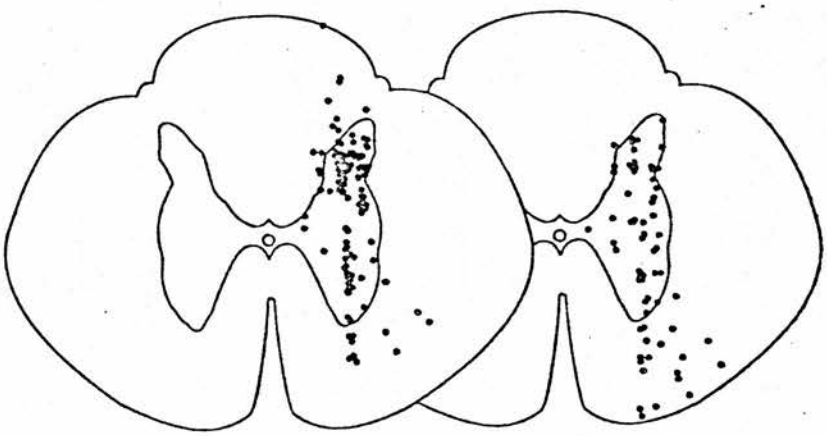


Figure 4. Recording sites of long latency responses in C2 and C3 segments following stimulation of neck muscle nerves. Left, unit responses with latencies between 6 and 15 msec. Right, unit responses with latencies exceeding 15 msec.



C 2



C 3

Figure 5. Convergence of cutaneous and muscle afferents on single units in the spinal cord and medulla. A. Unit recorded in C2 dorsal horn responding to train of stimuli to muscle nerve (top), and light touch to the face (bottom). B. Unit recorded in deep layers of cuneate responding to single pulse to biventer cervicis (top) and light touch to ipsilateral shoulder (bottom).

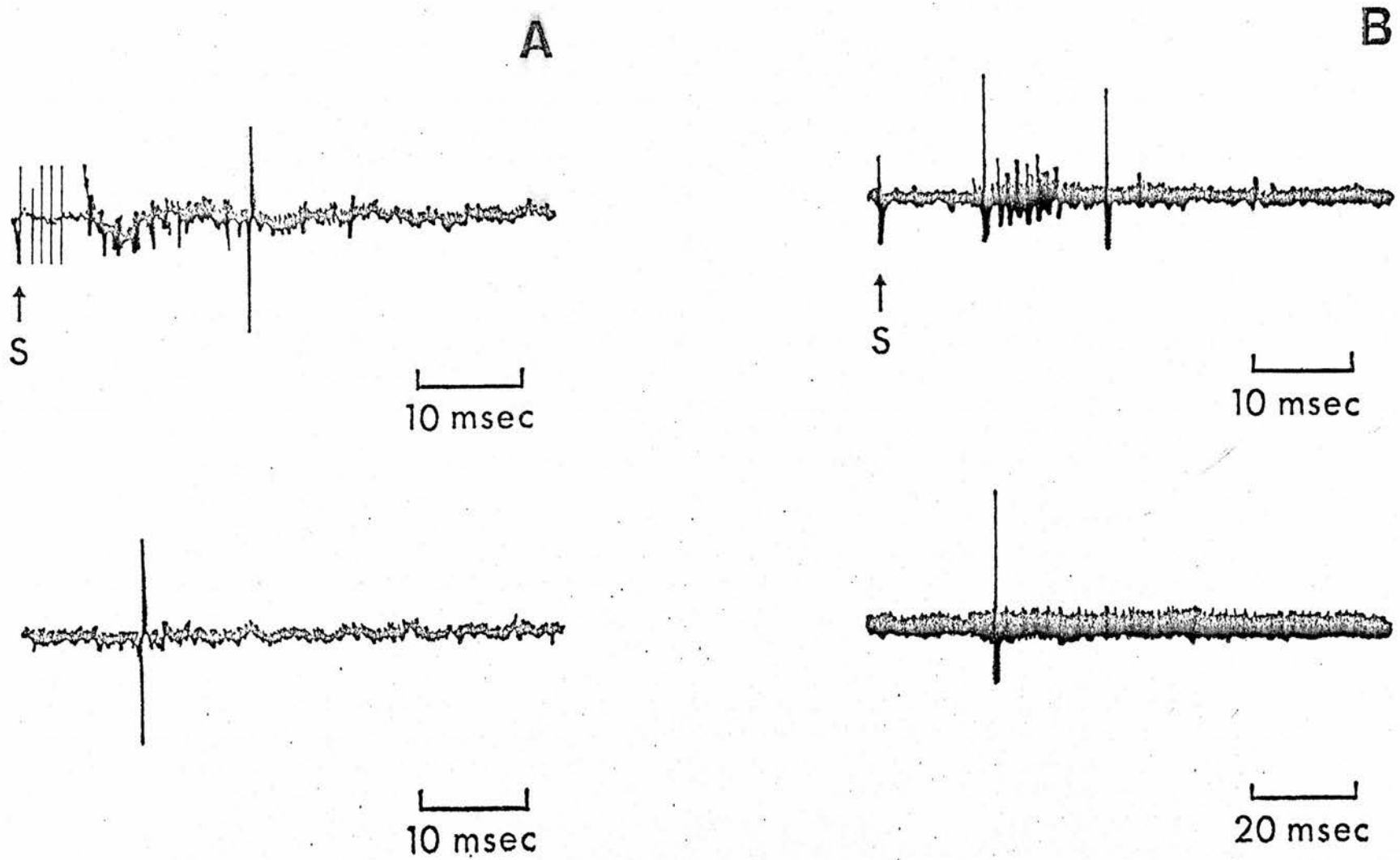
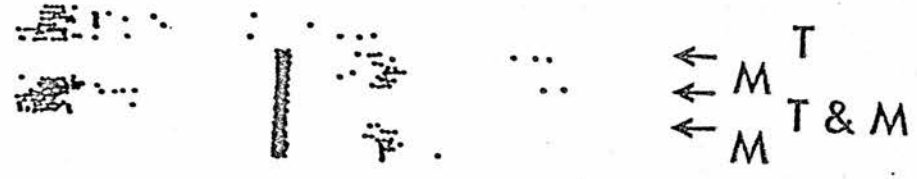

A
S
10 msec
10 msec
B
S
10 msec
20 msec

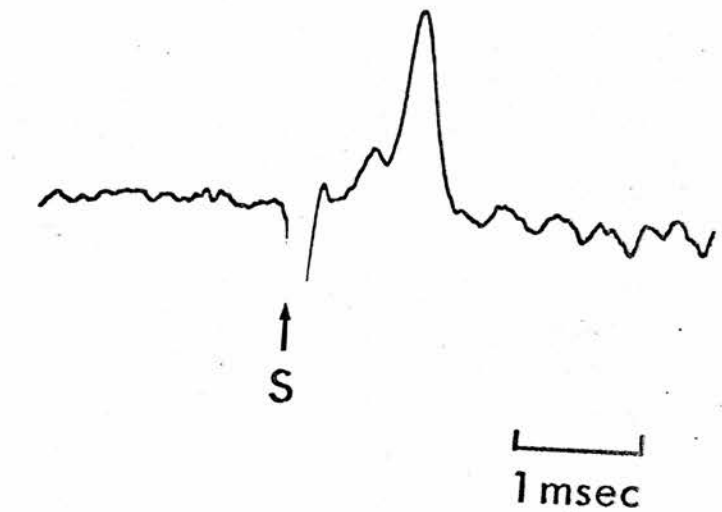
Figure 6. Raster diagrams to show interactions between unit responses to neck muscle afferent (M) and light facial stimuli in medulla (top) and upper cervical cord (bottom). The top illustration shows the inhibition of a unit response elicited by a train of 3 stimuli to the neck muscle nerve (M) by prior stimulation of the face (T & M). The response to facial stimulation alone is also shown (T). In the bottom illustration, prolonged inhibition of the response to neck muscle afferent stimulation (M) is produced by prior stimulation of the face (T & M).

10 msec



40 msec

Figure 7. Top: Antidromic potential recorded in ventral horn following biventer cervicis nerve stimulation. Bottom: same unit discharges to stimulation of central end of cut dorsal roots. S, stimulus artefact.


