ABSTRACT OF THESIS

Name of Candidate Douglas William Young,

Address

Degree Ph.D. *Date* 18th January, 1974.

Title of Thesis Tactile information processing within a trigemino-cerebellar pathway.

The maxillary sinus hairs or vibrissae of the cat appear to be used in searching and possibly orientation behaviour, especially in the dark. The presence of large numbers of different mechanoreceptors in the sinus hair follicle provides an opportunity to investigate the organisation of tactile responses in the central nervous system and the segregation of various functional components of the collective response. In this thesis special consideration is given to the slowly adapting responses of the sinus hair follicle and their possible involvement in cerebellar function. The primary afferent innervation of the maxillary sinus hair follicles were investigated in single fibres of the infraorbital nerve using controlled mechanical stimuli. Four main types of discharge were identified, two slowly adapting and two rapidly adapting. Statistical and qualitative criteria were used to separate the different responses and an attempt was made to relate these patterns of discharge to morphologically distinct receptors located within the sinus hair follicle. Single-unit activity in the trigeminal sensory complex was investigated using microelectrode recording techniques and controlled mechanical stimulation of the vibrissae. Responses obtained were considered in the light of previous results to determine the organisation of the primary afferent projections to the brain-stem and the functional significance of this organisation. The microelectrode recording in the cerebellar cortex showed that the discharge of Purkinje cells could be modified by movement of the maxillary vibrissae. Responses were phasic in nature and mediated by both climbing and mossy fibre pathways relaying in the inferior olive and lateral reticular nucleus respectively.

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TACTILE INFORMATION PROCESSING WITHIN A TRIGEMINO-CEREBELLAR PATHWAY.

by

Douglas William Young

THESIS

Submitted for the degree of Doctor of Philosophy
of the University of Edinburgh
in the Faculty of Veterinary Medicine.

January, 1974.

ACKNOWLEDGEMENTS.

I wish to thank Professor A. Iggo for providing the opportunity and facilities which enabled this work to be carried out. The experiments of Section II were conducted in collaboration with Professor A. Iggo and Dr. K.M. Gottschaldt who gave me considerable practical and theoretical guidance. The assistance given by Dr. R.L. Ramsey in the development of experiments described in Sections III and IV is greatly appreciated.

I am indebted to Mrs. L. Anderson for technical assistance and Mr. C. Warwick and Miss S. Fulton for help given to me during the preparation of this manuscript.

Financial assistance was provided by the Science Research Council and the Peter Wilson Foundation.

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The maxillary sinus hairs or vibrissae of the cat appear to be used in searching and possibly orientation behaviour, especially in the dark. The presence of large numbers of different mechanoreceptors in the sinus hair follicle provides an opportunity to investigate the organisation of tactile responses in the central nervous system and the segregation of various functional components of the collective response. In this thesis special consideration is given to the slowly adapting responses of the sinus hair follicle and their possible involvement in cerebellar function.

Section I.

Previous literature concerning somatic sensation, primary afferent innervation, spinal and trigeminal pathways and their cerebellar projections is reviewed. The innervation of the skin and the maxillary sinus hairs is considered in detail and the suggestion is made that this group of hairs acts as a composite sensory organ relaying tactile information to the central nervous system. Tactile responses in the spinal trigeminal and lateral reticular nuclei are investigated and the organisation and properties of these responses related to function. The relevance of tactile information to the operations of the cerebellar Purkinje cell is discussed and the spinal and reticular pathways carrying this information examined. Trigeminal homologues of these pathways are postulated.

Section II.

The primary afferent innervation of the maxillary sinus hair follicles were investigated in single fibres of the infraorbital nerve using controlled mechanical stimuli. Four main types of discharge were identified, two slowly adapting and two rapidly adapting. Statistical and qualitative criteria were used to separate the different responses and an attempt was made to relate these patterns of discharge to morphologically distinct receptors located within the sinus hair follicle.

Section III.

Single-unit activity in the trigeminal sensory complex was investigated using microelectrode recording techniques and controlled mechanical stimulation of the vibrissae. Responses obtained were considered in the light of the results of section II to determine the organisation of the primary afferent projections to the brain-stem and the functional significance of this organisation. The responses of these cells to electrical stimulation of the anterior lobe of the cerebellum was also investigated and units with a direct projection to the cerebellum identified as cells of the lateral reticular nucleus.

Section IV.

Microelectrode recording in the cerebellar cortex showed that the discharge of Purkinje cells could be modified by movements of the maxillary vibrissae. Responses were phasic in nature and mediated by both climbing and mossy fibre pathways relaying in the inferior olive and lateral reticular nucleus respectively.

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The results presented in this thesis are discussed in relation to the previous literature.

SECTION I

A review of relevant literature

A REVIEW OF LITERATURE CONCERNING SOMATIC SENSATION, PRIMARY AFFERENT INNERVATION, SPINAL AND TRIGEMINAL PATHWAYS AND THEIR CEREBELLAR PROJECTIONS.

The reflexive and conscious mechanisms by which an animal responds to changes in the environment receive information from a variety of sensory structures. These sensory structures or receptors are capable of transducing aspects of the impinging stimuli into coded signals which are passed to the central nervous system where some sort of processing takes place. Since the discovery by Adrian (1926) that the frequency of discharge in the sensory nerve fibre was proportional to the intensity of an applied stimulus and that the generation of impulses was all-or-nothing in nature, the code by which sensory information is transmitted to the central nervous system is assumed to be inherent in a measurable parameter of this discharge.

The physical basis of sensation was approached empirically by physiologists such as Sir Charles Bell and Francois Magendie, who drew the distinction between the dorsal (sensory) and ventral (motor) roots of the spinal cord. Later in the 19th century von Frey (1895) proposed what is now regarded as the classical theory of sensory specificity: that for each of the four modalities of cutaneous sensibility (touch, pain, warmth and cold) there is a specific receptor structure which responds only to the appropriate sensation.

The first challenge to this theory was that of Head & Sherren (1905) who observed that in cases of peripheral nerve injury the restoration of sensibility was always in two stages which followed a strict time sequence. They conclude that "the hairs receive a double innervation when all cutaneous nerves are divided and deep sensibility alone remains a hair can be plucked out without producing any sensation. But as soon as the hand becomes fully sensitive to prick and to the extremes of heat and cold, pulling the hairs produces pain, and stimulation with cotton wool evokes a peculiar radiating tingling sensation. Later when the hand has regained sensibility to light touch, this tingling quality disappears, giving place to the well localised sensation produced when normal hairs are gently moved." The generalised hypothesis derived from these observations was that two parallel cutaneous sensory systems existed, one subserving fine touch sensation (epicritic) and the other pain and temperature (protopathic). This notion has been carried over into neuroanatomy with the concept of medial lemniscal and anterolateral pathways which are the embodiment of the 'epicritic' and 'protopathic' systems respectively. Evidence for such a division draws heavily upon clinical observations. Analgesia and thermanaesthesia produced by anterolateral tractotomy and caudal medullotomy (Sjöqvist, 1938) has been interpreted as due to the interruption of a protopathic pathway. Darian-Smith, Rowe and Sessle (1968) in a study of information transmission in the medulla reported a loss of fine movement discrimination

in the caudal (protopathic) trigeminal nucleus but not in the rostral nucleus which has a demonstrable lemniscal projection. Precise lesion studies with minute examination of sensory loss have shown, however, that this simple duality is not the case. Gilman & Denny-Brown (1966) found that within six weeks of complete dorsal column section (at C2) monkeys could make detailed examination of their immediate 'personal space' and, for instance, pick up a pin, although co-ordinated movement was severely affected. In other experiments hind-limb sensibility was preserved by sparing the lateral columns in an otherwise complete bilateral section of the dorsal columns. This evidence points to spinal afferent pathways carrying fine touch information outside the dorsal column-medial lemniscal route, for example, the spinocervical tract (see A. G. Brown, 1968). An extreme position in the argument over sensory specificity was occupied by Weddell and his co-workers (Sinclair, Weddel & Zander, 1952) who stated that all modalities of sensation could be elicited from areas of the skin where no specific sensory structures could be located histologically. Weddel 's revision of these statements (Weddel , 1966) has isolated Wall (1960, 1970) as the neurophysiologist most committed to a formal defence of the non-specific hypothesis. He suggests an interaction of all modalities on a 'common carrier cell' located in the dorsal root entry zone of the spinal cord. A hypothesis to explain the transmission of pain sensation was put forward (the 'gate control theory'; Melzack & Wall, 1965) where afferent fibres of

different diameters interact to alter the excitability of cells in the substantia gelatinosa of the cord. Wall's view of sensory specificity is that "Pressure sensitive A fibres form a group within which threshold varies with fibre diameter. No justification can be found for the arbitrary subdivision of this group into light touch, deep touch or pain fibres."

This statement was made despite clear evidence to the contrary where many investigators (for example Adrian, 1926; Zotterman, 1939; Frankenhaeuser, 1949; Witt & Hensel, 1959) had established several categories of cutaneous receptors each with different sensitivities to touch and temperature stimuli. The predictions of the gate control theory have not been borne out by experimental investigations. An expected positive dorsal root potential and primary afferent hyperpolarisation following noxious stimulation was not found by Zimmerman (1968), Franz & Iggo (1968), Jänig & Zimmerman (1971) or Vycklicky, Rudomin, Zajac & Burke (1969) although differences in experimental technique makes the comparison of results from different laboratories difficult.

The work of Iggo and his collaborators has taken the classical theory beyond the limits set by von Frey in describing morphologically distinct receptor structures which respond consistently and differentially to identical stimuli. Examples of this are the type I and type II slowly adapting responses to sustained displacement of the skin (see Chambers, Andres, von Duering & Iggo, 1972).

Some of the arguments for specificity or non-specificity must rest with definitions of sensation, perception and modality and the distinction between the peripheral and central nervous system. The number of distinguishable sensations is clearly larger than the number of unique peripheral sensory structures which could account for each specifically if, for example, texture is regarded as a sensation. This implies organisation of sensory information from various sources in a fashion unique to each sensation. A scheme such as this would accommodate a spectrum of receptors activated by specific stimuli upon which 'modalities' are arbitrarily imposed stimulus categories. What is arguable is the resolution of this organisation in the peripheral and lower central levels of the nervous system and the width of the categories, or modalities, of primary afferent information which are ultimately manipulated.

Cutaneous Innervation.

The skin is served by a range of nerve fibres from the large myelinated A fibres to small unmyelinated C fibres. It has been established that in hairy skin there are two types of mechanoreceptor with large myelinated axons and a slowly adapting response to stimulation. The type I receptor or touch corpuscle has received considerable attention from neurophysiologists (Hunt & McIntyre, 1960; Tapper, 1965; Iggo & Muir, 1969; Mann, 1971). The corpuscle consists of an elevated dome of thickened epidermis under which lie up to 50 tactile cells each connected to a large A fibre by short branches myelinated

to within 2 - 10 μm. of the terminal (Iggo, 1969). The response of this structure (as measured in the afferent axon) to a maintained displacement of the skin is an initial high frequency burst of action potentials followed by an irregular discharge which ceases after some seconds or minutes. Munger, Pubols & Pubols (1971) describe a similar structure in glabrous skin which they call the 'Merkel rete papilla'. The slowly adapting type II receptor (Chambers & Iggo, 1967; Chambers et al., 1972) responds less vigorously at the onset of a sustained displacement but maintains a steady, highly regular discharge for, in some cases, several hours.

Hair follicle receptors show properties unique to the type of hair involved. The largest hairs (other than the sinus hairs) are the tylotrichs and these are associated with receptors which discharge only during hair movement and have fast conducting axons (54 - 78 m/sec. Straile, 1960; Brown & Iggo, 1967). The receptive fields of the tylotrich units recorded in the peripheral nerve are from 0·24 - 6·0 cm^2 with 2 - 7 follicles served by a single afferent axon (Brown & Iggo, 1967). The hairs of intermediate size, the guard hairs, have follicle receptors of variable sensitivity and have been separated into two groups, the less sensitive (G_1) and more sensitive (G_2) by Burgess, Petit & Warren (1968). The receptive fields of these units range from 0·5 to 6·0 cm^2 with a great deal of overlap between adjacent units and covering an area containing as many as 500 single hairs, although any one axon innervates only 10 - 20 individual guard hair follicles.

The small down hair follicles have receptors which are exquisitely sensitive to small movements of the hair and have receptive fields of 0·5 - 6·5 cm^2 oriented along the long axis of the limbs and trunk.

The sinus hairs will be considered as a separate group in a later section.

Non-myelinated C fibres in the skin have touch sensitive endings (Iggo, 1959) but are also represented by both warm and cold sensitive terminals (Hensel, Iggo & Witt, 1960; Iggo, 1969). The discharge patterns of the cutaneous 'cold' receptors are unusual in that the primary afferent response may be grouped into bursts at certain temperatures, which poses a special problem of how the stimulus information is coded by the receptor. In primates and in the cat infra-orbital nerve these properties are true of the discharge of some Aδ fibres.