S
1 msec

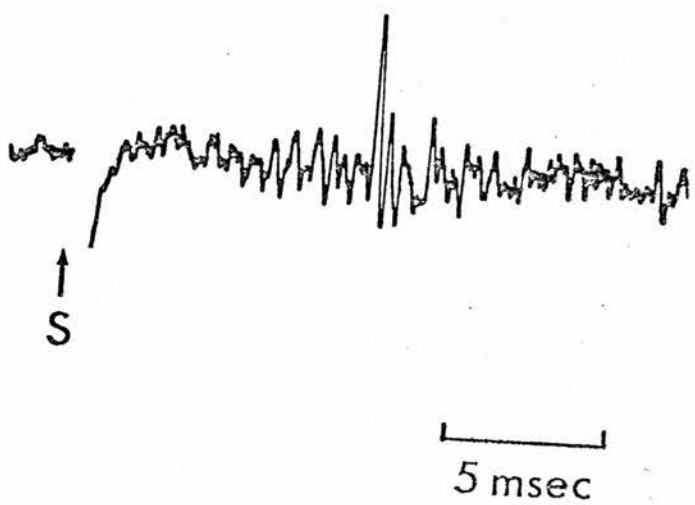

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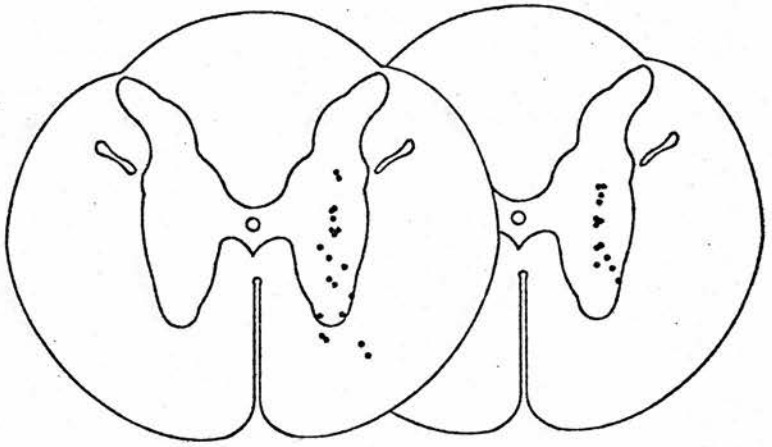
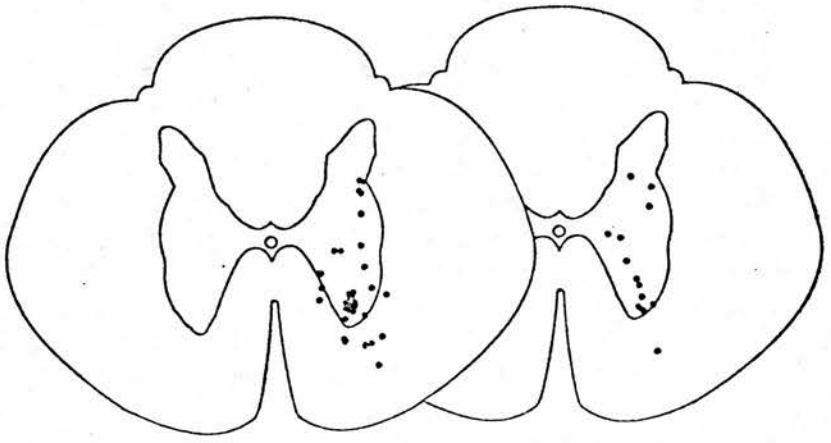
Figure 8. Diagram to indicate recording sites of antidromic action potentials assigned to motoneurons and their axons in C2 and C3 segments. Left, responses to biventer cervicis/complexus stimulation; right, responses to splenius stimulation.

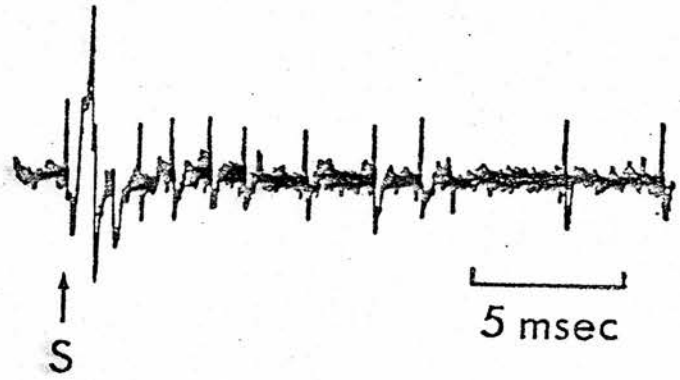


C 2



C 3

Figure 9. Response recorded in ventral root following stimulation of muscle nerve. Dorsal roots cut S, stimulus artefact. Large potential immediately following artefact is motoneuron antidromic potential. Note train of potentials following antidromic potential.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5 msec

THE MOTOR SYSTEM OF THE HEAD

A Short Review

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INTRODUCTION

The head movement system has a number of intrinsic specializations which separates it from other motor systems. The muscles that serve to move the head and the receptors of these muscles have distinctive characteristics. The upper cervical cord differs in essential aspects of its organization from the more familiar lumbosacral cord, and one specialized supraspinal structure, the superior colliculus plays a major role in the organization of head movement. This review will touch upon all these areas.

In primates, the position of the foramen magnum at the base of the skull means that many head movements are executed with little influence on the centre of gravity and therefore without the need for compensatory adjustments in other muscle systems. In quadrupeds, the head cannot be moved without some compensating postural adjustment. But in both primate and quadruped, head movements are executed about an extensible and retractable "universal joint" so that there is the possibility of horizontal and vertical rotation, flexion or extension (either alone or in combination).

Sensory Apparatus of Neck Muscles

Early considerations of head motor physiology dealt little with head movement per se, but mainly with the sensory role of the neck receptors. Experiments dating back to the 19th century showed that interference with neck muscles may produce deficits which are difficult

to attribute solely to interference with the motor functions of these muscles. Longet (1845) surgically damaged the neck muscles of a horse, dog, cat, rabbit and copybara and claimed that in all these species, damage to neck muscles produced an ataxia which closely resembled that which follows hemi-cerebellectomy. A similar deficit was reported by Claude Bernard (1865) to have been found by Magendie in experiments on rabbits. There are continuing reports of a disorder in man in which disturbance of gait, dizziness or disorders of the oculomotor system occompany damage to neck structures. The syndrome, sometimes called cervical ataxia or cervical nystagmus, has been attributed to damage to neck muscles (Weeks and Travell, 1955; Cope and Ryan, 1959; Jongkees, 1969), to disordered cervical sympathetic activity (Hinoki and Niki, 1975), or to altered cervical vascularity (Sandstrom, 1962). Of course, dizziness of great severity is a common accompaniment of "whiplash" injury (Finneson, 1969).

Gross ataxia may be produced in experimental animals by damage to the sensory apparatus of the neck. In 1961, Cohen showed that injection of the upper three cervical dorsal roots of monkeys with local anaesthetic led to gait being so disordered "that the animals walked as though drunk". In experiments in this laboratory, it was found that in the cat an ataxia followed local anaesthetic injection of the muscle mass of the neck or section of dorsal neck muscle nerves (Abrahams and Falchetto, 1969). The sensory origin of the ataxia

was shown by removing the C2 to C4 dorsal root ganglia. This causes postural and locomotor deficits as great as those that follow unilateral labyrinthectomy (Richmond, Anstee, Sherwin and Abrahams, 1976). For 7 to 21 days following operation, the animals, although free of nystagmus, have difficulty in standing and in walking, and particularly in attempting to turn.

The neck as a receptor has long been an important concept in physiology. The early work of Magnus and his colleagues (1926), showed that the decerebrate cat has tonic neck reflexes, which are not labyrinthine in origin but which are due to the activation of neck receptors. There was little systematic analysis of the receptors for tonic neck reflexes until the experiments of McCouch et. al., (1951). These workers showed that tonic neck reflexes of the decerebrate labyrinthectomized cat were not abolished when the muscle mass of the neck was sectioned but the reflexes were lost after section of a small nerve which entered the dorsal roots close to the vertebrae and which appeared to serve joint receptors. McCouch et. al., (1951) therefore suggested that the receptors responsible for tonic neck reflexes were in intravertebral structures. Examination of decalcified necks of cats has failed to show a specific class of intravertebral receptor in the upper cervical cord but has shown that the small intravertebral and perivertebral muscles contain an abundance of small spindles (Richmond, unpublished observation).

These small muscles may be served by the same nerve that McCouch et. al., (1951) sectioned and it is possible that these small spindles may be the receptors underlying tonic neck reflexes.

Spindles and Golgi tendon organs are abundant in the large dorsal neck muscles. A profusion of spindles in neck muscles has been described by Voss (1958) and Cooper and Daniel (1963), in human material, by Thompson (1970) in the rat, and by Richmond and Abrahams (1975b), in the cat. In cat skeletal muscle, the number of spindles per gm (spindle index) ranges from 5 to more than 120 (Matthews, 1972). Muscles which had been found to have a high spindle index were small in size, so that the total number of spindles per muscle was few. The neck muscles of the cat depart from this generalization, for not only are they spindle dense (47-107 spindles/g) but the muscles are relatively large (Richmond and Abrahams, 1975a), so that the total number of spindles per muscle is very great, often exceeding 200/muscle (Table 1). Richmond and Abrahams (1975b), cut serial sections of 4 major dorsal muscles of the neck so that a complete reconstruction could be made of the spindle organization within each of the muscles. Spindles were found to follow a relatively fixed distribution in any given muscle (Richmond and Abrahams, 1975b) and tended to be in association with these muscle fibres which have the histochemical characteristics of slow twitch muscle (Richmond and Abrahams, 1975a). This is particularly noticeable in splenius, which is arranged so that a sleeve of predominantly

fast fibres encircles a core of predominantly slow fibres. It is in this core that splenius spindles are to be found (Richmond and Abrahams, 1975b). It is likely that each spindle in a muscle carries unique information and that the nature of this information depends on the position of the spindle within the muscle (Meyer-Lohman, Riebold and Robrecht, 1975; Richmond and Abrahams, 1976a) and on the arrangements of motor units within the muscle (Binder, Kroin, Moore, Stauffer and Stuart, 1976). The high spindle density in neck muscles suggests that the spindle system can supply particularly "fine grain" information about the muscles.

Physiology of Dorsal Neck Muscles

Careful examination of neck muscles has shown a considerable internal complexity of fibre organization (Richmond and Abrahams, 1975a). The three large dorsal neck muscles, splenius, biventer cervicis and complexus, resemble rectus abdominus in that they are crossed by tendinous inscriptions (or as they are sometimes called, tendinous intersections). These inscriptions are connective tissue bands which incompletely intersect the muscles (Fig. 2). Tendinous inscriptions have excited virtually no curiosity among muscle physiologists and their occurrence in rectus abdominus has been dismissed as a remnant which reflects the segmental origin of the muscle (Johnston and Whillis, 1954). Tendinous inscriptions are an important functional element in the physiology of neck muscle and

probably also in other muscles in which they are present. Dissection of single muscle fibres after partial digestion of neck muscles has revealed that neck muscles are complexes of short and long fibres. Many of the short fibres extend only from one tendinous intersection to the next. Long fibres adhere to the tendinous inscriptions where they perforate it (Richmond and Abrahams, 1975a). Although tendinous inscriptions serve as insertion points for short fibres, they are not the only insertion point and large numbers of muscle fibres terminate by tapering and fusing with neighbouring fibres. The tendinous inscriptions not only form an attachment for muscle fibres, are also the location of many Golgi tendon organs and many of the spindles of neck muscle lie close to or perforate the inscriptions (Richmond and Abrahams, 1975b).

Each region of the muscle between inscriptions is innervated by a separate nerve which takes its origin in a different cervical root. It is not uncommon for a limb muscle to be innervated from a number of spinal roots, but usually there is fusion of the various branches in a plexius to form a common motor nerve. The innervation of neck muscles is also unusual in its fibre content. On the average as many as 76% of the myelinated fibres are sensory and in individual nerves this proportion can rise as high as 95% (Richmond, et. al., 1976). Relatively few Group I fibres exist and this is probably related to the fact that neck muscle spindles are in general supplied by smaller and slower fibres than hind leg spindles (Richmond and Abrahams, 1976c). The motor innervation of neck muscles includes a preponderance number of gamma fibres, ranging from 55 to 93% (Richmond et. al., 1976).

Even on superficial examination some obvious differences can be seen between the individual large dorsal neck muscles of the cat. Biventer cervicis has a narrow medially placed insertion on to the lamboidal crest which gives the minimum of leverage for head turning and the muscle probably functions as an elevator of the head. Splenius has a very broad insertion on the laboidal crest, so that it both supports and turns the head. Functional testing of these two muscles (Richmond and Abrahams, 1976a) confirmed the earlier histochemical observations (Abrahams and Rancier, 1973; Richmond and Abrahams, 1975a) that splenius is predominantly a fast muscle and biventer cervicis predominantly a slow twitch muscle. Complexus is dominated histochemically by neither fast nor slow fibres, (Richmond and Abrahams, 1975a). In functional testing, the fast fibres component of complexus dominates and its twitch and tetanus fusion frequency times are characteristic of a fast twitch muscle (Richmond and Abrahams, 1976a).

Splenius, complexus and biventer cervicis differ substantially in their passive extensibility (Richmond and Abrahams, 1975a); biventer cervicis is very extensible, splenius is relatively inextensible and complexus is intermediate in its properties. Active length-tension curves resulting from stimulation of the different nerves to the same muscle show different optimum lengths for the different sections of the muscle. Thus over a wide range of initial lengths when all the motor nerves are stimulated the overall length-tension

curve is flattened. A consequence of this is the likelihood that similar mechanical forces can be exerted on the head by these muscles regardless of initial head position. Thus the head can be moved with equal facility regardless of its initial position. The utilization of muscles of such complexity requires that the nervous system have available to it a great deal of information about the different parts of the muscles. Perhaps here lies part of the answer to the dense array receptors in those muscles.

Spinal and Medullary Projections of Neck Muscle Afferents

Very powerful descending spinospinal interactions take origin in afferents from neck muscle nerves (Abrahams and Falchetto, 1969; Abrahams, 1970, 1971). Such descending interactions were not demonstrable in the spinal or decerebrate animal and were thought to involve supraspinal structures, possibly including cortex (Abrahams and Falchetto, 1969; Abrahams, 1970). Cortical lesions restricted to the anterior suprasylvian gyrus, the cortical region known to receive a projection from neck muscle afferents (Landgren and Silfvenius 1968), abolished the descending interactions in acute experiments (Abrahams, 1970), but not in chronic experiments (Abrahams, 1972). It has not been possible to demonstrate a monosynaptic reflex of any significance in the dorsal muscles in the neck (Abrahams, Richmond and Rose, 1975). While there is no doubt that the cervical afferents are connected monosynaptically to neck motoneurons (Wilson and Maeda, 1974), the connection is synaptically weak and as previously mentioned

does not seem capable of activating motoneurons. The understanding of muscle spindle function that has been developed from the study of the hind leg and the lumbosacral cord (cf Houk) thus cannot apply in its entirety to the head movement system.

From the point of view of spinal motor organization there may be four basic types of organization. The lumbosacral cord contains those modifications most appropriate to a role in locomotion (even in quadrupeds, the main force of fast locomotion is developed by the hind legs). The thoracic cord contains those specializations especially suited to the motor task of respiration. The lower cervical cord must contain specializations that are unique to the operation of the forelimb and the hand and the upper cervical cord contains those specializations which fit it for the functional task of head movement.

The functional organization of the cervical cord is based on close relationships between neck muscle and trigeminal afferents. The spinal tract of the trigeminal system has excited the curiosity of physiologists for many years, mostly with respect to the perception of pain (for reviews see Darian-Smith, 1973). But there have long been suggestions that the spinal tract of the trigeminal nerve might play a motor role (Kerr and Olafson, 1961; Wall and Taub, 1962). Our experiments leave us in no doubt that the spinal tract of the trigeminal nerve must play a major role in the control of head movement.

Interneurons in the ventral horns of C1 to C4 are activated at short latency (2 msec or so) by electrical stimulation of the infraorbital nerve, a branch of the trigeminal nerve, and this same stimulation readily excites neck motoneurons (Abrahams, Anstee and Richmond, 1976).

The association between the trigeminal and neck motor system is not confined to the upper cervical spinal cord. Neck muscle primary afferents project to the caudal part of subnucleus caudalis of the spinal nucleus of $\underline{\overline{V}}$ at lower medullary levels and there is considerable convergence between neck muscle afferents and facial afferents within the spinal nucleus of the trigeminal system at this level (Abrahams and Richmond, 1975). It seems likely that the spinal nucleus of $\underline{\overline{V}}$ must play a role in the organization of head movements, perhaps in the organization of aversion motor responses to nociceptive facial stimuli. This kind of reflex could be expected to parallel the flexor reflex of the limbs.

The Superior Colliculus and Head Movement

Two tracts take origin in the superior colliculus and connect with a minimum disynaptic linkage on to neck motoneurons. These are the tectospinal tract (Altman and Carpenter, 1961; Nyberg-Hansen, 1964; Petras, 1967; Kawamura et. al., 1974) and the tectoreticulospinal system (Anderson,Yoshida and Wilson, 1971). The tectospinal system is a largely crossed system with fibres terminating in the spinal cord on interneurons in close proximity to neck motoneurons (Nyberg-Hansen, 1964).

The tectoreticulospinal system projects to pontine and medullary reticular nuclei and then activates reticulospinal neurons, some of which connect monosynaptically with neck motoneurons (Anderson, Yoshida and Wilson, 1971).

The existence of these descending spinal systems may explain why electrical and chemical stimulation of the superior colliculus so readily leads to head movement (Apter, 1946; Hess, Burgi and Bucher, 1946). What has not been generally recognized is the extent to which the superior colliculus is a proprioceptive receiving area, with substantial inputs from neck and extraocular muscle afferents (Rose and Abrahams, 1975; Abrahams and Rose, 1975a) and that the proprioceptive afferent systems can influence output to the motor system of the head (Abrahams and Rose, 1975a). For some time it has been apparent that extraocular muscles project to the superior colliculus (Cooper, Daniel and Whitteridge, 1953; Fillenz, 1955), but it has only recently been demonstrated that by the use of a simple index that this input may exceed input of retinal origin, (Abrahams and Rose, 1975b). Neck muscle afferents are not the only skeletal muscle projection and there is input from both hind and forelimbs (Abrahams and Rose, 1975b). Table 2 is a "score sheet" in which the relative efficacy of proprioceptive input to the superior colliculus is matched against retinal afferent input. It can be seen that it is the extraocular system, which supplies the largest input with neck muscle afferents a close second.

The cells of origin of the tectospinal system may be antidromically activated from the upper cervical cord (Abrahams and Rose, 1975a). Approximately 60% of all tectospinal cells so identified have been found to be influenced by retinal and neck muscle afferents. Clearly then, this is a supraspinal loop by which a great deal of sensory information can be utilized to influence head movement.

The nature of the information reaching the superior colliculus from extraocular muscles has now been examined in some detail (Rose and Abrahams, 1975b; Abrahams and Anstee, 1977). When the eye was passively moved nasally, a substantial population of units were found which fired only when the eye was moved at saccadic velocity. In the earlier investigation in which horizontal movements were examined (Rose and Abrahams, 1975) a major population was found and analysed that fired only when the eye was in transit past a fixed orbital point at a saccadic velocity. A second population was also found in these studies which responded to small eye movements regardless of initial eye position.

In the more recent study (Abrahams and Anstee, 1977) the technique of eye movement was improved to allow a wide range of movements to be applied in both directions in both the horizontal and the vertical plane. With this more readily controlled technique, three classes of units were now found. A population of units was now found in the superior colliculus which appeared to be sensitive to very low tension, for they responded after the application of

small forces to the eye and in some instances prior to that force eliciting detectable eye movement (about 10). In the course of these latter experiments the presence of a collicular loop was reaffirmed, for it was found that a high percentage of the cells in the superior colliculus activated by eye movements were cells of origin of the tectospinal tract.

Much work remains to be done to elucidate the overall picture of head motor physiology. The role of the cortex has received scant attention (Landgren and Silfvenius, 1968), the cerebellum some more (Berthoz and Llinas, 1974; Wilson, Maeda, Franck and Shimazu, 1976). Our experience has been that wherever we turn we find a new surprise - and huge information gaps, and yet the head motor system is of the greatest importance. After all, how does the monkey know where to put the manipulandum if it cannot move its head to see the cue?

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Table 1.

Spindle content and spindle index in dorsal neck muscles.

Muscle	Cat	Muscle Weight, g	No. of Spindles	Spindle Index
Occipitoscapularis	*A*	0.46	6	13.0
	D (R)	0.93	15	16.1
	D (L)	0.59	11	18.7
Splenius	*B*	3.18	148	46.5
	G	2.59	172	66.4
	E	2.99	189	63.2
Biventer cervicis	*B*	2.42	180	74.4
	A	0.91	68	74.7
	C	1.80	173	96.1
Complexus	*F*	2.66	190	71.4
	G	2.38	254	106.7
Rectus capitis major	*A*	0.62	30	48.4
	B (L)	0.75	44	58.7
	B (R)	0.62	38	61.3
	C	0.68	57	83.8

Spindle index measured as spindles per gram.