The perception of pain has always been at the centre of the specificity - non-specificity debate, for until recently very little information was available concerning the peripheral response to noxious stimuli. Zotterman (1939) and Burgess & Perl (1967) reported units in the hairy skin of the cat which only responded to high intensity squeezing or pinching of the skin and which were the terminals of axons in the A delta range. A group of thermal nociceptors which respond only to temperatures outside the response ranges of the warm and cold receptors has been identified by Iggo (1959) and Bessou & Perl (1969). The axons of these receptors are non-myelinated and are reported to synapse on large cells at the margins of the

dorsal horns of the spinal cord (Christensen & Perl, 1970).

It is apparent therefore that a varied population of receptors exists within the skin which provides information relating to the tactile and thermal environment. The responses of these receptors can be measured in the afferent nerve fibres and at higher levels of the nervous system, but to what extent do these measurements imply function?

The problems of measurement.

An attempt has been made (Werner & Mountcastle, 1965; Talbot, Darian-Smith & Kornhuber, 1968) to link the results of neurophysiologists and psychophysicists in the hope of assessing the operations of the central nervous system on receptor information. Some psychophysicists have tried to show that there is a general law relating the magnitude of an applied stimulus to the magnitude of the subjective response. Fechner, in 1860 (cit. Stevens, 1961) proposed that this relationship was logarithmic, that is:-

$$\text{Sensation } (S) = a \log_{10} \text{stimulus } (I) + b$$

where a and b are constants.

This equation was derived from Fechner's observation that the just noticeable difference between two adjacent stimuli (on any scale of magnitude) remains subjectively constant at any level of intensity. The mathematician Bernoulli had apparently derived this relationship previously (Boring, 1942) with his assertion that "fortune morale" was proportional to the logarithm of "fortune physique". Stevens' rejection of Fechner's law and his derivation of the power law has had a considerable

effect on both psychophysics and neurophysiology. This law states that subjective magnitude grows as a proportion of the stimulus magnitude raised to a power, or:-

$$\text{Sensation } (S) = a\ (I - I_t)^b$$

where I is the stimulus intensity and I_t the stimulus intensity at the subjective threshold. a and b are constants which differ according to modality (Stevens, 1957, 1958; Stevens & Stevens, 1960). The wide (but not general) applicability of this 'law' is probably due to Stevens' modification of the basic power law:- $S = aI^b$, which proved to be widely inaccurate at low intensities near threshold (Stevens, 1961). By removing the threshold intensity from the scale of intensity $(I - I_t)$ a third adjustable parameter is introduced into the equation (a,b,I_t) which makes the <u>power function</u> more adaptable to <u>any</u> data than either <u>linear</u> or <u>logarithmic</u> functions with only two adjustable parameters (a,b).

Stevens (1957) identifies two categories of sensory continua which are of importance to physiologists who have attempted to appraise their results by the canons of psychophysics. His two classes are:-

1) Prothetic. Sensory information which answers the question; How much?

2) Metathetic. Sensory information which answers the question; What kind, and where?

Examples of prothetic information are the intensity of sound or pressure on the skin, where progress along the intensive continuum takes place by adding excitation to excitation. Examples of metathetic continua are pitch

discrimination or localisation of touch on the skin, where progress along a continuum is by a substitutive rather than an additive process. Stevens (1957) defines methodological and mathematical limits to investigations of these continua, for example the prerequisite ratio scaling of intensity (relative to threshold or a maximum) on a prothetic continuum. Unlike von Frey or Head his two classes of sensory activity are based on methodological rather than physiological or neurological criteria. This work represents an attempt to classify sensation in a useful way, independent of modality.

Cutaneous sensory physiologists have found power relationships between stimulus intensity and the frequency of discharge in primary afferent axons (Werner & Mountcastle, 1965; Brown & Iggo, 1967; Chambers et al., 1972) although it has not been established that the data does not fit other functions equally well (Campbell & Kulikowsky, 1972). The question of the accuracy of the power function has been discussed critically in a recent review article (Kruger & Kenton, 1973) and will be presented with the results of Section II.

The problem is compounded by the inaccurate typing of receptors involved in these investigations and inappropriate descriptions of their responses to stimulation. For example, Werner & Mountcastle (1965) confused the two types of slowly adapting receptor and presumed a role in the perception of touch (c.f. Harrington & Merzenich, 1970 and Mann, 1971).

The hypothesis of a power function relating stimulus

intensity to the frequency of discharge in the primary afferent receptor led Mountcastle (1967) to predict that no further transformation of sensory information takes place in the route through the central nervous system to the efferent response (subjective estimate). This sort of conclusion may be critiscised on several points. Any investigation of the operational properties of a particular sensory system or group of receptors must recognise the possibility of parallel processing of not only the stimulus information (by separate groups of receptors) but the primary afferent information (by different pathways in the central nervous system for different functional ends). For example, the representation of a single group of receptors in both the spinothalamic-posterior thalamic and the medial-lemniscal - ventrobasal somesthetic pathways (Poggio & Mountcastle, 1963). It may also be necessary to treat different aspects of the primary afferent response, such as the phasic and tonic response of slowly adapting receptors, as contributing information to functionally separate operations of the central nervous system. Moreover, even if a specific receptor type is shown to play an unequivocal role in cutaneous sensation there is no reason to treat subjective perceptual phenomena as somehow describing the underlying physiological process on the assumption that the two processes are similar in form, or on the even more hazardous assumption that they are numerically related (Mackay, 1971).

As an argument for the continued measurement of the stimulus-response relationship it can be said that however statistically capricious (see Section II) there is consistent non-linear, monotonic relationship between stimulus intensity and the primary afferent response of cutaneous mechanoreceptors. This relationship may prove useful in identifying the nature of the process which transforms stimulus energy into action potentials (Braitenberg, 1965; Lipetz, 1971) and in assessing the extent to which receptors can discriminate between different stimulus levels and therefore their ability to transmit information relating to stimulus intensity to the central nervous system (Werner & Mountcastle, 1965; Darian-Smith et al., 1968; Walloe, 1968). To this extent the assessment of stimulus-response relations is guide to the functional specificity of a particular afferent unit and makes possible the comparison of data from different laboratories in a quantitative fashion.

In order to approach the general problem of how tactile information is acquired and to what use it is put by the animal it might be fruitful to investigate a cutaneous structure which appears to act as a specific sensory organ. The group of large hairs with complex follicle structure, the sinus hairs, are individually sensitive to a wide variety of mechanical stimuli and in certain locations seem to act as a composite tactile apparatus.

The Sinus hairs and Maxillary Vibrissae.

Many animals, such as rodents, dogs and cats, possess hairs which form an anatomically and physiologically distinct group, the sinus hairs. These hairs are most commonly found in the maxillary region of the face and are arranged in rows, the number and extent of which varies from species to species. The rat generally has 5 rows of 3 - 7 hairs (Zucker & Welker, 1969; Waite, 1973a), the opossum 5 rows of 3,3,5,6 & 7 hairs in each row from dorsal to ventral respectively (Pubols, Donovick & Pubols, 1973). The array of hairs in the cat is somewhat less regular but commonly consists of 4 rows of 4 - 7 hairs 4 to 7 cm. in length(Fig1). In addition there are 2 rows of smaller (1·5 - 3 cm.) perioral hairs (Iggo, 1968). In all these species the largest hairs occupy a caudal position in the group. Cats also have a group of 4 - 7 carpal and 2 - 3 supraorbital sinus hairs.

Uncertainty over the distinctive structure and innervation of the sinus hair has led to considerable confusion in nomenclature. The maxillary sinus hairs are usually termed vibrissae (Vincent, 1913; Fitzgerald, 1940; Hahn, 1971; Patrizi & Munger, 1966 and Zucker & Welker, 1969), or whiskers (Waite, 1973a,b.). Other terms used for sinus hairs are sensory hairs (Winkelmann, 1959) and tactile hairs (Nilsson, 1969 ; Melaragno & Montagna, 1953). Merkel (1880) and Andres (1966) use the term sinus hair describing the exclusive morphological structure of the follicle, which includes the vibrissae as well as other hairs at different locations on the body,

and which does not anticipate hitherto undefined functional properties. The obvious involvement of the maxillary sinus hairs in feeding and searching behaviour has suggested to most authors that this group of hairs function as a specialised sensory apparatus used in the acquisition of fine tactile information and the control of delicate head movements.

Anatomists and physiologists have been attracted to the sinus hairs expecting, and finding, a rich source of information concerning the structure and function of sensory receptors.

Sinus hair morphology.

In attempts to resolve the contemporary debate over the correspondence between form and function several anatomists of the late 19th century undertook to study the morphology of the sinus hair follicle and its innervation in great detail (Gegenbauer, 1851; Dietl, 1872; Bonnet, 1878; Merkel, 1880; Ostroumow, 1895 and Botezat, 1897). Their observations have been extended considerably by Andres' (1966) electronmicroscopical study of the sinus hairs of the rat, cat and rabbit, establishing that the sinus bodies have a complex internal structure and contain several types of nerve terminal. The sinus hair root sheath (of epidermal origin) bears proximal and distal thickenings, the former is surrounded by branched lanceolate nerve endings embedded in dermal tissue, and this contained within a spongy blood sinus. The distal thickening of the root sheath is encircled by a blood-filled, ring sinus and the entire structure

enclosed by a capsule which may be moved relative to the skin surface by striated muscle. The inner hair follicle is separated from the outer, dermal tissue by a 'glassy membrane' of connective tissue. This forms the substrate onto which two types of nerve ending are attached. On the inner surface of this membrane is a band of Merkels' discs which encloses most of the distal root thickening. Opposed to this on the outer surface of the glassy membrane are the straight lanceolate nerve endings which lie in a palisade around the thickening. In the spongy dermal tissue surrounding the lower half of the root sheath are embedded lammelated corpuscular nerve endings (Fig 2). Detailed study of the lanceolate endings revealed that the terminal is sheathed in two Schwann cells at the margins of which, along the mid-line, fingerlike processes extend 0·5 - 3μm. into the collagenous connective tissue in which the ending is suspended. Andres suggests that deformation of these processes could give rise to the generator potential which initiates the impulse discharge.

Andres (1966) reports no significant differences between the sinus bodies of the different species he investigated. Nilsson & Skoglund (1965) showed that the carpal sinus hairs are similar in structure but without the lammelated nerve endings of the lower root enlargement. These sinus hairs were always, however, associated with 5 - 13 Pacinian corpuscles which lay around the sinus body, embedded in the dermis.

Physiological Investigations.

Physiological responses of sinus hairs to tactile stimulation were first recorded by Fitzgerald, (1940) who found a preponderance of slowly adapting directionally sensitive responses to displacement of the cats' vibrissae. His finding that there was maximal excitation of receptors when the hair was moved toward the centre of the group of hairs, has not been sustained by later investigations. Zucker & Welker (1969) and Hahn, (1971) observed that individual afferent units in a single hair follicle would respond maximally to movements in different directions.

The classification of response characteristics of sinus hair receptors is dependent on the laboratory of origin. Zucker & Welker (1969) identify 5 groups of responses; double threshold slowly adapting, moderate velocity threshold slowly adapting, low velocity rapidly adapting, high velocity rapidly adapting and miscellaneous units with less well defined adaptive properties and thresholds. These categories are based solely on responses to a variety of stimuli, and no attempt was made to relate these findings to morphological features of the sinus body. Pubols et al. (1973) describe two classes of slowly adapting responses based upon the presence or absence of resting discharge, making up 53% of the sample of trigeminal ganglion responses. The remaining 47% were either high or low velocity threshold rapidly adapting responses. The classification of Hahn (1971) was based on responsiveness to sinusoidal stimulation and revealed rapidly adapting and two types of slowly adapting responses,

although this stimulus is clearly unsuitable for tonic receptors. All investigators have reported a preponderence of slowly adapting responses ranging from 50 - 70% of the samples of afferent units.

Iggo (1969), while investigating the thermoreceptors of the dog snout, encountered numerous slowly adapting responses originating in the sinus hair follicles of the vibrissae and perioral hairs. Some of the slowly adapting responses could be maintained for several minutes after the displacement of a hair and the pattern of discharge was highly regular. The regularity of the afferent discharge has been used with effect to distinguish between the types of cutaneous slowly-adapting receptors described previously (Iggo & Muir, 1969; Chambers & Iggo, 1967; Chambers et al., 1972) and it was felt that similar properties might be exhibited by the slowly adapting receptors located within the sinus hair follicle.

The Vibrissae as a Sensory Organ.

Under natural conditions several of the maxillary sinus hairs may be moved when an object comes in contact with the side of the face. The barrage of afferent information from the group of hairs will be a complex mixture of various rapidly and slowly adapting impulses trains, which will reflect various aspects of the impinging stimulus. Waite (1973 a,b) reports that there is a somatotopic projection of the maxillary vibrissae of the rat to the contralateral ventrobasal complex. This nucleus is a thalamic relay in the pathway from the facial afferents to the somatosensory cortex. Cells responding

to movement of the vibrissae show velocity and directional sensitivity but no graded response to different deflection amplitudes and no slowly adapting behaviour. These findings suggest that dissociation of the functional components of the vibrissae input may take place at the trigeminal level and that this dissociation may be revealed by differential projections of afferents to or from the nuclei of the fifth cranial nerve.