TABLE 2. Efficacy of visual and muscle afferent stimulation in eliciting unit discharges in the superior colliculus (this table includes data from Abrahams & Rose, 1975)

Muscle afferent input	N_t	N_v	N_m	N_v/N_t	N_m/N_v
Extraocular	93	68	91	0·73	1·34
Neck	145	107	124	0·74	1·16
Forelimb	106	77	93	0·73	1·21
Hind limb	72	54	51	0·75	0·95

N_t = total number of units.
N_v = visually excited.
N_m = muscle afferent excited.

TECTOSPINAL AND TECTORETICULAR CELLS. A COMPARISON OF THEIR DISTRIBUTION WITHIN THE SUPERIOR COLLICULUS AND THEIR AFFERENT CONNECTIONS

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Abstract

Experiments on chloralose anaesthetized cats have shown that cells in the superior colliculus may be antidromically activated either from the pontomedullary reticular formation or the ventral cervical spinal cord, or from both sites. This has provided a basis for differentiating between cells of origin of the tectospinal tract and the tectoreticular system within the superior colliculus. The distribution of the two cell populations within the superior colliculus has been found to be dissimilar but a comparison of some of their afferent input shows no differences in the inputs to the two cell populations. Afferent input to the superior colliculus is often inhibitory, the inhibition being more commonly observed in tectospinal cells than tectoreticular cells. Evidence has also been obtained that the tectoreticular system, in part, consists of collaterals of the tectospinal tract.

Summary and Conclusions

1. A technique is described whereby cells of origin of the tectospinal and tectoreticular systems may be identified within the superior colliculus of cats anaesthetized with chloralose.

2. Cells of the tectospinal and tectoreticular systems were found to be differentially distributed within the superior colliculus. Tectospinal cells were unevenly distributed antero-posteriorly, with most cells in mid-regions of the superior colliculus. In contrast the antero-posterior distribution of tectoreticular cells was relatively even. In general tectoreticular cells were found in deep layers of the superior colliculus and underlying tegmentum, but tectospinal cells were also commonly found in intermediate layers.

3. Afferent connections to tectospinal and tectoreticular cells from the retina and from neck muscle and extraocular muscle afferents are numerically similar.

4. Afferent stimuli can produce a strong inhibition which is more commonly observed in tectospinal than tectoreticular cells and which, in its duration, is a characteristic of the cell and not the stimulus.

5. An examination of latencies of antidromic activation has provided evidence that many tectoreticular axons are collaterals of tectospinal axons.

Introduction

Neck motoneurons receive input from two descending systems that take origin in the superior colliculus, the tectospinal tract (TST) and the tectoreticulospinal pathway (4). The fibres of the TST leave the brainstem without synaptic junction to terminate in the ventral horn of upper cervical segments (15, 16). Cells of origin of the TST in the superior colliculus may therefore be antidromically excited by electrical stimulation of the contralateral ventral upper cervical cord (1). The tectoreticular (TR) fibres have both an ipsilateral and a contralateral projection (10, 16) and the initial course of TR fibres projecting contralaterally is thought to be similar to that of TST fibres (10, 16). Both TST and TR fibres cross the midline ventral to the central gray matter. TST fibres, together with those TR fibres which terminate in the contralateral pontomedullary reticular formation, then travel in a midline region ventral to the medial longitudinal bundle and close to the raphe nuclei (10, 16).

These anatomical arrangements suggest that stimulation in or close to the medial longitudinal bundle in pontine regions should antidromically excite axons of both TST and TR cells. This could provide a basis for distinguishing between TST and TR cells in the superior colliculus. Both pathways should be excited by midline pontine stimulation, but only TST axons will also be excited by contralateral upper cervical cord stimulation. This technique has in practice proven reliable. With its aid a comparison has been made of the density and distribution of TST and TR cells and axons within the superior colliculus and tegmentum and their sensory inputs have been compared.

Methods

Experiments were performed on 21 cats weighing from 2.4 to 3.8 Kg., anaesthetized with 60 mg/kg chloralose i.v. The methods used for electrophysiological recording have been previously described (1, 2). Both biventer cervicis C3 nerves (18) were prepared for stimulation. One eye was enucleated in 15 experiments and the abducens (VI) nerve and branches of the oculomotor (III) nerve prepared for stimulation. In 9 cats, the trochlear (IV) nerve was also prepared for stimulation. A C1 to C4 laminectomy was performed in all experiments so that a biopolar stimulating electrode (David Kopf, type SCE-100) could be introduced into the spinal cord to antidromically excite the TST. Bone overlying the cerebellum was also removed so that a bipolar stimulating electrode could be introduced into pontomedullary reticular structures. Stimuli to the neck and extraocular muscle nerves consisted of single rectangular pulses 0.05 msec in duration. Because muscle afferent projections to the superior colliculus are largely from smaller fibres (1, 2, 18), pulses were usually 5 to 10 V in magnitude, although the nerve to the inferior oblique muscle occasionally required voltages above this for excitation of

collicular units. Stimulating current to brain and spinal structures was regulated by a Tektronix type 2620 constant current unit with the electrode core as cathode. Visual stimuli consisted of 2 msec light flashes presented by a pre-focussed bulb subtending 2 degrees of arc, which could be placed anywhere in the visual field (1). The eyes were protected with contact lenses.

On completion of surgery, the animals were paralyzed with gallamine triethiodide (Flaxedil, Poulenc) and then artificially ventilated. To reduce respiratory movements, a bilateral pneumothorax was performed. The temperature of the animal was continuously monitored and was maintained between 37° and 39°C using a heated operating table. Extracellular unit activity within the superior colliculus was recorded using stereotaxically introduced tungsten microelectrodes selected from commercial stock (Haer, type 25-10-3). Conventional amplification and display techniques were used. In the initial experiments antidromically activated units in the superior colliculus were identified while stimulating the contralateral medial reticular formation once a second with a 500 μA 50 μsec stimulus pulse. In later experiments a TST unit was first found by stimulation of the ventral cervical cord with a 500 μA stimulus. A second stimulating

electrode was then introduced into the medial pons to a site from which the same TST cell could be antidromically activated. In this way the pontine location of the descending pathway was quickly established. Once a suitable site was found the pontine electrode was kept in the same area, and stimulation repeated once per second. The recording electrode in the superior colliculus was then moved to locate new antidromically activated units. This procedure greatly increased the yield of units. At the end of each recording electrode penetration, the deepest point was marked by a small lesion made by passing 50-100 μA through the recording electrode for 1 to 2 seconds.

The criteria used to identify antidromic activation of cells in the superior colliculus included short constant latency (not varying by more than 10%), ability to follow paired stimuli at 2 to 5 msec intervals, and fractionation of the antidromic potential into IS and SD spikes (5). Positive potentials which were monophasic, or symmetrical biphasic positive-negative were attributed to axons (5). Potentials attributed to cell bodies had a distinct discontinuity of their negative wave and

usually had a large late positive component that failed during repetitive stimulation at a frequency of more than 20/sec (1, 5, 6).

Inhibition of TST and TR cells by afferent stimuli was examined by determining the effectiveness of an orthodromic stimulus in preventing antidromic invasion elicited by pontine or spinal stimulation. The interval between conditioning and test stimuli was varied from 10 to over 700 msec in 10 or 20 msec steps. At least 4 seconds was allowed to elapse between each pair of conditioning-test stimuli.

At the end of each experiment the brain was perfused with saline and then with 25% formaldehyde in saline. Serial 30μ coronal sections of the mesencephalon were cut on a freezing microtome to locate microelectrode recording sites. The pons, medulla, and upper cervical cord were cut in the sagittal plane to locate the placement of stimulating electrodes. All sections were stained for fibres and cells (11). The location of each recording site was established with respect to the lesion identifying the terminal point of each track. The units in the superior colliculus were assigned to the major laminar division previously described (1). Their locations were plotted on four representative coronal sections of the midbrain.

Results

As was to be expected from the anatomy it was possible to find 2 populations of units within the superior colliculus, one of which fulfilled the criteria for TST cells, the other fulfilling the criteria for TR cells. TST cells alone were antidromically excited by stimulation of the contralateral ventromedial upper cervical cord, but both TR and TST cells could be antidromically excited by stimulation in contralateral medial pontomedullary structures. One hundred and eleven units were found in the superior colliculus and underlying tegmentum which could be antidromically excited by stimulation in pontomedullary structures. Fifty-two of these units could also be antidromically excited from the upper cervical cord and fulfilled the criteria for TST cells. Thirteen units were found which could be antidromically excited from spinal cord stimulation but not from pontine stimulation. These were regarded as TST cells with axons some distance removed from the reticular stimulating electrode. The remaining 46 units were positively identified as TR units.

Latency of TST and TR Units

Figure 1 is a histogram of the latencies of TST and TR unit discharge following pontomedullary stimulation. It can be seen that TR units had latencies varying from 0.2 to 4.9 msec but the distribution was skewed so that although the median latency was 0.7 msec, the mean latency was 1.14 msec. TST unit latencies were not skewed in their distribution and their latencies ranged from 0.2 to 1.5 msec with a median of 0.5 msec and a mean of 0.59 msec. The skewing seen in TR latencies is due to the fact that the system contains substantial numbers of slow fibres with latencies in the range of 1 to 4.2 msec. Unlike TR axons, TST axons form a relatively homogeneous fast fibre tract. No relationship could be found in the distribution of TR units within the superior colliculus on the basis of axon size. Twenty-four TR units could be excited by stimulation of medial pontine structures and of these, 16 (67%) had latencies of less than or equal to 1.0 msec. Of the 35 TR units that were excited by stimulation of more lateral sites, 17 (49%) had latencies of less than or equal to 1.0 msec suggesting that more rapidly conducting TR axons travel medially in the pons.

When recording from antidromically activated TST units, it is to be expected that the conduction time from the reticular stimulation to the superior colliculus should be related to the conduction time from the spinal cord to the reticular formation in a way that reflects the difference in conduction distances. In Figure 2 expected conduction times have been plotted together with the actual conduction times of the 52 TST units examined. Measurements based on the positions of stimulating and recording electrodes showed that the distance from the spinal stimulation site to the reticular site was approximately 19 mm and from this site to the superior colliculus approximately 21 mm. Only a few points fall on the line predicted by these measurements. When a generous estimate is made for errors in distance measurement and for the possibility of inconstant conduction velocity, an envelope can be constructed (limits indicated by lines B and C in Figure 4). Many data points still lie outside this envelope.

The proportion of aberrant points was related to the distance of the reticular stimulating electrode from the midline (Table 1). Close to

the midline, 1 of 15 units had a conduction time from the spinal cord to the reticular formation below reasonable limits, and 8 had conduction times which were unexpectedly long. Between 1.2 and 1.9 mm from the midline 13 of 19 units now had unexpectedly short latencies, and 3 of the 19 had unexpectedly long latencies. Overall, only 15 of the 52 units fell within the anticipated limits. The most likely explanation of these data is that a significant proportion of the axons of the TST that are stimulated from reticular sites are not the same as those stimulated from the spinal cord, but are collaterals with a different conduction velocity.

Distribution of Tectoreticular and Tectospinal Neurons and Axons Within the Superior Colliculus

The distribution of antidromically excited units within the superior colliculus is shown in Figure 3. In constructing this figure, a distinction has been made between potentials believed to be of axonal origin and those believed to be due to cell bodies. Axonal potentials in the superior colliculus were, as expected, largely confined to medial

and deep brain stem regions just lateral to the central grey. Confirming previous studies, (1, 4, 12) the cells of origin are both the TST and TR were found to be widely distributed throughout the intermediate and deep layers of the superior colliculus and underlying tegmentum, although an occasional TST cell was found in superficial layers. Because of the presence of the bony tentorium, exploration of the superior colliculus tended to concentrate on more lateral regions. Thus, the lateral concentration of antidromically excited units in Figure 3 may not be entirely due to a reduced number of cells in more medial areas but to the small number of penetrations in more medial areas.

TR and TST cells are not uniformly distributed throughout the superior colliculus and there are differences in their distribution in the various laminae of the superior colliculus and in anterior and posterior regions (Tables 1 and 2). It can be seen that TR cells are more commonly located in the deep layers of the superior colliculus and the tegmentum than are TST cells. Sixty-five percent of TR cells were found in the deep layers of the superior colliculus but only 43% of

TST cells were in these layers (significant at the .05% level, χ^2 test). TST cells are largely confined to the mid-region of the superior colliculus and those TST cells in anterior regions were in the deep layers. In consequence, all TST and TR cells in anterior regions are in the deep layers and underlying tegmentum.

Anatomical Localization of TR and TST Axons in the Pons and Medulla

Some idea of the course followed by TR and TST axons was obtained by plotting the points where the minimum stimulus strength set up antidromic potentials in the superior colliculus. Ninety-four of the 111 ponto-medullary sites which excited superior colliculus units were situated dorsally, close to the midline in or close to the medial longitudinal bundle and neighbouring reticular structures. The 94 units excited from these sites were almost exactly divided between the two systems and 46 fulfilled the criteria for TST units and 48 fulfilled the criteria for TR units. The stimulus sites excited by both these systems overlapped almost completely.

Seventeen pontine stimulus sites were well removed from the medial longitudinal bundle, mostly located ventrally. Anteriorly these sites were in or close to the tegmental reticular nucleus and posteriorly close to the nucleus of the trapezoid body. Eleven of these latter sites activated TR units, and 6, TST units. No evidence was found that axons projecting to more ventral structures took origin in any particular region of the superior colliculus and units antidromically excited from these regions were widely distributed throughout the superior colliculus.

Retinal, Neck and Extraocular Muscle Afferent Projections to TST and TR Cells

We have previously described projections to cells of origin of the TST from neck muscle and extraocular muscle afferents as well as from the retina (1). TR units in the superior colliculus are very similar to TST units in having a multiplicity of afferent inputs (Table 3). Although some differences in sensory input may seem to exist between TST and TR units, statistical analysis with the χ^2 test fails to support this supposition. About 1/3 of the TR units identified in the present

experiments were found to receive input from the neck muscle, biventer cervicis and also to receive input from extraocular muscles. Retinal, extraocular and neck muscle afferents frequently converged onto single TR and TST units and slightly over one-half of the orthodromically excitable cells were excited by all three projections (11/19 TST; 7/13 TR). The neck muscle afferent projection to TR, like that previously described to TST was usually bilateral (15/18 TST; 11/15 TR) and the extraocular muscle afferent projection almost always originated from both the lateral rectus and superior oblique muscles (14/14 TST; 6/8 TR). The inferior oblique nerve stimulation was less effective than other extraocular muscle afferents and only excited a total of 10 TR and TST units. Ten of 29 TST units and 18 of 31 TR units did not have convergent input and were excited by either retinal or muscle afferents but not by both.

The firing patterns of both TST and TR cells following stimulation of neck and extraocular muscle afferents were similar to those reported previously for units in the superior colliculus (1, 2). Both TST and TR

neurons either fired at a short latency (less than 40 msec, median 20-30 msec) at a long latency (50-140 msec, median 70-90 msec) or at both a short and long latency. The frequency with which these firing patterns were encountered was similar for both TR and TST neurons and as previously reported, (1), short latency responses were especially frequent as a result of stimulation of extraocular muscle afferents. All TR (9/9) and most TST (11/14) cells had latencies of less than 30 msec following stimulation of extraocular muscle afferents. The shortest latency observed was 8 msec for a TST cell which was excited from lateral rectus muscle afferents. The majority of TST (11/18) and TR(9/14) cells fired within 30 msec of neck muscle afferent stimulation.

We have previously shown that TR axons have a wide range of conduction velocities. No differences could be found in the sensory input to those cells based on their axonal conduction velocities. For example, retinal and extraocular and neck muscle afferent projections to TR neurons with an antidromic latency greater than 1.0 msec were similar to those of TR neurons with an antidromic latency less than or equal to 1.0 msec.

Inhibition of TR and TST Neurons

Most studies of projections to the superior colliculus have been concerned with the excitatory effects of such stimuli. These projections also have strong inhibitory actions and failure of the antidromic action potential to invade TR and TST cells often followed prior retinal, extraocular muscle, or neck muscle afferent stimulation (Figure 4). The onset of the inhibition was characterised by either a failure of invasion (Figure 4E), a marked reduction in the SD spike so that only a small IS spike was seen, or an increase in the duration of the antidromic action potential and a marked inflexion on the negative potential. These latter effects are attributed to a failure or slowing of the soma-dendritic invasion. There followed a period in which no antidromic potential could be elicited (Figure 4F). At the end of this period of complete inhibition a small negative potential (IS spike) usually returned, followed then after a further short interval by an abrupt increase in size of the spike. When an antidromic potential was tested which was positive-negative biphasic or positive monophasic

no inhibition could be obtained. This is consistent with the view that such potentials originate in axons. Units exhibiting the properties of axons have not been considered in evaluating the frequency with which inhibition was observed.

Inhibition following orthodromic stimulation was most evident in TST units. Stimulation of extraocular or neck muscle afferents led to inhibition of almost all TST neurons tested but only approximately one-third of the TR neurons (Table 4).

Inhibition was independent of axon size and was present in TR cells with latencies ranging from 0.4 to > 2.0 msec. Retinal stimulation was also more effective in inhibiting TST cells than TR cells. These differences between TR and TST neurons are highly significant and the χ^2 showed significance at the 5% level. All TST (13/13) and most TR (9/10) cells tested that showed inhibition were inhibited by at least 2 groups of afferents and 11 of 13 TST and 6 of 10 TR cells were inhibited by neck and extraocular muscle afferents and by visual stimuli.

There was a large variation in the duration of the inhibition in different cells. Some cells were inhibited for only brief periods, usually less than 20 msec while others were inhibited for prolonged periods of up to 600 msec. The duration of the inhibition was not related to the source of the input or whether the neuron belonged to the TST or TR system, but was a characteristic of the individual cell. Inhibition tended to be of the same duration regardless of the nature of the afferent stimulus which provoked inhibition. For example, the duration of the inhibitory period of a TST neuron following a brief flash of light was 450 msec. The same neuron was inhibited for 550 msec by stimulation of afferents from either the ipsilateral or contralateral biventer cervicis muscle, for 450 msec by stimulation of afferents from the lateral rectus muscle, and for 400 msec by stimulation of afferents from the superior oblique muscle.

Like the inhibition itself, the time of onset of inhibition also appeared to be more characteristic of a given unit than to the nature of the stimulus employed to produce the inhibition. This is shown in

Figure 5 in which data obtained from 10 TST and 6 TR neurons is plotted. All units in this group were inhibited by both neck muscle and extraocular muscle afferents. Each point in the plot was obtained by plotting the latency of inhibition following neck muscle afferent stimulation against the latency of inhibition following extraocular muscle afferent stimulation. Most of the points lie along the 45° line that represents equal onset times. Similar results could be obtained by plotting data for any 2 stimuli. Inhibition combined with excitation was most commonly seen while recording from TST cells. TR cells were usually either excited or inhibited by an afferent input and combined excitation and inhibition were rarely seen in TR cells. When a combination of effects was obtained from either TST or TR cells, excitation almost always preceded inhibition regardless of the source of the input (39 of 46 tests for TST neurons and 7 of 7 tests for TR neurons). Excitation preceded the inhibition by 20-120 msec and only occasionally did the inhibition commence immediately after excitation.