THE TRIGEMINAL SENSORY COMPLEX.

Somatosensory information from the mandibular, maxillary and opthalmic regions of the face is transmitted via the trigeminal nerve to an array of morphologically and functionally separate tracts and nucleii in the brainstem which constitutes the trigeminal sensory complex. This composite structure extends from the cervical spinal cord to the mid-brain and is bordered by the nuclei of the cranial nerves III to XII, the ascending and descending reticular formation and the bulbar relays of spinal pathways to the thalamus. The diversity of ascending projections from the nuclei and the disparate results of physiological investigations suggest that the sensory trigeminal complex must be viewed as a region where dissociation of the separate functional components of the afferent barrage of cutaneous sensory information takes place.

Olszewski (1950), working on cat and human tissue, identified three cytoarchitectonically distinct nucleii within the spinal trigeminal nucleus. This questioned the validity of the conventional view that the spinal nucleus was an uninterrupted cranial extension of substantia gelatinosa of the spinal cord up to the level of the main sensory nucleus of the trigeminal nerve in the Pons. The corollary of this assumption is that there exists a unity of function over the whole length of the spinal nucleus. Olszewski described the nucleus caudalis, with subnuclei marginalis, gelatinosa and magnocellularis and which was structurally indistinguishable

from the dorsal horns of the cervical cord. The nucleus interpolaris, at the level of the obex, consisted of two cell types, one of which was similar to those cells found in the nucleus cuneatus. Rostral to the nucleus interpolaris, at the level of the inferior olive, lies the nucleus oralis, consisting of small cells with long dendrites. This nucleus extended to the main sensory nucleus, bordering it at the region of the VIIth nerve entry zone. Olszewskis' interpretation of these findings was that the functional organisation of the trigeminal sensory complex was not, as previously supposed, a simple division between the rostral (fine touch) and spinal (pain, temperature) nucleii (Sjöqvist, 1938) but that each morphologically identifiable region might subserve a particular aspect of tactile sensation.

An early attempt by Darian-Smith and Mayday (1960) to find a rostrocaudal somatotopic organisation which might reflect these morphological observations led to the proposition that there was a dual rostral and caudal representation of potentials evoked by electrical stimulation of the face, with no apparent projection to the region of the nucleus interpolaris.

Single-unit investigations of somatotopic organisation have yielded conflicting results. There is a general agreement that on any transverse plane of recording within the nucleus there is an inverted mediolaterally reversed projection of the whole face (Kruger, Siminoff & Witkovsky, 1961; Kruger & Michel, 1962; Gordon, Landgren & Seed, 1961; Darian-Smith, Phillips & Ryan, 1963; Darian-Smith, Proctor &

Ryan, 1963; Wall & Taub, 1962; Nord, 1967).

The concept of a rostro-caudal variation of functional properties, suggested by the early division of the trigeminal nucleus into the rostral and caudal subnuclei , has been challenged by several workers who have observed that cells with similar receptive fields and response characteristics may be found at all levels of the brainstem trigeminal complex. Gordon et al. (1961), investigating the caudal nucleus (1 - 7 mm. caudal to the obex) under nembutal anaesthesia, describe 'A' cells with small receptive fields and early response to stimulation, and 'B' cells with large receptive fields and a longer response latency than the 'A' cells. They found no responses of either type of cell to small thermal increments and no differences in the axial distribution of these cells. Systemic variations in receptive field sizes, which might reflect aspects of function, have been investigated in barbiturate anaesthetised reptiles; Kruger & Witkovsky, 1961, decerebrate cats; Kruger & Michel, 1962 a,b, Wall & Taub, 1962, Eisenman et al., 1963, in chloralose anaesthetised cats; Darian-Smith et al., 1963b, barbiturate anaesthetised cats; Kruger et al., 1961 and in decerebrate rats; Nord, 1967. In reptiles, Kruger & Witkovsky found no rostro-caudal differentiation of response properties and in the cat (Kruger, Siminoff & Witkovsky, 1961) concluded that the rostral nucleus was the trigeminal equivalent of the dorsal column nuclei , and also that the spinal nucleus was similarly organised with respect to receptive fields, adaptive properties and the proportions

of hair and pressure responses of its neurones. Kruger & Michel (1962) re-iterated earlier findings that there was no differentiation of the rostro-caudal axis in terms of receptive field sizes but that the receptive field dimensions were related to the density of innervation at the face rather than the position of the cell in the nucleus. For instance, the perioral skin and vibrissae projected to large areas of the nucleus but they could not assign functional criteria to morphologically distinct subnuclei . They summarise their results as follows:- "The implication to be drawn from our findings that the entire face is represented at virtually all levels of the entire sensory trigeminal complex, with no apparent spatial differentiation in the anteroposterior plane, is that each region of the face projects to a column of cells running from the rostral pole of the principal nucleus of V to the substantia gelatinosa of the upper cervical cord." Darian-Smith, Proctor & Ryan (1963) found that whilst the oral and caudal nuclei displayed the reverse inverted somatotopy described by Kruger et al., (1961) with both large and small receptive fields (c.f. Gordon et al., 1961), there was a degree of rostro-caudal organisation in which the apical portions of the face projected largely to the nucleus oralis and the pre-auricular (temporal/ear) regions of the face to the nucleus caudalis. Cells of the nucleus interpolaris, extending 1 - 2 mm. on either side of the obex showed no somatotopic organisation, in the sense that receptive fields generally covered the whole of the ipsilateral face.

Further controversy was introduced by Wall & Taub (1962), who described (in decerebrate preparations) cells with large receptive fields in the oral and caudal nucleii and cells with small, perioral and maxillary receptive fields in the region of the nucleus interpolaris. These results contradict those of Darian-Smith et al. (1963b) with regard to the sizes of receptive fields in the nucleus interpolaris, but might be due to the differences in anaesthetic procedures. The exacting histological and physiological studies of Eisenman, Landgren & Novin (1963) revealed a cytoarchitectonic organisation on the longitudinal axis of even greater complexity than that described by Olszewski (1950) but could show no organisation of function related to this. From these results it is clear that receptive field size and distribution is an unsuitable measure of functional specificity and more exacting criteria have been searched for.

Wall & Taub (1962) pointed out that N. interpolaris lies at the dorsal root entry zone of the trigeminal nerve, a zone where large myelinated afferent fibres enter the nuclear mass. Unmyelinated fibres penetrate to the caudal pole of nucleus caudalis. Myelinated fibres of the descending tract divide at the level of the obex sending branches rostrally and caudally. In the cat 80% of these fibres terminate in or near the rostral pole of N. caudalis and only 10% of these fibres reach the caudal pole of this nucleus at C2. The fibres which extend from the entry zone taper, resulting in a deceleration of impulses travelling along these axons (Wall & Taub, 1962,

Darian-Smith, Mutton & Proctor, 1965).

The changing spectrum of afferent fibre diameters and their subsequent synaptic organisation on the nuclear cells has been put forward as a means by which modality segregation might be achieved. With this model in mind, Wall & Taub suggest that the descending tract of the trigeminal nerve is the facial equivalent of the dorsal columns and the nuclei caudalis and interpolaris equivalent to the dorsal column nuclei , that is, the brain-stem relay for fine tactile sensation projecting to the contra-lateral ventrobasal complex of the thalamus via the medial lemniscus. This function was conventionally ascribed to the rostral, rather than the caudal nuclei .

Investigation of the synaptic organisation within the spinal trigeminal nucleus (Erikson, King & Pfaffman, 1961; Darian-Smith, 1965; Darian-Smith et al., 1965) have shown that excitability changes recorded after conditioning stimuli follow a time course identical to that of primary afferent depolarisation and which are probably presynaptic in origin, but with no variable distribution along the rostro-caudal axis. Young & King (1972) found no primary afferent hyperpolarisation with toothpulp or C fibre input which might be predicted if 'gate control' were operating in the nucleus caudalis. Neither did noxious conditioning stimuli significantly reduce antidromic test excitation in this region.

The apparent absence of functionally distinct cell groupings in the spinal nucleus has led to the search for a coding scheme by which information relating to the

intensive, temporal and spatial patterning of the impinging stimulus could be organised and transmitted to higher centres of the nervous system. To a certain extent, such information may be coded by the discharge of a single primary afferent, secondary or tertiary cell pathway (Werner & Mountcastle, 1965; Walloe, 1968; Darian-Smith et al., 1968; Kruger & Kenton, 1973; Waite, 1973b) and there is no a priori reason to expect specific aspects of function to be represented differentially in separate groups of cells, at least not at the level of the trigeminal nucleus. Alternatively, the criteria upon which functional specificity has been defined may require revision, and this approach has been made in a number of recent studies, especially those of Darian-Smith et al., (1968) using the notional concept of information transmission developed by MacKay & McCulloch (1952) as it relates to the number of spikes in response to one dimension (in this case intensity) of the applied stimulus.

An important aspect of the trigeminal nucleus, recognised by Olszewski (1950), Carpenter & Hanna (1961), Wall & Taub (1962) and Darian-Smith, Phillips & Ryan (1963), is that along its rostro-caudal axis the adjacent brain-stem structures change in nature. The assumption must therefore be made that if such structures subtend substantially different functions, there is justification in supposing that the trigeminal cells which project to these structures carry information related to these functions.

The work of Olszewski (1950) established that cells of the nucleus caudalis of the spinal nucleus of V were

continuous with, and morphologically similar to, the substantia gelatinosa of the 1st cervical segment. It was assumed that these adjacent structures function in a similar manner, that is, in transmitting information concerning pain and temperature to the thalamus. The receptive field organisation and response properties of cells in the oral and principal nuclei have suggested to some workers (Kruger & Michel, 1962) that these nuclei are organised in a fashion similar to that of the nearby cuneate and gracile nuclei and in fact are the facial components of a whole body representation at the level of the brain stem for somatosensory information relaying, via the medial lemniscus, to the contralateral ventrobasal complex. Darian-Smith, Phillips & Ryan (1963) found that by antidromically activating cells from the contralateral arcuate nucleus, a pathway with a conduction time of approximately 2 ms. existed, also a polysynaptic pathway of latency 2 - 4 ms. The short latency pathway is thought to be monosynaptic and 'lemniscal' in its properties. The most significant contribution to this pathway, as measured by the number of cells activated from the contralateral thalamus with a latency of less than 2 ms., is from the region of the nucleus oralis of the spinal trigeminal complex. The activation of cells in the region of the nucleus interpolaris was largely via a long-latency polysynaptic pathway and this would seem to preclude this nucleus from involvement in 'medial lemniscal' function, as suggested by Wall & Taub (1962). Gordon et al. (1961) and Nord (1967) assumed that the caudal

nucleus was involved in the 'lemniscal' pathway, but did not make this test of contralateral thalamic stimulation. Of the cells found in the nucleus interpolaris that did not respond to stimulation of the contralateral thalamus directly (Darian-Smith, Phillips & Ryan, 1963) a large number were found which could be directly activated from the anterior lobe of the cerebellum. Darian-Smith and Phillips (1964) showed that 38% of these cells were located in the region of the nucleus interpolaris, but subsequent histological examination showed that "the cell bodies of most of these cells lay within the lateral reticular nucleus." No investigation was made of the functional properties of these cells.

Evidence from degeneration studies has suggested that there is a direct pathway (Carpenter & Hanna, 1961) and an indirect pathway via the reticular nucleus (Brodal, 1949, 1953), between the trigeminal nucleus and the anterior lobe of the cerebellum. A substantial spino-reticulo-cerebellar pathway exists, the properties of which are at least partly known. A possible homology may exist between the spino-reticulo and trigemino-reticulo-cerebellar pathways, the implications of which would be important in the interpretation of functional divisions at the trigeminal level.

The position, structure and function of the Lateral Reticular Nucleus.

The involvement of the reticular nucleus (LRN) in a trigemino-cerebellar pathway opens up several possibilities for the segregation of function at the level of the brain-stem.

Histological and physiological studies of the Lateral reticular nucleus have established a powerful role in the projection of spinal and trigeminal proprioceptive and exteroceptive information to the cerebellum. An early paper of Blakeslee, Freiman & Barerra (1938) states that all projections of LRN terminate in the cerebellum. Afferent fibres entering the nucleus were said to originate in the dorsal and ventral spinocerebellar tracts as well as the homolateral dorsal column nuclei . Brodal (1949) extended these results in some detail with degeneration studies, making clear the distinction between architectonically distinct groups of cells contained within LRN. In any transverse plane the nucleus consists of a ventral portion containing the pars magnocellularis (ventrolateral) and the pars parvicellularis (ventromedial), and a separate dorsal portion the pars subtrigeminalis. These regions do not share identical afferent input. Lesions of the lateral funiculus at the second cervical level (C2) produced considerable degeneration of synaptic boutons in the pars parvicellularis and magnocellularis but not in the subtrigeminal region. Ventrolateral funicular section at C1 produced degeneration of fibres in the dorsal and lateral funiculi and revealed bundles of fine fibres passing through the nucleus and medio-lateral to it. In general, ascending fibres from the thoracic and lumbosacral divisions of the cord were shown to terminate in ventrolateral, superficial areas of the nucleus. Fibres projecting from the cervical divisions of the cord terminated in the medio-central areas, suggesting that the

subtrigeminal portion of the nucleus received afferents from regions rostral to C1, possibly the medullary cranial nucleii.