Discussion

Anatomical accounts of descending projections from the superior colliculus suggest that there are two major outputs directed to the contralateral brainstem. The majority of fibres from the superior colliculus terminate in reticular and raphe nuclei (10), but a small number of fibres which travel in or close to the medial longitudinal fasciculus to form the tectospinal system, and continue on to the spinal cord. The existence of two descending projections from the superior colliculus to motoneurons of the upper cervical cord was demonstrated physiologically by Anderson et. al. (4). In those experiments it was found that the TST has synaptic actions on neck muscle motoneurons that are much weaker than those exerted by a pathway with a reticular synapse, the tectoreticulospinal system.

In the present experiments we have confirmed the existence of two separate populations of output cells in the superior colliculus by antidromic excitation. However, the antidromic latency characteristics of a large percentage of these neurons excited from both regions suggests that within these two populations there exists a third population in which tectospinal and tectoreticular axons both take origin from the same cell.

To understand the arguments for this, it is necessary to refer to Fig. 2. In constructing this figure, the assumption was made that the overall conduction distance from spinal cord to superior colliculus was 40 mm. If the conduction velocity in each TST axon was constant and the distance estimates correct, all points should lie on line A, with their precise position determined by the conduction velocity of individual axons. The lines B and C in Fig. 2 are the limits to be expected if there were errors of distance measurement of $\pm$ 10% (that is either an over-estimate or under-estimate of 4 mm). Even allowing for such a error, 37 of 52 units (71%) lie outside these limits. There are several factors that could contribute to this. The measurement error could be greater than allowed for, or there could be additional sources of error. For example the assumption that conduction velocity along the axon is constant may not be valid. Lloyd (13), showed that Group I fibres entering the dorsal columns have a slowed conduction velocity and Clough, Kernall, and Phillips (7) showed a 25% slowing of conduction velocity in the proximal portion of axons of the brachial plexus of the baboon. However, no sources of error that we have considered can explain the many

points that lie below the envelope in Fig. 4. We have previously shown that the tectospinal fibres have a wide spectrum of conduction velocities, ranging from 11-125 msec with a mean of 54 msec (1). Assuming a conduction distance from the spinal stimulation site to the reticular stimulation site of 2 cm, this would give an expected range of conduction times to the reticular site of 0.16 to 1.82 msec. It is unlikely that the lower conduction time can be substantially reduced, for the lower latency already represents a conduction velocity of 125 m/sec. The data in Fig. 2 shows that if the same axons are stimulated at both reticular and spinal sites then a substantial number of axons have a calculated conduction time from the spinal cord to the reticular nuclei of 0.1 msec or less, corresponding to conduction velocities in excess of 200 m/sec. While there might be a few fibres with such extremely high velocities, the most likely explanation of the points that lie below the envelopes in Fig. 4 is that the initial assumption in constructing the figure was incorrect. The data are more readily explained if spinal stimulation excites one TST axon branch and reticular stimulation another, and that these two branches have substantially different conduction velocities.

Degeneration following collicular lesions has been reported in the medial longitudinal fasciculus at pontine levels (3, 10, 16) and it is to be expected that this is where the bulk of TST fibres lie. If, however, all TST fibres and their collaterals are in the medial longitudinal fasciculus at pontine levels, great difficulty would be experienced in explaining the present data. If two branches of the TST, one fast and one slow, lie close together in the medial longitudinal fasciculus, it is to be expected that the fastest fibre would have a lower threshold to electrical stimulation and thus be activated prior to the slower. The measured latency of antidromic excitation in the superior colliculus from stimulation in the medial longitudinal fasciculus would then be due to conduction in the faster fibre. Thus it seems likely that the two branches were separated by some distance at the pontine stimulating level. This possibility is supported by the incidence of aberrant latencies when stimulating in the pons at various distances from the midline. When the stimulating electrode was more than 1.2 mm from the midline the majority of TST axons were found to have unexpectedly low conduction times to the superior colliculus. This data is consistent with the view that small collaterals

separate from the TST early, perhaps just caudal to their decussation and these small collaterals travel somewhat lateral to the medial longitudinal fasciculus.

Such a laterally placed collateral could form part of the collicular projection to paramedian pontine regions. Since cells in this area are concerned with eye movement (8, 9) these branched TST axons may be part of a substrate for the coactivation of head and eye movement. The presence of substantial numbers of tectospinal branches occupying a lateral position in the pons might explain the experimental data of Anderson et. al., (4) which led to their conclusion TST has only a minor synaptic effect on neck motoneurons. Anderson et. al., (4) examined the effects of stimulation of the superior colliculus on synaptic potentials recorded in neck motoneurons in cats before and after midline medullary cuts. When such cuts were made in two cats low in the medulla, they "had no consistent effects on the amplitude of contralateral EPSP's. In some cells EPSP amplitudes after the cuts were smaller and more variable than usual". In two experiments when cuts were made high in the medulla, EPSP's were substantially reduced and delayed.

Since Anderson et. al., (4) believed their caudal lesion to interrupt the tectospinal system, the obvious inference was that the tectospinal system had only small effects on neck motoneurons. It can be seen that an alternative explanation for the data now exists if branching of TST fibres is widespread; that is that a significant number of TST axons may give rise to branches which lie more laterally than has been assumed from anatomical experiment and were not interrupted by the low medullary cuts of Anderson et. al. (4).

The locations of both TR and TST cells found in these experiments re-emphasize the fact that output from the superior colliculus arises both from intermediate as well as deep layers (10, 14). The differences in the distribution of TST and TR cells within the superior colliculus may mean that, given the organization of sensory input to the superior colliculus (19, 20) that there are differences in the intrinsic content of sensory information leading to output in the two systems.

It remains to be seen in more precise terms what are the differences and similarities in sensory input, not only to TST and TR cells, but also to collateralized TST cells, and indeed it would be of great interest

to determine the connections of reticular cells excited by these collaterals, for they must be directly related in some way to the head motor system.

The presence of powerful inhibitory connections to cells of origin of the TST in particular would suggest a difference in intrinsic collicular connections between the TR and TST. Because inhibition is unrelated to the stimulus modality used, but is a function of the cell itself it is likely due to some form of recurrent inhibition. The fact that inhibition is most marked in TST neurones suggests that the inhibition is functionally related to the role of the TST in head movement. Perhaps the TST subserves a particular role in the initiation of head movement rather than in the execution of a movement, once initiated.

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TABLE 1

Incidence of aberrant conduction times from spinal cord to reticular formation in TST units, related to different penetration sites.

Latency Difference	Total No. TST Units	Relationship of Stimulating Sites to Midline			
		M to 0.3mm	0.3 to 1.2	1.2 to 1.9	1.9 to 2.9
Less than anticipated	24	1	5	13	5
Within anticipated limits	15	6	4	3	2
Greater than anticipated	13	8	2	3	0

TABLE 2

TST and TRT Cell Distribution in the SC

Region of SC	Number of Cells Encountered		Ratio of TRT Cells to TST Cells
	TST	TRT	
Posterior	7	12	1.7
Mid	45	23	.5
Anterior	3	13	4.3
	--	--	
Total	55	48	.9

TABLE 3

Retinal and Muscle Afferent Stimuli and the Excitation of TRT and TST Neurons

	Source of Input											
	Ipsi Neck			Contra Neck			Extraocular			Retina		
	Number Tested	Number Excited	%	Number Tested	Number Excited	%	Number Tested	Number Excited	%	Number Tested	Number Excited	%
TRT	43	14	33	43	12	28	34	9	26	40	16	40
TST	44	15	34	43	18	42	31	14	45	40	22	55

TABLE 4

Occurrence of Afferent Inhibition in TRT and TST Neurons following Retinal and Muscle Afferent Stimulation

	Source of Input											
	Ipsi Neck			Contra Neck			Extraocular			Retina		
	Number Tested	Number Inhibited	%	Number Tested	Number Inhibited	%	Number Tested	Number Inhibited	%	Number Tested	Number Inhibited	%
TRT	23*	8	35	23**	6	26	23*	10	43	23**	9	39
TST	16*	12	75	16**	13	81	15*	12	80	16**	14	87

* $P < .05$

** $P < .01$

FIGURE 1: Latency histogram for TST (—.—.—) and TR (———) cells following antidromic stimulation from pontomedullary structures.