The relation of LRN to other brain-stem structures.

Some confusion has existed as to the localisation and role of the lateral reticular nucleus in relation to other brain-stem structures, notably the ascending and descending reticular formation and the nuclei of the cranial nerves V, VIII and XII. Brodal (1953) pointed out that the term 'reticular formation' was coined by anatomists and the term 'reticular activating system' by neurologists, and that the two terms rarely describe the same structure or process. He stated (1953) that the lateral reticular nucleus described by Walberg (1952) (sometimes referred to as the nucleus funiculus lateralis) is a clearly defined nucleus relaying proprioceptive and exteroceptive information to the cerebellum. Another discrete structure is the nucleus reticularis tegmenti pontis which lies rostral to LRN as a continuation of the pontine grey and which has been identified as a cerebro-cerebellar relay (Brodal & Brodal, 1971). These nuclei lie within a less clearly defined mass of cells and fibres collectively known as the reticular formation. These masses are largely concerned with extra-pyramidal motor function and send ascending and descending collaterals to numerous bulbar, pontine and thalamic structures. LRN is then, a well-defined nucleus with an intrinsic morphology visible in any transverse plane and divided (Brodal, 1949, 1953) into three regions along the anterior - posterior axis.

These divisions are:- the superior nucleus at the level of the inferior olive; the middle nucleus at the root of n. VIII; the inferior nucleus at the level of descending tract of n.V.

Spino-Reticulo-Cerebellar pathways.

Spino-reticulo-cerebellar pathways have been investigated in some detail and the function properties of afferents to this system have been tentatively established (Grant, Oscarsson & Rosen, 1966; Oscarsson & Rosen, 1966; Rosen & Scheid, 1973 a,b,c.). Recording in the lateral reticular nucleus and the ipsilateral restiforme body the ascending tract of this system was identified as the bilateral ventral flexor reflex tract (bVFRT) containing fibres of the flexor reflex afferents (Lundberg & Oscarsson, 1962). These afferents are defined as the group II and III muscle and joint afferent fibres; high threshold joint afferent fibres and cutaneous afferent fibres (Eccles & Lundberg, 1959). This definition however, has proved to be inadequate, especially in the distinction between the various types of cutaneous afferent fibres and the possible inclusion of unmyelinated C - fibres (Franz & Iggo, 1968). Activation of cells in this pathway by electrical stimulation of nerves of all four limbs was equally effective (Grant et al., 1966). Rosen & Scheid (1973b) have found that bVFRT axons with cell bodies in cervico-thalamic regions of the cord respond to both fore and hind limb afferents and similarly that axons of bVFRT with cell bodies in lumbar region respond to forelimb stimulation too. They thus explain the great convergence recorded in

cells of LRN (Rosen & Scheid, 1973a) by convergence at segmental levels before transmission to the nucleus. The afferent fibres ascend in the lateral funiculi and originate at all levels of the cord (Brodal, 1949), terminating in the parvi and magnocellular regions of the nucleus. These 'spinal' nuclear cells send axons via the ipsilateral restiforme body to the vermis, terminating as mossy fibres (Brodal, 1949, Szentagothai, 1964).

Functional properties.

Functional analysis of the reticulo-cerebellar pathway showed that LRN received information concerning the flexor-reflex patterns involving all four limbs and the trunk (Combs, 1956; Grant et al., 1966; Oscarsson & Rosen, 1966; Rosen & Scheid, 1973 a,b,c). Grant et al. (1966) showed that group I muscle afferents were not effective in evoking an LRN response and that manual stimulation of cutaneous receptors was only weakly effective, requiring vigorous stroking and tapping. Rosen & Sheid (1973c), investigating the cutaneous input to cells of LRN, found a general convergence of different types of cutaneous receptors. This convergence took the form of either inhibition or excitation, with latencies of between 8 - 20 ms. Tap responses, however, were generally excitatory and showed a latency of about 9 ms. Weights applied to the skin often produced a tonic inhibition for the duration of application, an inhibition which could suppress a superimposed tap response. Receptive fields were very large and showed no somatotopic organisation

within the parvicellular and magnocellular portions of the nucleus. Similarly, no discrimination could be made between cells activated from hindlimb and forelimb areas of the cortex (Rosen & Scheid, 1973a). An important feature, observed by Oscarsson (1967a) and Rosen & Scheid (1973c), is that cutaneous stimulation is more effective in decerebrate preparations than in anaesthetised animals. The conclusion has been drawn that activity in this tract is subject to control by descending systems at both the segmental and brain-stem levels (Oscarsson, 1967a).

Such convergent and labile activity provides no modality specificity and only crude spatial information. The Oscarsson group suggest that the reticulo-cerebellar pathway subserves the general function of altering the background activity of cells in the cerebellar cortex, ultimately relating to the 'transmittability' of segmental neurones participating in spinal reflexes.

The spino-reticulo-cerebellar mossy fibres have actions on the molecular layer Purkinje cells which are distinct from those originating in the dorsal and ventral spino-cerebellar tracts (DSCT & VSCT). Szentagothai (1964) observed that reticular mossy fibres terminated on the granule cells which make a more superficial contact with the Purkinje dendritic tree whereas DSCT and VSCT mossy fibres terminated on those granule cells which synapsed at deeper layers of the Purkinje arborisation. The distribution of these intrinsic fibres of the cerebellar cortex has been shown to be of critical importance in

the control of cerebellar outflow (Eccles, Ito & Szentagothai, 1967). A detailed account of the cutaneous projections to the cerebellum via these pathways is presented in a later section.

Trigemino-Reticular projections.

Information relating to the trigeminal afferents of LRN is less than abundant. In a study of trigemino-cerebellar projections Carpenter & Hanna (1961) described an indirect pathway relaying in the ventral cell group of the contralateral reticular nucleus (Brodal's pars magno-cellularis and parvicellularis). These results are confirmed and extended by Stewart & King (1963) who reported ipsilateral projections to LRN from the nucleus caudalis of the spinal trigeminal nucleus. These axons were, in the main, secondary fibres originating as cells of the trigeminal nucleus, not first - order sensory axons of the trigeminal nerve. Physiological evidence for a trigemino-reticular tract was put forward by Darian-Smith and Phillips (1964) who discovered that most of their trigemino-cerebellar cells lay within the lateral reticular nucleus.

The Functional properties of trigeminal nuclear cells.

The functional properties of the cells projecting from the trigeminal sensory complex to other structures are less well known than cells of spinal pathways. In a preliminary investigation of the information-transmitting properties of trigeminal cells which respond to movement of the vibrissae, Darian-Smith et al., (1968) found that cells of the nucleus caudalis could transmit only one bit

of information concerning the extent of the hair deflection, that is, only the information that the hair had been moved was conveyed. In cells of the nucleus oralis, however, 80% of the information transmitted by the cutaneous receptor was conveyed. This, in conjunction with earlier findings that the nucleus has a large projection to the contralateral thalamus (Darian-Smith, Phillips & Ryan, 1963) suggested a traditional role in relaying information concerned with fine touch discrimination to the thalamus and ultimately the somatosensory cortex.

Attempts to classify the types of responses in the trigeminal nucleus to tactile stimulation, in terms of the categories of cutaneous receptors observed (Iggo & Muir, 1969; Brown & Iggo, 1967; Chambers et al., 1972) have not been made in a systematic manner until very recently (Mosso & Kruger, 1973). Darian-Smith et al. (1968) described both types of slowly adapting and rapidly adapting responses in the spinal nucleus; Kruger & Michel (1962) identified rapidly adapting and the slowly adapting type I response in the caudal nucleus; Wall and Taub (1962) mention hair responses of the "non-adapting, stretch receptor type" which we must attribute to the sinus body type II (SAII) response described by Gottschaldt et al. (1972), activated only by the movement of the maxillary vibrissae. Mosso & Kruger (1973) investigating the afferent input to the nucleus caudalis, found slowly adapting vibrissae units with directional sensitivity, types D, G_1 and G_2 hair responses and Pacini-type vibration sensitive units. The slowly adapting responses were

capable of fine gradation by altering hair deflection amplitude, contrary to the results obtained by Darian-Smith et al. (1968) and indicating that fine touch sensitivity might be mediated by the caudal as well as the rostral nucleus.

Investigations into the primary afferent responses to mechanical stimulation of facial skin and hair, especially the vibrissae, have shown that approximately 50% of these vibrissal responses are slowly adapting in nature (Fitzgerald, 1940; Hahn, 1971; Gottschaldt et al. 1972). The proportion of slowly adapting cells in the second and third order cells of the trigeminal complex and its projections is markedly lower, and this has been observed in both decerebrate and anaesthetised preparations. Kruger, Siminoff & Witkovsky (1961) found only 13 of 117 cells in the spinal nucleus had slowly adapting responses and Waite (1973b) recording in the contralateral ventrobasal complex, could find no slowly adapting responses to movement of the maxillary vibrissae. She suggested that slowly adapting responses might be 'filtered out' or diverted elsewhere in the nervous system.

The responses of hindlimb-activated mossy fibres in the cerebellar cortex have been shown by Eccles et al (1972a) to be largely slowly adapting, with considerable activation from the foot-pads and having both excitatory and inhibitory effects on the subsequent Purkinje cell discharge. Mann (1971) identified the slowly adapting SAI receptor in DSCT mossy fibres projecting to the anterior lobe of the vermis. A functional pathway might then be postulated

which conveyed information relating to the tonic activation of facial sinus hair receptors to the cerebellum. This putative pathway could ascend via the trigemino-cerebellar relays investigated by Darian-Smith & Phillips (1964) and Carpenter & Hanna (1961) and would involve mossy fibres entering the cerebellum via the ipsilateral restiforme body. The possible spinal homologues of this pathway and their actions in the cerebellar cortex have been investigated in some detail.

THE PROJECTIONS OF CUTANEOUS AFFERENT RECEPTORS TO THE CEREBELLUM.

Cerebellar responses to cutaneous stimulation have been described by several authors (Adrian, 1943; Snider & Stowell, 1944; Carrea & Grundfest, 1953; Combs, 1954; Eccles, Rosen, Scheid & Taborikova, 1972; Eccles, Sabah, Schmidt & Taborikova, 1972a,b,c; Leicht, Rowe & Schmidt, 1973 a,b). Combs (1954) suggested a dual, specific and non-specific representation of cutaneous afferents which may be revealed under different anaesthetics. The non-specific projections become apparent in barbiturate anaesthetised preparations, the specific projections in the decerebrate preparation. In general, mid-line structures project to the anterior lobe, peripheral structures to the hemispheric portions of the anterior lobe and the paramedian lobules (Adrian, 1943; Snider & Stowell, 1944; Combs, 1954). In particular, receptors activated by movements of the vibrissae project to a circular area of the anterior lobe extending two folia either side of the fissura prima.

The detailed structure of the cerebellar cortex is well known, and the operational relationships between the two ascending fibre systems, the mossy and climbing fibres, and the five main types of cortical neurones have been established over the past decade (see Eccles, Ito & Szentagothai, 1967; Eccles, 1969; Eccles, Faber, Murphy, Sabah & Taborikova, 1970; Eccles, 1973).

The Purkinje cell discharge is the only output from the cerebellum and these cells are subject to direct

afferent excitation by climbing fibres (Eccles, Llinas & Sasaki, 1966a) and indirect excitation by parallel fibres via the mossy fibre granule cell relay (Eccles et al., 1966 c,e). The Purkinje cell discharge may be directly inhibited by the actions of stellate and basket cell axons. These cells are activated by the same band of parallel fibres passing through the dendritic tree of an active Purkinje cell. The axons of the basket and stellate cells inhibit the discharge of Purkinje cells lateral to the band of active parallel fibres. The strip of excited Purkinje cells would then be approximately 2mm long (the length of the parallel fibres) with about 600 μm. of inhibition on either side - the extent of the basket and stellate cell axons (Palkovits, Magyar & Szentagothai, 1971). The activation (not the discharge) of Purkinje cells may also be indirectly inhibited by the post synaptic actions of Golgi cell axons on the granule cell dendrites. Inhibition occurs where the dendrites make contact with the mossy fibre terminal boutons in the granule layer glomeruli (Eccles et al., 1966d). This inhibition prevents the discharge of parallel fibres and the subsequent activation of Purkinje cells (Eccles et al., 1966e). Postsynaptic Golgi cell inhibition of the mossy fibre/granule cell relay ensures that weakly excited granule cells are inhibited whilst those granule cells strongly activated by a mossy fibre barrage will discharge, so 'sharpening' the afferent activation of Purkinje cells (Eccles et al., 1970). The action of adjacent Purkinje cells upon each other is also inhibitory, mediated by the recurrent

collaterals of the Purkinje cell axons. The degree of convergence upon the Purkinje cells and the highly integrated nature of the efferent outflow from the cerebellum is indicated by the estimated convergence of 60 - 120,000 parallel fibres onto one Purkinje cell and the passage of a single parallel fibre through the dendritic arborisations of approximately 460 Purkinje cells (Eccles et al., 1967). The parallel fibres act therefore as a diffuse input which may be modified and shaped by Golgi inhibition, whereas the climbing fibres (which are outnumbered by mossy fibres in the ratio 600:1) provide a powerful, specific excitation (Eccles et al., 1966a).