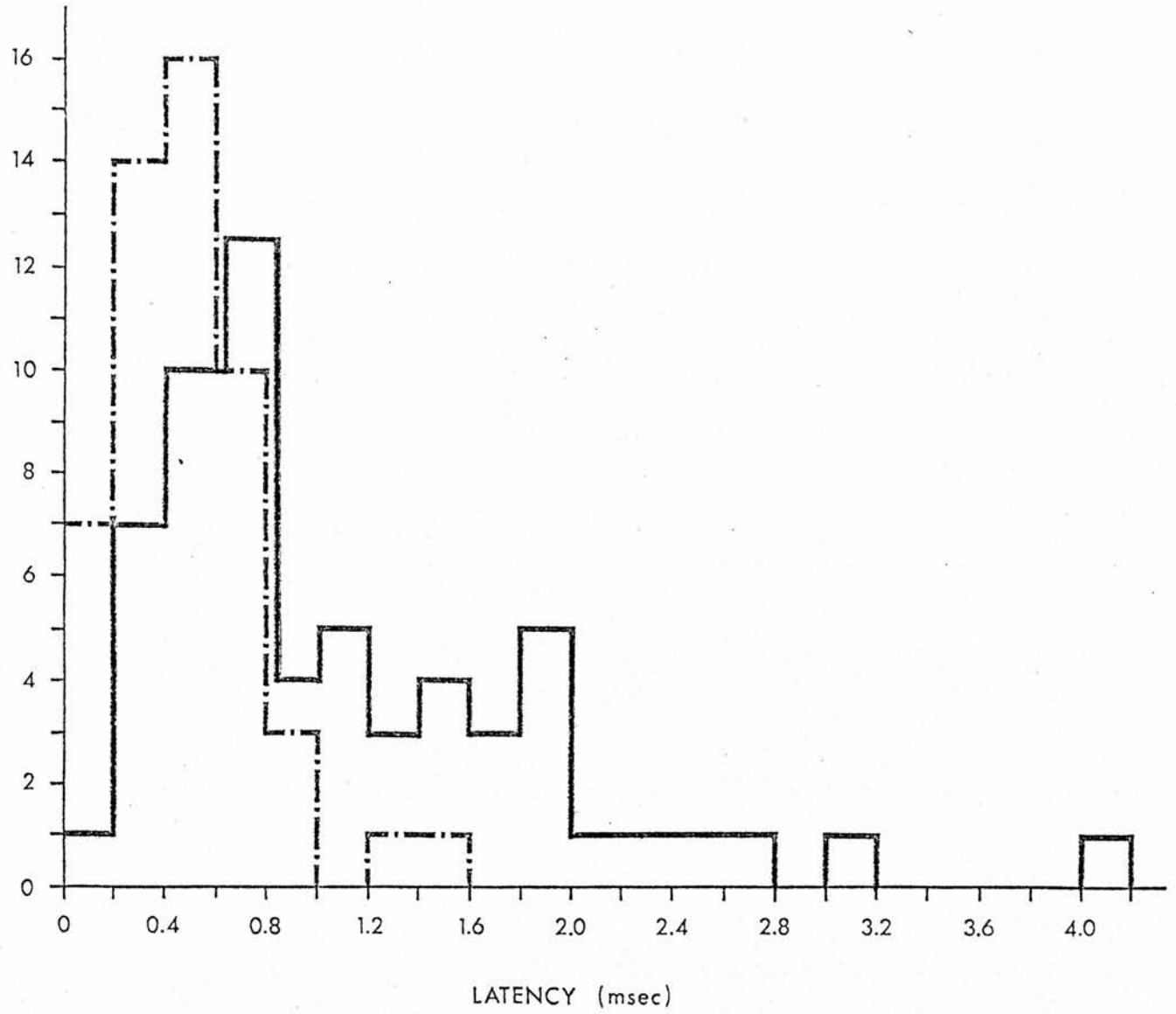

16
14
12
10
8
6
4
2
0
0
0.4
0.8
1.2
1.6
2.0
2.4
2.8
3.2
3.6
4.0
LATENCY (msec)

FIGURE 2 Plot to examine the relationship of conduction times in two segments of TST axons. Conduction time from reticular stimulating site to the superior colliculus has been plotted against the conduction time from the spinal cord to the reticular nuclei for each TST cell examined. The diagram on the right shows the arrangement of the stimulating and recording electrodes. Line A is where all the points would be expected to fall if the overall conduction distance from spinal cord to superior colliculus was 40 mm. The envelope formed by lines B and C allows for distance measurement errors of about ± 25% (see discussion).

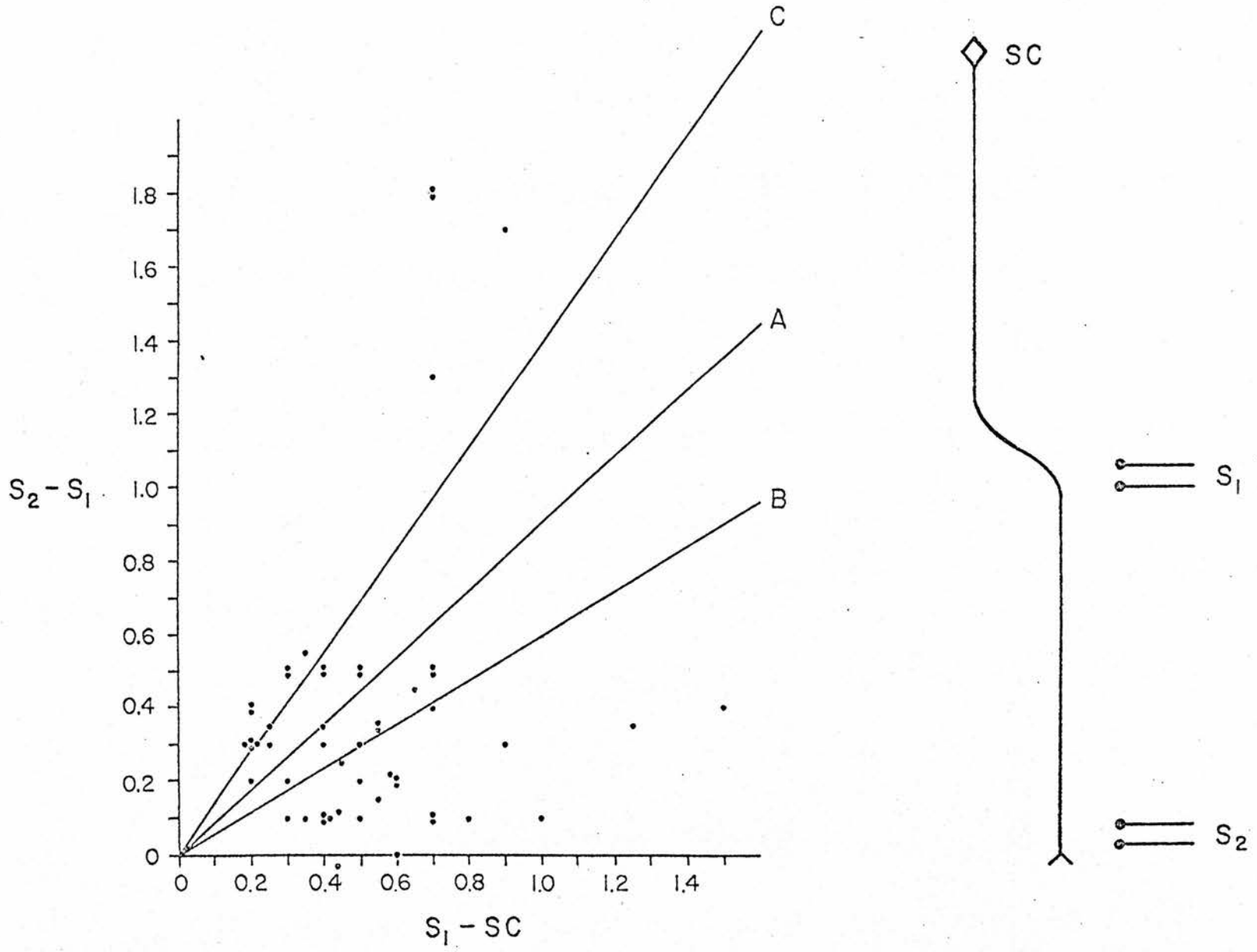

C
A
B
SC
S_1
S_2
$S_2 - S_1$
$S_1 - SC$
0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
1.6
1.8

FIGURE 3: Location of TST and TR cells and axons within the superior colliculus and underlying structures. Caudalmost sections at top of diagram.