Spinocerebellar pathways.

Spinal pathways to the cerebellar cortex convey information from muscle, joint, tendon and cutaneous receptors. This information is involved in the detection of relative movement between different parts of the body (proprioceptive) and between parts of the body and external space (exteroceptive), where external space in this context is the immediate tactile environment. Oscarsson (1973) has reviewed the functional organisation of the spinocerebellar projections and concluded that there are at least 12 functionally distinct pathways involved. Specific cutaneous afferents are strongly represented in the dorsal and cuneocerebellar tracts and the spino-olivo - cerebellar tract. More convergent cutaneous information is transmitted via the reticular nucleus of the brain-stem and directly to the cerebellum

as part of the so called 'flexor reflex afferents' defined previously.

Identification of the exteroceptive component of the afferent input to the cerebellum, and its subdivision into information from functionally and morphologically distinct receptor groups has been achieved to a limited extent (Lundberg & Oscarsson, 1960; Mann, 1971; Eccles et al., 1972 a,b,c; Leicht et al., 1973).

The direct pathways.

Lundberg and Oscarsson (1960) describe two main classes of axons within the dorsal spinocerebellar tract (DSCT) originating in cells of Clarke's column which carry information exclusively from cutaneous receptors. One group responded to light pressure on the footpad with an initial high frequency followed by a slower, sustained response, and was evoked over a small (1-20 cm^2) peripheral receptive field. Another group responded to light touch over an extended receptive field ($>$100 cm^2) and pinch or pressure over a restricted field. In addition, a group of axons was found which responded both to light touch and muscle movement. Antidromic activation of these axons from the cerebellum confirmed the assumption that the tract terminated in the anterior lobe of the vermis. Mann (1971) identified units of DSCT which responded only to stimulation of the SA I touch corpuscle.

The forelimb equivalent of DSCT, the cuneocerebellar tract (CCT), originates from cells within or near the rostral part of the cuneate nucleus and, like DSCT, carries information from slowly adapting pad, fast and slowly

adapting hairy skin receptors and joint and muscle proprioceptors. This tract also terminates in the anterior lobe but within lobule V as opposed to the termination of DSCT within lobule IV (Oscarsson, 1973).

The ventral spinocerebellar tract originates in cells of Clarke's column in the ventral horn of lumbar segments three to six (L3 - L6; Burke, Rudomin, Vyklicky & Zajac, 1971). Fibres of the tract cross the mid-line at lumbar levels and ascend in the contralateral ventral cord entering the cerebellum via the brachium conjunctivum and terminating bilaterally within lobule IV of the anterior lobe. This tract contains segmentally organised information largely from the group Ib tendon organ with the flexor reflex afferents weakly represented (Carrea & Grundfest, 1954; Oscarsson, 1957) by generally inhibitory actions. The cutaneous input to this pathway is highly convergent and can be suppressed at spinal levels (Oscarsson, 1967a). The latency of activation of this tract from the hindlimb to the cerebellum is 6 - 8 ms. (Carrea & Grundfest, 1954).

The forelimb equivalent of this pathway, the rostral spinocerebellar tract (RSCT) does not cross the mid-line at the segmental level and receives both inhibitory and excitatory action from ipsilateral fields and unlike VSCT group Ib tendon organ activation is polysynaptic and highly convergent (Oscarsson, 1964). Cutaneous input is restricted to the convergent tactile component of the flexor reflex afferents. The termination of this tract on the anterior lobe is bilateral and extends over lobules IV and V. The latency of action of this pathway is 4 - 6 ms.

The Indirect Pathways.

The indirect spinocerebellar pathway via the lateral reticular nucleus has been discussed in relation to this brain-stem structure which lies close to the spinal trigeminal nucleus.

A second indirect spinocerebellar pathway relays in the olive, the spino-olivo-cerebellar pathway (SOCP) and has a latency of action upon cerebellar Purkinje cells of 15 - 30 ms. This pathway has been described by several authors (Grant & Oscarsson, 1966; Oscarsson, 1967a; Armstrong, Eccles, Harvey & Matthews, 1968; Crill, 1970). The tract has been identified as the contralateral ventral flexor reflex tract (cVFRT) containing group I and high threshold (group III) muscle afferents and cutaneous afferents, projecting as climbing fibres to the molecular layer of the vermal cortex. Leicht et al., (1973a), investigating the climbing fibre input to the cerebellum mediated by cVFRT, found both contralateral and ipsilateral receptive fields with a predominantly phasic cutaneous input, probably from Pacinian corpuscles.

The interactions of these various inputs on the intrinsic cells of the cerebellar cortex have received considerable attention, particularly that of Eccles and his collaborators.

Identification of the afferent pathways by electrical Stimulation.

Using electrical stimulation of peripheral nerves under chloralose or chloralose and urethane anaesthesia Eccles et al. (1968a) investigated the evoked field

potentials within the cerebellar cortex, in an attempt to differentiate between transmission in the mossy fibre and climbing fibre pathways. An early potential was found in the granular layer at depths of 350 - 500 μm beneath the cortical surface at the synapse of mossy fibres and granule cells. A second depolarising wave approximately 8 ms. later (for hindlimb nerves) was observed in the molecular layer and was attributed to electrical activity generated by the activation of Purkinje cells by climbing fibres. The stability of the early response was good at high frequencies of stimulation (up to 600Hz), whereas the later evoked response in the molecular layer was abolished by stimulation at frequencies of greater than 300Hz (Eccles et al., 1968 a,b). Earlier, Thatch (1967) had recorded single unit response in the cerebellar cortex and observed two forms of Purkinje cell discharge, complex; consisting of a variable burst of 4 to 7 action potentials, and simple; a regular, high frequency train of spikes, often showing apontaneous activity at approximately 100/sec. Eccles et al., (1971b) attributed the complex discharge of the Purkinje cells to the powerful activation of the dendritic tree by climbing fibres, and the simple discharge to the depolarisation generated by a band of active parallel fibres traversing the dendritic tree at right angles, the parallel fibres in turn having been activated by the mossy fibres acting upon the granule cells in the granular layer (Eccles et al. 1966 c,e). The definitive features of the mossy and climbing fibre input to the cerebellum have been described

by Eccles et al. (1971 a,b) using barbiturate or decerebrate preparations. The short latencies of mossy fibre activation, the capacity to follow high frequencies of stimulation and the presence of bursting activity originally observed by Lundberg & Oscarsson (1960), suggested that these fibres were monosynaptically activated via DSCT or, in fact, that mossy fibres were the axons of DSCT. Using electrical stimulation no distinction could be made between the proprioceptive and exteroceptive components of DSCT described by Lundberg & Oscarsson (1960). Climbing fibres were identified by Eccles et al. (1971b) as axons of neurones in the inferior olive; the long delay at this synapse was put forward as the reason why these fibres were unable to follow frequencies of stimulation higher than 300 Hz. A later discharge of Purkinje cells in the simple pattern was attributed to a mossy fibre pathway via the lateral reticular nucleus, a pathway postulated by Brodal (1953) and Carpenter & Hanna (1961) on consideration of fibre degeneration studies.

The actions of cutaneous input on the cells of the cerebellar cortex.

Direct stimulation of cutaneous receptors of the foot pad and hairy skin was undertaken by Eccles et al. (1972,a,b,c) in order to determine the adequate stimuli for activation of the two exteroceptive afferent pathways to the cerebellar cortex. The mossy fibres were shown by Eccles et al. (1972a) to respond to both phasic and tonic stimulation of the foot pad. The assumption was made that with stimuli of rise times less than 50 ms. 90% of the response

was due to the discharge of Pacinian corpuscles, and with stimuli of durations longer than 500 ms. any response at this time after stimulus onset originated from the slowly adapting type I epidermal mechanoreceptors. The pathways involved were taken to be the dorsal, ventral and rostral spinocerebellar tracts. These results are in agreement with those of Mann (1971) who carefully stimulated SAI receptors and recorded responses in axons identified, by antidomic stimulation of the vermis, to be those of the DSCT. The Purkinje cell response to mossy fibre activation under identical conditions of peripheral stimulation is either an increase or a decrease in the firing rate, according to the particular integrated actions of the inhibitory or excitatory synapses which attend the discharge (Eccles et al., 1970, 1972b). Both phasic and tonic responses were recorded and the thresholds for hair and skin movements were approximately 0.2 mm. with a latency of $\simeq$11 ms. The activation of Purkinje cells by climbing fibres is predominantly phasic in nature with a latency of between 20 and 35 ms. for hindlimb stimulation. Thresholds for mechanical skin stimulation were recorded as low as 0·02 mm. and the maximum repetition rate at which a 1:1 response could be obtained was 16/sec; this compares with 40-50/sec for mossy fibre evoked Purkinje cell discharge. The longer latency of the climbing fibre response and its inability to follow high frequencies of stimulation was attributed to the long lasting IPSP set up at the inferior olivary neurones (Armstrong et al., 1968; Crill, 1970). This series of

experiments was performed on decerebrate animals or animals under light barbiturate anaesthesia.

An examination of the interaction between mossy fibre and climbing fibre evoked response in the Purkinje cells to cutaneous stimulation (Eccles et al., 1972c; Leicht et al., 1973a) has revealed that former tend to be tonically activated and the latter activated by phasic stimuli. Mossy fibre driven responses were grouped spatially with overlapping projections (on the hypothetically unfolded surface of the cerebellum) according to a very patchy somatotopic organisation with a great deal of convergence from different cutaneous sources, obviously representing some sort of functional rather than purely topographical subset of the afferent input. Leicht et al. (1973a) describe three classes of receptive field for climbing fibre evoked Purkinje discharges; restricted to the distal areas of one limb only, circumscribed receptive fields which are discontinuous at the distal portions of two or four limbs and extended fields continuous over the whole body. The climbing fibre projections also, therefore, emerge as organised in a complex, functional distribution with great convergence from spatially separate peripheral receptors. Within any one cluster of active Purkinje cells, separated from other clusters by zones of poor response, adjacent cells respond differently to stimulation of a common receptive field according to the extent of inhition effected by locally active basket and Golgi cells (Eccles et al., 1972d).

Common to most theories of cerebellar function

(see Llinas, 1970) is the interplay of mossy and climbing fibre input, of tonic and phasic input, projecting back via the Purkinje cell axon to shape, by inhibition, the ongoing motor activity at spinal and supra-spinal levels. The dramatic motor disturbances following cerebellar lesion emphasise this motor role, but may obscure a specific sensory function, suggested by Snider & Stowell (1944).

The possibility exists then, of a projection of slowly and rapidly adapting tactile information acquired by the vibrissa organ to the cerebellum via the trigeminal sensory complex. This projection is additional to the considerable vibrissal component of the thalamus-somato-sensory cortex projection.

The properties of the primary afferent and second order activity projecting to the cerebellar cortex provides information concerning the contributions of this pathway to the integrative behaviour of the Purkinje cell. On the basis of physiological investigations of spinocerebellar pathways there are four possible trigemino-cerebellar projections which could exist as functional homologues of the four main spino-cerebellar pathways. The distribution of cutaneous activity within these pathways may then be postulated:-

1) Slowly adapting mechanoreceptive afferents including the cutaneous SA I and possibly the sinus body St I, the activity of which may be distinguishable at the second-order level. This projection is homologous with the cutaneous component of DSCT and CCT terminating within the anterior

lobe of the cerebellum as mossy fibres activated at short latency (4 - 10 ms.) from the facial region.

2) Rapidly adapting cutaneous and sinus body afferents relaying in the olive. This projection is the equivalent of the contralateral ventral flexor reflex tract (cVFRT) which has a predominantly contralateral component ascending from the olive as climbing fibres which terminate in the molecular layer of the anterior lobe. The latency of activation from the face is approximately 10 - 40 ms.

3) Unspecific, convergent, bilateral mechanoreceptive afferents which project to the cerebellum via the lateral reticular nucleus would form part of the facial equivalent of the bilateral ventral flexor reflex tract (bVFRT) which terminates in the cerebellar cortex as mossy fibres. The latency of activation from the face is generally longer (7 - 30 ms.) than that of the directly activated mossy fibres.

4) A short latency (5 - 10 ms.) mossy fibre pathway carrying highly unspecific and convergent mechanoreceptive information with a bilateral projection to the anterior lobe representing the facial homologue of VSCT and RSCT.

Summary of the issues raised by the literature.

A) Primary afferent information from the sinus body nerve may be tentatively assigned to morphologically distinct receptor structures within the sinus hair follicle. The organisation of the sinus hairs into an array which effectively extends the tactile surface several centimeters

beyond the facial profile suggests that the vibrissae may act as a composite sensory apparatus.

B) The properties of the primary afferent outflow may be preserved to some extent in the activity of second order cells of the spinal trigeminal sensory complex. The distribution of this activity within the complex and its efferent projections may reveal significant functional groupings.

C) The afferent fibre system projection to the cerebellum offers an alternative pathway for almost all types of mechanoreceptive afferent information which may or may not ascend in parallel pathways to the somatosensory cortex or take part in segmental reflexes.

D) The effects of these mechanoreceptive afferent projections on the activity of the cerebellar Purkinje cells are quite specific and identification of the less well known trigeminal component of this projection is of importance to the continuing debate on the nature of cerebellar function.

A series of investigations have been undertaken to test statements (A) and (B) above, to identify the trigemino-cerebellar pathway proposed in (C) and the effects of sinus hair stimulation on the activity of cerebellar Purkinje cells referred to in (D).

SECTION II

The primary afferent innervation of the sinus hair system.

Introduction.

The discharge of impulses in afferent fibres dissected from the infraorbital nerve was recorded during controlled movements of the maxillary sinus hairs. No attempt was made to investigate the discharges from common hair follicles or skin mechanoreceptors. Four main types of afferent units were identified, two slowly adapting and two rapidly adapting, on the basis of qualitative and quantitative criteria. Relating the findings of this study to the results of other anatomical investigations of the receptor structures in the sinus hair follicle a correlation between the distinguishable afferent responses and the morphologically identifiable nerve endings has been proposed.

METHODS.

Preparation of the animal.

The experiments were performed on 44 cats of both sexes, weighing 1·7 to 4·5 kg. Anaesthesia was induced with ethyl chloride and ether, followed by 80 mg./kg. chloralose i.v.. If necessary, subsequent doses of pentobarbital were administered through an intravenous cannula. The trachea was intubated and breathing supported with a pump if required. Rectal temperature was maintained at 36-38^{o}C using a thermostatically regulated electric blanket.

The dorsal surface of the head and neck was shaved, the cranium exposed by medial incision, the temporal muscles reflected and the bone cleaned with a solution of

hydrogen peroxide (B.P.) in water. Four holes were drilled into the skull using a dental burr and a 25 x 25 mm. steel plate fixed to the surface with self tapping screws and a commercial dental cement (de Trey, London). A steel bar fixed to this plate and clamped to the experimental table rendered the head immobile and allowed unrestricted access to the right side of the forehead. The right eye was carefully enucleated and then removed and the cavity of the orbit extended to approximately 6 x 3 cm. by removing the surrounding bony rim and the masticator muscles. The infraorbital nerve was gently freed from enveloping connective tissue and the infraorbital artery was separated from the nerve without disturbing the continuity of the blood supply to the nerve. A black plastic dissecting plate was placed beneath the nerve and fixed rigidly to the experimental table. The skin flaps surrounding the orbit were sewn onto a metal ring to form a pouch which was filled with warm mineral oil (paraffin oil, B.P.). Using the conventional equipment for micro-preparation, small strands were dissected from branches of the infraorbital nerve and lifted onto bipolar silver/silver chloride electrodes.

Mechanical stimulation.

A particular sinus hair giving rise to unitary action potentials was at first identified by manual exploration of the face. Quantitative stimulation of the sinus hair was achieved in several ways:

1) An "angle stimulator" (Fig.3) was designed which fulfilled the requirements for sinus hair stimulation

detailed in the results section. This instrument enabled the hair to be bent in all cardinal directions (forward, backward, upward, downward) as well as in any direction resulting from the combination of adjacent cardinal directions. In addition, the hair could be rotated around its long axis independently of any angular deflection by fastening the hair shaft in the eye of the stimulator head (for detailed description see legend of Fig.3). By mounting the angle stimulator on a micromanipulator, longitudinal displacements of the hair were made possible. The amount of manually applied movement was controlled by reading the deflection or rotation angle on the appropriate protractors through an operating microscope. The actual deflection of the hair, which depended on the point of attachment of the stimulator head along the hair axis, could be determined using a 'calibrator' which indicated the real hair deflection at any position of the angle stimulator. The advantage of using the angle stimulator in this was the versatility and large amplitudes of possible movements. The limited precision in controlling the velocity of movement with this device was a drawback.

2) An electro-mechanical stimulator (Brown & Iggo, 1967) could either be attached directly to the hair or, by means of a coupling, to the angle stimulator. Thus it was possible to superimpose electronically controlled movements of up to 8 degrees onto the angle stimulator. Due to the inertia of the angle stimulator the fastest rise time without distortion of the superimposed angle movement was 50 ms. corresponding to an angular velocity of 160°/sec.

3) Sinusoidal stimulation was achieved by driving the electro-mechanical stimulator or a piezoelectric bender element (Brush-Clevite, polymorph) with a sinewave generator. Since the necessary sinewave frequencies lay far above the limits of the available amplitude control mechanisms, it was not possible to measure the amplitudes of an oscillatory movement satisfactorily. Therefore comparisons of amplitudes were made by using the output voltage of the oscillator.

4) Electrical stimulation: The conduction velocities of sinus hair follicle afferent fibres were calculated from conduction times, measured from sharp bipolar stimulating electrodes in the skin overlying the sinus body, and an estimate of the conduction distance. Electrical stimuli, triggered by the master clock, were delivered from a Grass SIU stimulator.

Recording.

Preamplified biological signals, trigger and time marker impulses from a Devices digitimer and the monitored mechanical stimulus were all adjusted to a 1 volt maximum amplitude by amplifiers of a Tektronix plug-in unit. The output was displayed on a Tektronix 565 oscilloscope, simultaneously stored on a 7-channel F.M. tape recorder (Bell & Howell) or fed directly, via a real time clock, to a PDP 12 computer. Photographs were taken using a Grass camera. Spike numbers and discharge frequencies could be collected and were displayed by an electronic counter (Venner TSA 3336).

Data collection.

Impulses of sinus hair follicle units were led to the real time clock of the PDP 12 digital computer and the time intervals between successive events measured and stored. Two modes of data collection were used: A) each interspike interval was measured separately and stored sequentially in the core memory; B) the latency of each spike was measured relative to a marker impulse delivered through the second channel of input to the real time clock. The clock counter could be set to different sample periods, appropriate to the rate of discharge. Normally a sample period of 1 ms. was used, which enabled measurements of interspike intervals between 1 and 500 ms. duration to be made. Longer intervals were counted but not stored. Alternative sample periods of 0·1 ms. and 10 ms. were available under programme control or an external time base (from the Digitimer) could be fed into a third time clock channel to provide any desired sample period. The sample period is identical with the "bin width" of the interval histograms and determines the time resolution of the measurements. All measurements were transferred to the core memory and displayed on a cathode ray oscilloscope. In the case of data collection mode A, the data were displayed either as a curve of successive interval lengths or as an interspike interval distribution histogram. In the data collection mode B, sequential spike latencies relative to a stimulus marker impulse were displayed as responses to successive stimulus presentations or as post-stimulus time distribution.

histograms. By delaying the stimulus marker impulse it was possible to fractionate long responses into successive portions of 500 ms. duration, maintaining a resolution of 1 ms. over long observation times. The whole response was then reconstructed by reassembling the fractions.

Data processing.

All data collected in the course of an experiment was analysed "off-line" by a number of specially written programmes. The record of intervals stored on magnetic tape was displayed in blocks of 512 on the computer oscilloscope. A programme displaying two vertical and two horizontal, adjustable cursors was written both to analyse the data within any portion of the memory, and to display it on the C.R.O.. Latencies and interval lengths of a desired number of data collected in mode A or B could be read off directly as the difference between the vertical or horizontal cursors or could be processed arithmetically. A separate programme could generate interval histograms from small portions of successive interval records stored on magnetic tape. Graphical representation of raw and processed data displayed on the oscilloscope could be drawn by a Complot incremental plotter (Houston Instruments, Houston, Texas).

RESULTS

The gross morphology of the sinus hair follicles.

The size of the sinus hair follicles (sinus bodies) varies with the size of the hair shaft and may appear as just visible structures (≙0·2 mm. in diameter) or may be as large as 4 mm. in length and 2 mm. in diameter. The upper half of the sinus body is embedded in the subcutaneous tissue while the lower half is enclosed by striated muscles attached to the follicle capsule. This arrangement allows the hair follicle a certain mobility during active and passive movement while its lower pole is held firmly in the connective tissue of the maxillary fascia. These features are presented diagrammatically in figure 1.

Physiological results.

A total of 141 single-unit responses from facial sinus hairs were recorded from fine filaments dissected from the infraorbital nerve. About 66% of these were slowly adapting, the remainder rapidly adapting. This sample of single fibres probably represents the true distribution of rapidly and slowly adapting responses in the sinus body nerve supply, although in any one experiment only a minute proportion of the total nervous outflow of the maxillary sinus bodies into the infraorbital nerve was sampled.

A. Slowly adapting units.

The 100 slowly adapting responses recorded were divided into two main groups on the basis of discharge pattern, adaptation behaviour and other response

characteristics. These two groups showed characteristics similar to the type I (SA I) and type II (SA II) cutaneous mechanoreceptors previously described (Iggo & Muir, 1969; Chambers et al., 1972) and are therefore referred to as the Sinus type I (St I) (47 units) and the Sinus type II (St II) (53 units) responses.

The classification of a particular slowly adapting unit was to a certain extent subjective, usually based upon several criteria, as in many cases no single parameter was definitive. The variability of interspike intervals, the adaptation behaviour and directional sensitivity provided sufficient information in most cases but the presence or absence of resting discharge, the 'critical amplitude' and the second order properties of the discharge were also considered, as described below.

Differences in the discharge patterns of the slowly adapting responses.

The most obvious difference between the slowly adapting St I and St II unit responses was the regularity or irregularity of the afferent discharge. The St I discharge is characteristically irregular with impulses distributed randomly in time and the appearance of both long and short interspike intervals at all rates of discharge. The St II discharge is typically regular with very little variation in the length of time between successive impulses. Examples of these discharges are displayed as trace inserts to figures 6B (St I) and 6A (St II), photographed from the oscilloscope. The difference between these units is illustrated in two different ways in figure 4. The left-hand column of graphs show 250 successive intervals collected by the PDP-12 computer under mode A of the data collection programme as described in the methods section. The right-hand column of graphs is the distribution of these intervals in time, showing the amount and direction of scatter about the mean for each type of response. To facilitate comparison, unit discharges which are firing at approximately the same frequencies have been used in this figure. The

irregular scattering of the St I unit discharge and the regular, modal discharge of the St II discharge can be seen clearly. The central pair of graphs in this figure show a discharge which is typically modal, but which shows longer and shorter interspike intervals clustered symmetrically about the mean. These responses were otherwise similar to the St II response so the very regular responses (39 units) was designated St II(A) and the less regular (14 units) St II(B). A simple measure of variability, the coefficient of variation, averaged at 0·75 (range 0·52 - 0·86) for 10 St I units, at 0·085 (range 0·02 - 0·17) for 12 St II(A) units and at an intermediate value of 0·49 (range 0·07 - 0·83) for nine St II(B) units. This agrees with the ranges of coefficients of variation for the SA I and SA II cutaneous receptors described by Chambers et al., (1972).

Another difference between the St I and the St II discharge patterns is illustrated in figure 5 where the three types of slowly adapting receptors were subjected to a sustained displacement of the sinus hair and the discharges recorded over several minutes after the initial onset response had subsided. The interval histograms collected and plotted are an estimate of the probability of any interval length occuring in the discharge, or the probability density function f(t) of the distribution of intervals in time. Theoretical probability density functions (p.d.f.'s) were calculated by the computer using parameters (S.D. and/or Mean) derived from the experimental data and the goodness of fit to the interval histograms tested

with the chi-square test. Outside a variable functional refractory period of 10 - 20 milliseconds the distribution of the St I response appears to be random, with a Poisson p.d.f.. Outside the refractory period or dead time the shape of this distribution should be that of an exponential, of the form:-

$$f(t) = \lambda e^{-\lambda t}.$$

where t is the time between successive impulses and λ a rate constant. Figure 5A shows the interval histogram of an St I unit recorded from the 10th to the 100th second of hair displacement during which adaptation was not significant (mean = 47·76 ms. $\pm$ 34·97). The superimposed exponential function fits the observed histogram closely (p = 0·05). Deviations from the theoretical distribution occur in the 'tail' of the histogram, presumably due to a slow increase in the mean interspike interval which is not, however, statistically significant (t - test). Figure 5B shows the discharge of an St II(A) unit during steady discharge (mean = 22·0 $\pm$ 2·3 ms.) over a period of 260 seconds. A Gaussian distribution fits this histogram with a high degree of accuracy (p = 0·001). The interval distribution of an St II(B) discharge (Fig.5C) fits neither an exponential of Gaussian function of the same parameters unless the prevailing stimulus conditions produce a discharge of the St II(A) type as in figure 5D, recorded from the same unit. Although these results do not rule out other functions which may fit the data equally well, or better (for example Gamma functions), a reliable distinction between the two discharge types may be drawn

by this procedure. The St II(B) discharge appears to be fundamentally Gaussian, as revealed under certain conditions, with variability introduced from a source other than that which produces the regularly spaced impulses. In the case of the St II(A) discharge, shorter samples tend to produce a much better fit to the theoretical Gaussian function than do larger samples. This may be due to low amplitude, cyclical rate variations with a period of approximately 50 seconds. Figure 6A illustrates this where a histogram showing the distribution of 4584 interspike intervals does not fit the superimposed normal distribution although the shape is somewhat similar. Inset to this figure is a second histogram of the same unit discharge consisting of 3577 intervals. This histogram fits the theoretical function with a high degree of accuracy ($p = 0{\cdot}05$), indicating the importance of selecting a STATIONARY sample of interspike intervals before proceeding with statistical analysis. Another St I unit histogram consisting of 1577 intervals fits the exponential function closely ($p = 0{\cdot}05$) but again, showing the tendency for 'extra' long intervals as in figure 5A. This suggests that the process which generates the sustained, slowly adapting discharge is subject to different influences in the two main types of slowly adapting unit.