Cell bodies - ● axons - A

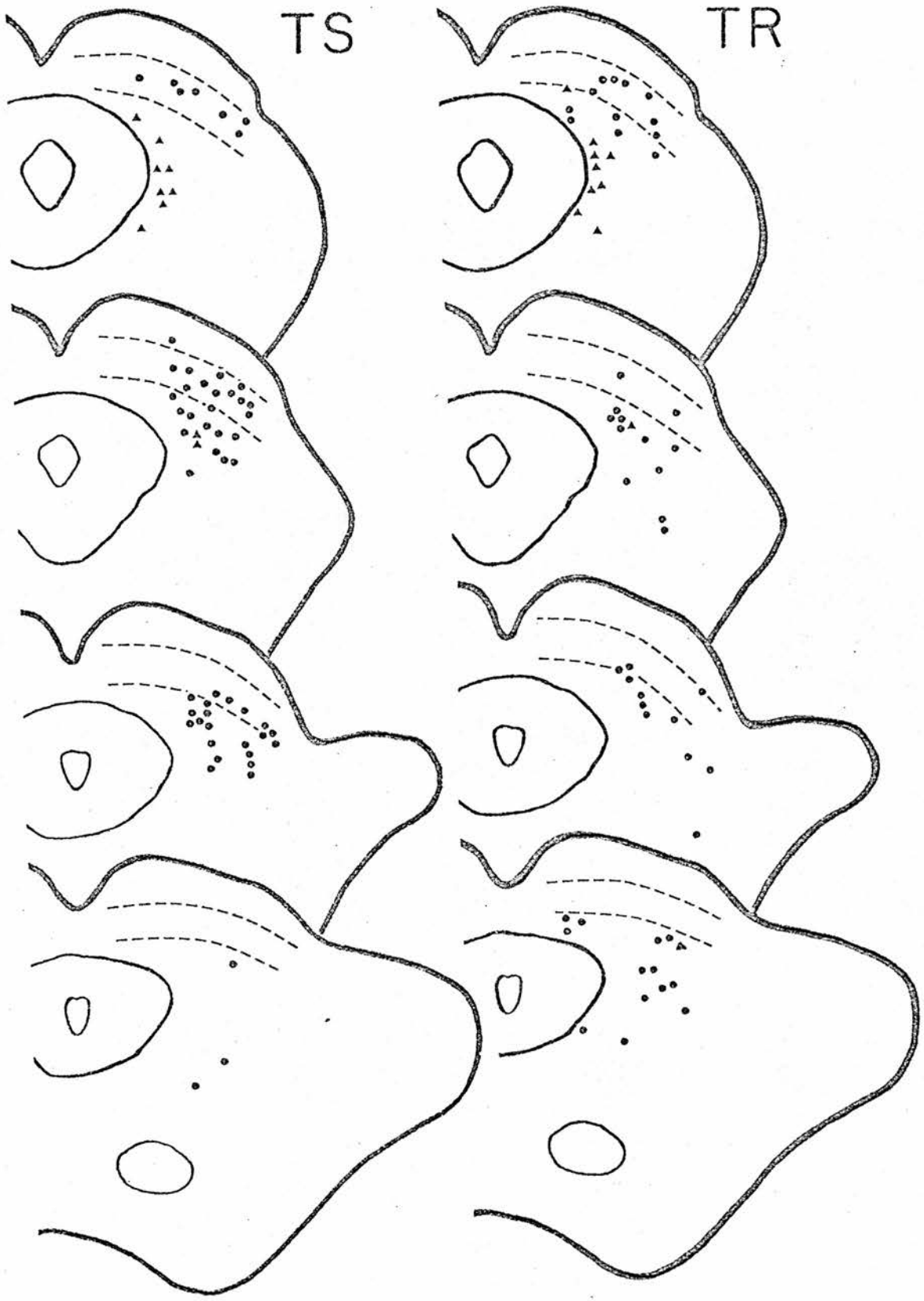

TS
TR

FIGURE 4 : Inhibition of antidromic invasion of cells in the superior colliculus provoked by orthodromic sensory stimuli. A. TST cell antidromically activated from reticular stimulation (early potential) and spinal cord (late potential). B. 2 superimposed responses of the same unit to pairs of spinal stimulation at 2 msec intervals. Note loss of SD response in one late stimulus. C. Same cell as A & B. Superimposed sweeps at 4 sec. intervals; cell antidromically activated by a stimulus, delayed by additional 10 msec in each sweep. D. Same cell orthodromically activated by stimulation of nerve to superior oblique nerve. E. Antidromic stimuli as in C combined with orthodromic stimuli as in D. F. Superimposed fast sweeps showing normal antidromic potential and inhibition produced by superior oblique stimulation 100 msec prior to antidromic stimulation.

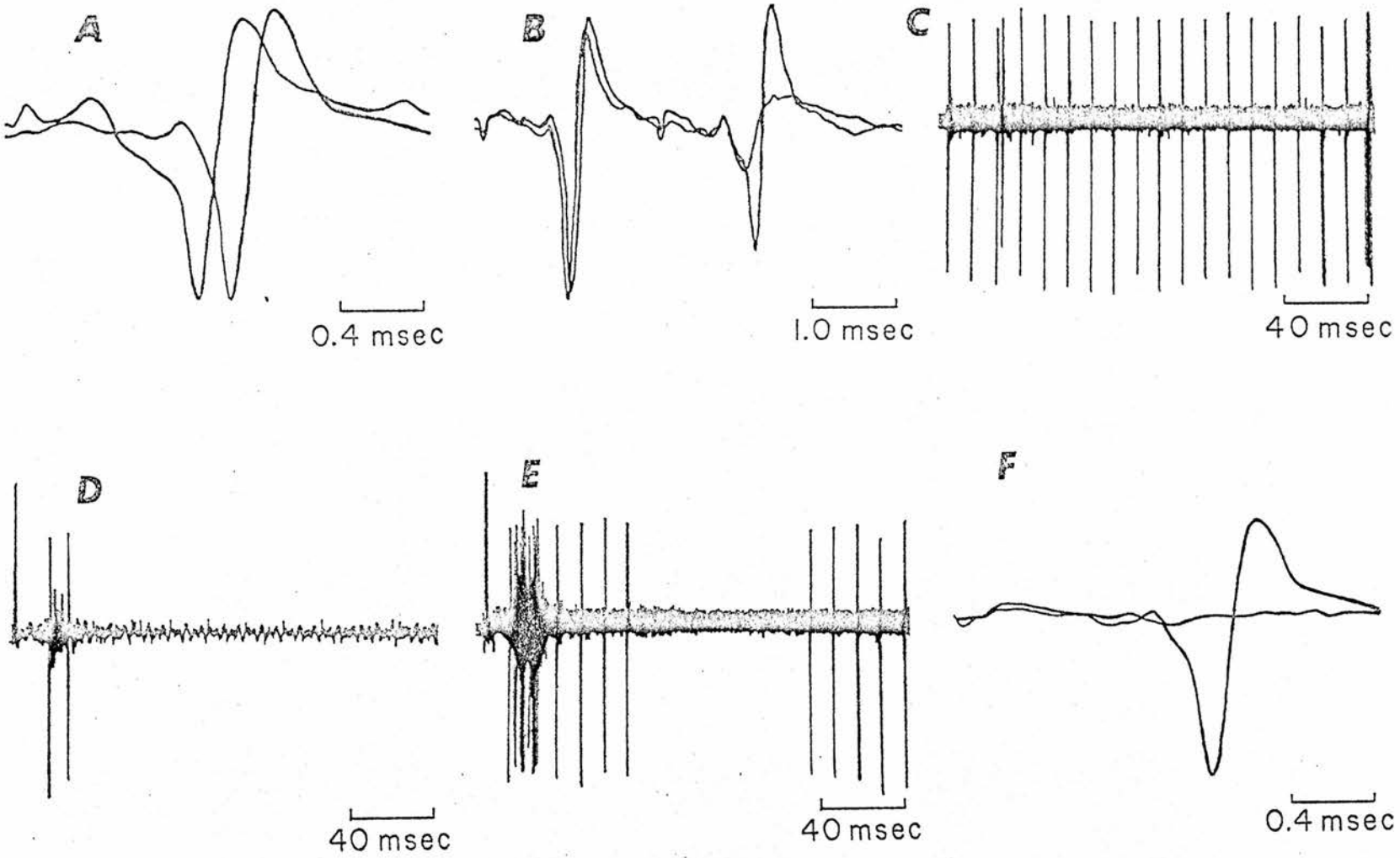
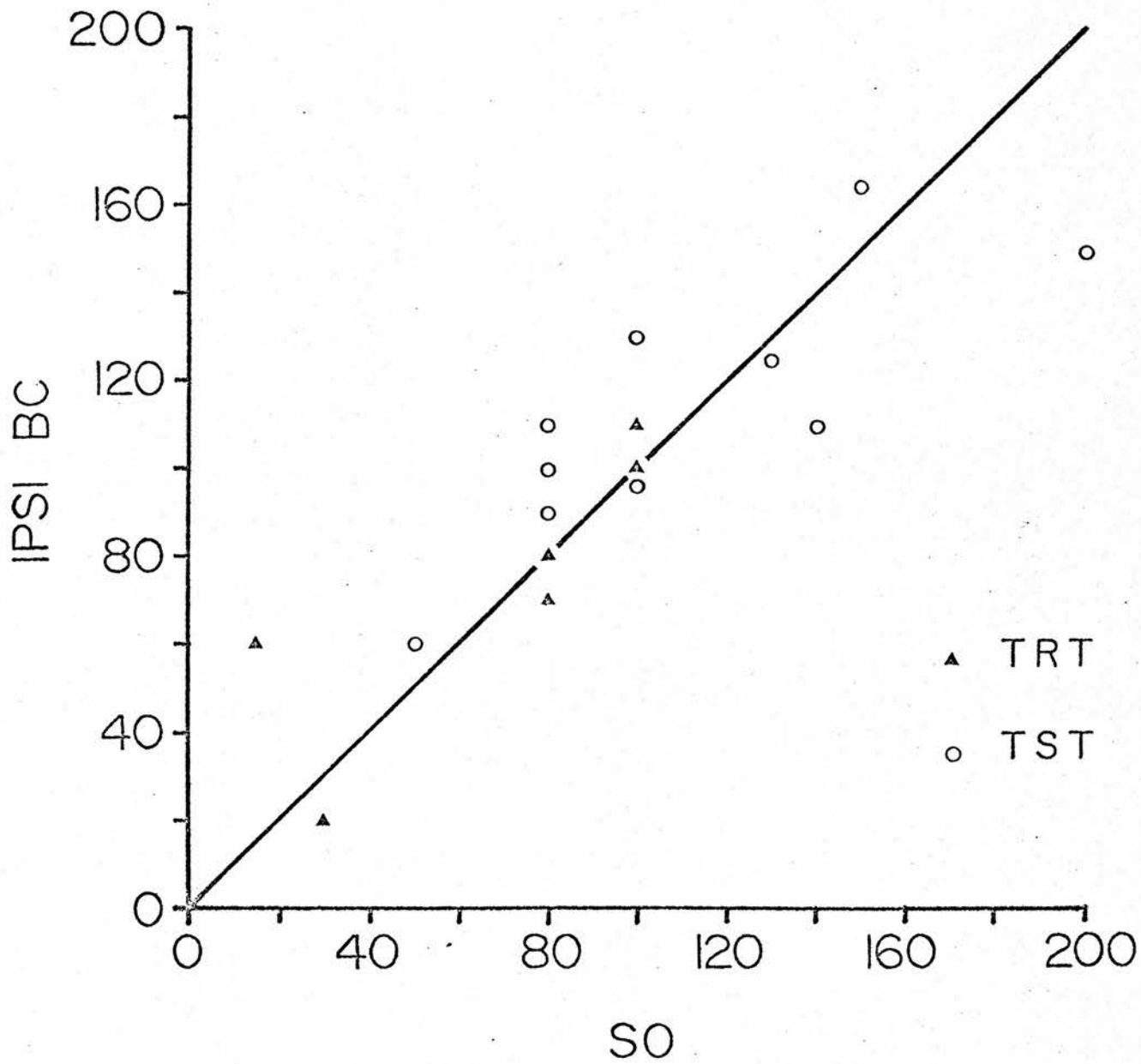

A
0.4 msec
B
1.0 msec
C
40 msec
D
40 msec
E
40 msec
F
0.4 msec

FIGURE 5 : Plot to compare the latency of inhibition in msec in individual TR & TST cells to two different sensory stimuli. Open circles, TST cells; triangles, TR cells. Ipsi BC, stimulation of biventer cervicis nerve ipsilateral to recording microelectrode. SO, stimulation of nerve to superior oblique.


200
160
120
80
40
0
IPSI BC
0
40
80
120
160
200
SO
TRT
TST