Second-order characteristics of the discharge.

Statistical measures so far described have depended upon properties of the distribution of intervals in time and have not considered properties which are due to

interactions between adjacent and nonadjacent interspike intervals. Such measures are termed order-dependent and may be useful for a number of reasons:

(i) Given sufficient evidence, inferences may be tentatively drawn as to the nature of the generator process which produces the discharge (Braitenerg, 1965; Firth, 1966; Junge & Moore, 1966).

(ii) These statistical measures may themselves be powerful descriptive or functional criteria upon which to separate different types of receptor activity, or in the central nervous system, different types of synaptic interactions (Perkel, Gerstein & Moore, 1967a,b).

(iii) Limits may be set to the samples of spike trains taken for analysis in other procedures by the identification of non-stationary or fluctuating data which would confound normal tests of significance and which are extremely common in biological systems (Sclabassi, 1971).

The relationship between the length of an interspike interval and its position in the spike train is illustrated in figure 7 for the three types of unit discharge. The best-fitting regression line is drawn through the point of each graph. In all three cases there is no significant correlation between serial order and interspike interval although the regression line of the St I discharge figure 7C shows a slight positive slope. In this graph the intervals are scattered randomly about the regression line with long and short intervals appearing at any time. By contrast, the St II(A) discharge (7A) shows very little deviation from the line and the St II(B) discharge

a similar clustering of interspike intervals with some scattering (7B) although the mean interspike interval is about half that of the St II(A) discharge in 7A which one might expect to produce more variability. The clustering of both the St II discharges around the mean indicates that some sort of constraint is operating which does not permit a random spike generating process.

An indication of this constraint is given in figure 8 where each of the interspike intervals (t_i) plotted in figure 7 have been plotted against the successive interval (t_{i+1}) in a graph which portrays the JOINT INTERVAL DENSITY (j.i.d.) of the data (Rodieck, Kiang & Gerstein, 1962; Perkel et al., 1967a). This measures the degree of dependence of each interval on the previous interval and one parameter of this joint interval relationship is the SERIAL CORRELATION COEFFICIENT of order one (ρ_1) (Cox & Lewis, 1967). For a completely random appearance of spikes in time the density of points in the scattergram should be even over the range of interval lengths plotted. This condition is met only by the St I discharge (Fig.8A), indicating a random generating process with no preferential probability of any length of interval being followed by any other. This is clearly not the case for the St II(A) discharge (Fig.8B) where a tight clustering of points around the common mean of both axes indicates that an interval of length t will be followed by an interval which is very close to t in length. The St II(B) discharge (Fig.8C) also shows a dense clustering of points around the common mean but with symmetrical, non-random

scattering of points over a limited area of the scattergram suggesting that this irregularity of the discharge is subject to some sort of constraint as are the regular St II(A) and St II(B) discharges in general.

Extending this investigation to non-adjacent intervals in an attempt to reveal dependencies between intervals over longer periods of time yielded the results presented in figure 9. In this figure serial order (j) is plotted against the serial correlation coefficient (ρ_j) of intervals (t_i), j intervals apart. The serial correlation coefficient of order j is defined as the ratio between the variance of all intervals j intervals apart (the serial covariance) to the variance of the whole train of spikes. A more thorough description of this statistic and its calculation is given in the legend to figure 9.

This is a test which can detect changes in the dependence between intervals which may be due to adaptation (all intervals tend to be followed by longer intervals) or cyclical changes in the generator process at the receptor terminal, (Perkel et al., 1967a; Mathews & Stein, 1969).

Studying the serial correlograms of figure 9 quickly establishes that, as expected, the St I discharge (Fig.9A) is subject to very small serial dependencies which are never significantly different from zero, the case of no dependence. The serial correlation coefficients tend to be larger in the St II(B) discharge, with a few widely spaced significant values, although the dependence at order 1,

as estimated by the joint interval density plot in figure 8C is not significant at the 5% level. The serial correlogram (Fig.9C) of the St II(A) discharge displays a large positive serial correlation between adjacent intervals and strong evidence of a cyclical change in serial dependency with a period of 13 - 16 interspike intervals, appearing as a damped oscillation of the serial correlogram.

Similar results were obtained with 9 other slowly adapting units, the St II(A) discharge showing a consistently large 1st order serial correlation and a variable periodic fluctuation thereafter.

The major problem of this analysis is to establish the level at which an observed serial correlation coefficient becomes significant. With large data samples ρ is normally distributed and the 1·96 x S.E. lines are drawn on the correlograms to indicate the 5% significance limit for these samples of 200 intervals used in this analysis. Matthews & Stein (1969) showed that with large samples however, the serial dependence under investigation (especially non-stationarity including adaptation) may destroy the underlying distribution of the statistic and therefore the test of significance. This is especially true of the St I discharge and care must be taken to identify this non-stationarity by inspection of the j.i.d. scattergram and the serial correlogram. The sample distribution of ρ is not known for small samples (Cox & Lewis, 1967), and caution must be exercised in the interpretation of these results. In the data subjected to this analysis there was no significant difference in the

means at either end of the data samples (i.e. between the first 150 and the last 150 intervals) and it was assumed that the correlational analysis was valid for the limited purposes of this investigation.

In the terms of renewal theory (Cox, 1962) the St I discharge may then be described as a renewal process (without underlying serial dependencies) subject to non-stationarities (in particular adaptation) and the St II(A) discharge as a stationary, non-renewal process with strong cyclical departures from independence among interspike intervals.

These conclusions, drawn from investigation of the first and second order properties of the two types of slowly adapting units, may reflect substantially different generator mechanisms at the receptor level, which in turn may be implicit in the structure and location of the receptors.

The Adaptation of slowly adapting responses.

During the period of adaptation to a sustained deflection of the sinus hair the discharge of an St I unit declines steadily until either the firing ceases completely or a very low discharge rate is reached ($\simeq$ 5 impulses/sec), with a correspondingly large variation in the length of interspike intervals (i.e. the coefficient of variation $\simeq$ 1). This is illustrated in figure 10A where the increasing interspike interval is matched by an increasing standard deviation over 2000 interspike intervals. By contrast, figure 10C shows the increasing interspike interval of an St II(B) unit discharge coupled

with decreasing interspike interval variability. The discharge is initially irregular and as adaptation proceeds the variance reaches a maximum after 450 intervals (Mean = 35.1 ± 10.5 ms.,C.V.=0.29). After 2500 intervals (~10 sec.) the mean interval length has increased but the irregularity has greatly decreased (Mean = 40.8 ± 3.6 ms.,C.V.=0.09). The discharge now exhibits the essential features of the St II(A) unit, an example of which is shown in figure 10B. Quantitative measures of the variability during adaptation must include considerable error due to the inclusion of a changing mean rate into the calculation of scatter about an averaged sample mean. In the early stages of adaptation this error may account for up to 40% of the measured variance. Inspection of figure 10, however, indicates that units previously designated St II(A) or St II(B) on the basis of discharge pattern adapt in a similar fashion, unlike St I units.

The time-course of adaptation.

An investigation into the time-course of the declining frequency may be carried out on the assumption that the rate at which the frequency drops is proportional to the frequency at any chosen instant in time or, in fact, that an exponential relationship holds between the firing frequency and the time after stimulus onset. Figure 11 is a plot of the firing frequency against the time after stimulus onset for the three types of slowly adapting unit. The St II(A) adapting response is plotted in figure 11A and shows a high frequency onset response followed by a quick decline and then a long, slow drop to a firing rate roughly double the rate of spontaneous activity (shown in the early part of the curve before the stimulus was applied). The St II(B) discharge (Fig.11B) also slows to a steady firing rate after about 50 seconds although

this is slower than that of figure 11A and there was no previous spontaneous activity. Short-term fluctuations in the firing frequency are present in the St II(B) discharge and this is preserved in the frequency plot by calculation over a short sampling period (500 ms.). The St I discharge (Fig.11C) adapts to a very slow firing rate and shows considerable fluctuations in the firing frequency calculated over 500 milliseconds. The shape of these adaptation curves has suggested to other authors (Iggo & Muir, 1969; Chambers et al., 1972) that the relationship between frequency and time is exponential. Chambers et al., (1972) report up to four distinct phases of the adaptation in cutaneous SA II receptors each governed by an exponential relationship with differing time-constants (TCA1 - TCA4). In the present study early adaptation phases were seen but only the two later phases, corresponding to TCA 3 and TCA 4, were investigated in an attempt to draw comparisons between the cutaneous and sinus slowly adapting responses.

In figure 12 the adapting discharges of both main types of slowly adapting sinus body responses indicate that in the period of 10 to 180 seconds after the initial displacement of the hair there are two time constants of adaptation. This data satisfies an exponential of the following form, which assumes independent and additive time-constants:-

$$f = f_1 . e^{-t/\tau_3} + f_2 . e^{-t/\tau_4} ,$$

where f is the frequency of discharge at any instant in

time t seconds after stimulus onset and f_1 and f_2 are the components of the initial frequency at time t = 0, determined by the time constants τ_3 and τ_4 corresponding to TCA 3 and TCA 4 of Chambers et al. (1972). The effects of τ_3 and τ_4 are additive therefore as t increases the effect of the shorter time constant (τ_3) decreases and becomes negligible, the exponential decline governed only by τ_4. The time-constant (τ_3) has been calculated by subtracting the extrapolated line of the later part of the curve from the earlier part of the new curve for τ_3 plotted as an inset to figure 12. This is the standard 'peeling off' procedure described in detail by Perl (1960). For St I units τ_3 ranged from 20 to 70 seconds and τ_4 from 100 to 200 seconds. For St II units τ_3 ranged from 40 to 500 seconds and τ_4 was very long, often approaching infinity, indicating that no adaptation is occuring. These results agree with the results obtained by Chambers et al. (1972) for the SA II receptor suggesting that the receptor structures in the skin and the sinus bodies may be quite similar. The cutaneous type I receptor has not been subjected to this analysis in detail but the indications are (Iggo & Muir, 1969) that the time constants of exponential decline are similar to those observed in the sinus bodies, again suggesting a similarity in receptor structure.

<u>The effective mechanical stimulus for slowly adapting receptors</u>.

The sinus hair transmits mechanical energy from the point of contact with an object to the receptor structures in the sinus body. This transfer of energy may not be

linear, due to bending of the hair and also to the fact that the force applied in one direction may be dissipated along vectors as a result of the curvature of the hair. This may be seen to occur when the tip of a long sinus hair is moved under the dissection microscope and although the movement of the tip is, say, in a horizontal plane, the predominant movement at the base of the hair is an axial rotation around the long axis of the hair. Depending on the amount of curvature of the hair shaft movement in one direction at the hair tip may cause rotation of the hair base in the other direction. In addition, the direction of rotation may change as the deflecting stimulus is applied at different points on the hair. Some units displayed strong directional or rotational sensitivity (see below) and the interaction of excitatory and inhibitory (wrong direction) movements at the receptor level could produce confusing results. Once this was realised care was taken to move the hair in the appropriate direction of displacement or rotation by use of the angle stimulator which could provide stimuli around three axes at any orientation. As the different stimulus components could act in an antagonistic or complementary manner it was usual to restrict movement as much as possible to one plane only, although this may be a poor simulation of the hair movement under natural conditions.

Directional sensitivity.

Directional sensitivity has been reported by all previous investigators of the slowly adapting sinus body

mechanoreceptors (Fitzgerald, 1940; Zucker & Welker, 1969; Nilsson, 1969; Hahn, 1971). Directional sensitivity is defined as the response to movement of the hair in a given direction combined with the cessation of discharge following movement in the opposite direction. Only the St I units meet these conditions, responding strongly to movement in one or two adjacent cardinal directions and ceasing to respond after wholly or partially returning the hair to its resting position. Movement towards the resting position in the sensitive direction was also effective, although generally less so than movement in the sensitive direction from the resting position. Most St II units of both types responded to movements in three or four cardinal directions away from the resting position but firing would always cease for a few seconds, the silent period, when the hair was moved back to the resting position from any other position, in contrast to the St I units. Figure 13 illustrates the different directional sensitivities of the two main types of slowly adapting unit recorded from the same sinus hair follicle. In both cases the hair movement, applied via the angle stimulator, was identical. The high frequency St I discharge stops immediately the direction of movement is reversed (directional sensitivity) whereas the St II discharge continued for 500 ms. with a decreasing firing rate as the hair approached the resting position.

The extent of these differences may be illustrated by plotting the unit responses to movement in different directions on polar co-ordinates where zero represents

the resting position of the hair and the four axes movements in the four cardinal directions; rostral, caudal, dorsal, ventral. Figures 14A, B show the directional sensitivity of two St I units with a high frequency response in one direction only (but a different direction in the two units shown) and very weak responses to movements in other directions. The sensitivity of the St II(A) units (14C,D) are approximately uniform, as is that of the St II(B) unit (14E) where movements in all directions elicit a substantial response. Such responses could provide no information as to the direction of a stimulus against the sinus hair, only information that the hair had been moved from rest by a certain amount (as described in a later section).

The 'silent period'.

When the hair was moved towards the resting position St II(A) and St II(B) units normally ceased firing altogether for a few seconds. Fitzgerald, (1940) proposed that the duration of this silent period was proportional to the number of impulses discharged in the previous response and was independent of the frequency of discharge. This hypothesis was not confirmed in this series of experiments. In general there was great variability in the duration of the silent period amongst the large population of type II receptors investigated.

Spontaneous activity.

A unit which discharged in the absence of an experimentally applied stimulus was termed 'spontaneously active'. Only two out of 35 slowly adapting St I units

were spontaneously active, with a very slow firing rate (≤ 2 impulses/sec.) and high irregularity. Of 39 St II(A) units 13 were spontaneously active, as was a similar proportion of St II(B) units (5 of 14). Nine St II units became spontaneously active in the resting position only after the hair had been vigorously manipulated. The rate of spontaneous activity could both increase and decrease over a recording period of several seconds and occasionally the firing rate changed very abruptly. Since in some animals there was very little spontaneous activity, while in others almost every type II unit carried a resting discharge it was assumed that the presence of this discharge was dependent on the influence of undefined factors. Some spontaneously active cells showed a strong pulse synchrony, indicating that blood pressure might play some part in maintaining a resting discharge.

Critical amplitude.

If a sinus hair was deflected by controlled movements of the angle stimulator a relationship was found between the velocity of angle deflection (varying the rise time, keeping the final deflection angle constant) and the 'critical amplitude' at which the first spike was elicited (Fig.15). For the St I units the critical amplitude tended to decrease at higher movement velocities while for both types of St II unit this amplitude increased, i.e. at higher movement velocities higher amplitudes were required to cross the threshold for the first spike. The critical amplitudes for individual units differed

greatly, but these relationships held for 21 units tested, without exception. This was a valuable aid in deciding the class of a slowly adapting response.

Stimulus-Response relationships.

A quantitative investigation of the slowly adapting responses to hair deflection was made. The frequency of discharge in the afferent nerve fibre was related to the amplitude and velocity of the deflection and an attempt was made to describe this relationship mathematically.

Amplitude variations were obtained by moving the 'angle stimulator' through angles which corresponded to deflections of the hair in 5 degree steps from 5 to 50 degrees. Rotational movements about the hair axis were also applied in the same way. Each trial was begun at the zero position of the hair and two series of increasing and decreasing amplitudes run. The first 50 interspike intervals of the response after the completion of hair movement were selected and the mean of these intervals calculated. The average of the two runs was plotted.

Angular deflections of the hair at different velocities were applied by the electromechanical driver, via the angle stimulator. Post-stimulus time histograms were collected by the computer in mode B, in the manner described in the methods of this section. Analysis was performed on at least 10, usually 20, consecutive responses to any one velocity of stimulation. The velocity response was measured during the last 10% of the hair movement. Iggo & Muir (1969) and Chambers et al. (1972) have shown that the frequency of discharge changes within the dynamic

phase. Choosing the last 10% of the response for analysis reduces the effect of a changing population on the statistical procedures employed. The amplitude of the stimulus was kept constant when investigating the velocity of the hair movement (Brown & Iggo, 1967; Iggo & Muir, 1969; Chambers et al., 1972).

Statistical considerations.

In this series of experiments the number of stimulus levels presented was usually between 6 and 10 and the mean of 2 (for static) or 20 (for dynamic) responses were plotted against them. This imposes severe limitations upon the amount of statistical inference which may be made. In order to test the goodness of fit of a theoretical function to the observed data a large sample must be collected. Werner and Mountcastle (1965) found it necessary to present thirty stimulus levels a total of over 600 times before assigning a particular function to a set of data. Tests of goodness of fit depend on identifying that portion of the total variance of the measurements which covaries with the stimulus, independent of the biological variability of the system. Fisher's variance ratio or F-test may be applied to large samples by comparing the sum of variances around each point with the variance around the fitted line, thus giving a measure of the degree of dependence of the response on the stimulus. This dependence may be different for different transformations of the same data and the effectiveness of the transformation in improving the goodness of fit may be tested INDIRECTLY by comparing the values of F obtained

with the significance limits of F. This test will not distinguish between two significant or two insignificant F values, only values which fall on opposite sides of the significance limit.

The correlation coefficient (r) has been used as a measure of the goodness of fit (Werner & Mountcastle, 1965; Mountcastle,1967) although this has been criticised (Kruger & Kenton, 1973). The sample distribution of r must range from -1 to +1 and is a normal distribution ONLY when the population r equals zero. In these experiments sample r's were greater than 0·9 in all but one case and in all cases significantly different from zero at the 1% level. Comparisons of different r's may be made by transformation to Fisher's Z, a statistic which is normally distributed. No significant differences in Z values were observed following various transformations of the data. This is unquestionably due to the small sample sizes used in estimating r. Different Z transformations are compared by obtaining a z-score (not Fisher's Z), defined as;

$$\frac{Z_1 - Z_2}{\text{S.E. of } Z_1,\ Z_2.}$$

The standard error of Z_1 and Z_2 is entirely dependent on sample size and a small sample yields a large standard error and a correspondingly small z-score, which has to exceed 1·96 to be significant at the 5% level. In the samples of data presented here (Figs.16, 17 & 18) N was usually between 6 and 10 and z-scores between 0·21 and 1·06.

Given these limitations it was felt that goodness of fit could be determined as accurately by eye as by any statistical procedure based upon insufficient data. Inferences drawn from the designation of a particular function as that which best describes the data have been limited to comparisons with results obtained elsewhere.

The effects of various transformations on Stimulus - Response relations.

The relationship between the velocity of hair deflection and the mean response frequency of both types of slowly adapting units following various transformations is presented in figure 16. Each graph illustrates the effect of trying to fit the data to a particular function, which would result in a straight-line relationship between the two variables if the function were an accurate description of the S - R relationship. These functions are:-

Linear (Fig.16A) $R = b.S+A$. The relationship between velocity and response frequency is distinctly curvilinear for both the St I and the St II units with correlation coefficients (r) of 0.9802 and .9829 respectively.

Logarithmic (Fig.16B) $R = b.\log S + A$. Plotting the response frequency against the logarithm (natural) of the stimulus produces a curvilinear relationship with a positively accelerating slope unlike that of figure 15A. rI(St I) = 0.9776, rII(St II) = 0.9804.

Power. (Fig.16C) $R = A.S^b$. Plotting log R against log S yields fairly straight lines with a small inflection at low stimulus levels. rI = 0.9987, rII = 0.9932.

Normalised power function. (Fig.16D) A transformation designed to remove the effects of differences in threshold between different units. Plotting % log R against % log S (where % log R is the percentage of the log of the response to the maximal stimulus) produces straight lines similar to those of figure 16C with almost identical slopes and intercepts. rI = 0·9976, rII = 0·9953.

Power normalised function (Fig.16E) A transformation similar to that of figure 12C suggested by Werner & Mountcastle, (1965). Plotting log % R against log % S yields straight lines with identical slopes. This corresponds to the function $R = 100^{(1-b)} . S^{b}$. whenever the BEST FITTING line passes through the point (100,100) as is the case for the StI line (upper). rI = 0·9983, RII = 0·9569.

The values of A,b,r and the standard errors of the estimate Y/X and the slope b are given for all these lines in Table II.

The effects of these transformations on the slopes and intercepts of the best fitting lines is considerable, especially when comparing units with substantially different thresholds, (Kruger & Kenton, 1973). This is shown clearly in figures 17 and 18 where data from units with widely different thresholds and sensitivity are subjected to the same transformations as those in figure 16, where the two units showed broadly similar response characteristics.

In general the response of all types of unit to sustained displacement was less consistent than their responses to a range of ramp velocities. This was

probably due to the wide range of deflections used and the less controllable manual movement of the hair. This was particularly true of the velocity at which the hair was moved which has a considerable effect on the frequency of the early part of the response. In addition, the effect of adaptation of the response to a maintained displacement over the first 50 intervals may vary considerably from unit to unit, as described in this section, page 10. Every attempt was made to recognise and reject data which included parts of the initial dynamic response.

Figure 17 A, plotted on linear axes, shows the relationship between the response in impulses per second to a deflection of the hair, measured over the first 50 intervals following the completion of hair movement. The St II unit represented by the filled symbol (●) has been subjected to a rotational stimulus around the hair axis and fits the linear relationship particularly well.

When the logarithm of the stimulus is plotted against the response (figs. 17 B,C) a wide dispersion of the four lines appears with no apparent improvement in the goodness of fit. Plotting the logarithms of both the stimulus and the response (fig. 18A), corresponding to a power function, the fit of all units (with the possible exception of the rotated St II unit) to the regression

lines is subjectively better. Normalising the power or logarithmic relationship with respect to the maximal stimulus and its response (Figs. 18 B.C), produces no advantage in terms of subjective goodness of fit and introduces a certain compression of the graphical display around the maxima. The statistical parameters of all these relationships are presented in table II.

It is felt that plotting the logarithmic transforms of the data has two advantages; (i) Subjectively less scatter about the regression lines - tentatively confirmed by the correlation coefficients shown in table II, and (ii) the ability to compare these results with previous findings plotted on logarithmic axes on the ASSUMPTION of a power relationship between the stimulus and response. No such assumption is made here as the statistical evidence is equivocal.

Figure 19 presents data collected during the static phase of responses to sustained deflections of several sinus hairs separated into the type I and the type II responses for clarity and plotted as logarithms. The two St I responses of figure 19A show minimal scatter around the best-fitting regression lines with exponents of 0·38 and 0·72 (as estimated by the slope of the regression line). The St II responses plotted in figure 19B show somewhat more variability with two units displaying exponents of greater than one.

The range of exponents of the power functions relating response to stimulus (0·28 - 1·79) is similar to that reported by

Werner & Mountcastle (1965), Brown & Iggo (1967); Chambers et al. (1972) for mechanical displacement of cutaneous and hair mechanoreceptors.

The dynamic responses of several sinus hair units have been plotted against velocity, after logarithmic transformation, in figure 20. The responses have been divided into two groups (Figure 20 A,B) for clarity, but all types of slowly adapting dynamic response are presented in both graphs. No distinction can be drawn between any of the different responses in terms of the goodness of fit to the power function or the exponent of this function. The slopes of these lines lie between 0·28 and 0·88, slightly wider than that reported for the velocity response of hair follicle receptors by Brown & Iggo (1967) who found exponents in the range 0·54 - 0·89 for these rapidly adapting units.

Thus a power function may be used to describe both the dynamic and static responses of the sinus hair slowly adapting receptors over a wide range of stimuli. Chambers et al (1972) report that the S - R relationship of the cutaneous type II receptor may be described by both a power and a logarithmic function whereas the slowly adapting type I receptor could only be accurately described by a power function. This was not found to be the case for the sinus hair receptors when frequency and not inter-spike interval was used as the dependent variable.

Lipetz (1971) has suggested that the log hyperbolic tangent function (log R = log tanh S) describes the stimulus-response relationship of a sensory receptor well

over a wide range of responses. This function is a sigmoid but if the asymptotic extreme ranges are not included the mid-range may be described by a power function. If the inflection of the sigmoid is included in the data then other functions may describe this portion of the curve more adequately. Chambers et al. (1972) use the interspike interval as the dependent variable in establishing the stimulus response relationships of the cutaneous SA II receptor. This alters only the sign of a power relationship and the exponents found in this series of experiments are directly comparable. The transformation of interspike interval to frequency may have different effects at different points of the general sigmoid relationship proposed by Lipetz (1971) and may explain the observation of Chambers et al. (1972) that the response function of the SA II receptor may be described both by a logarithmic and a power function.

Responses from the isolated sinus hair follicle.

Nineteen sinus bodies were isolated by microdissection during continuous recording of the afferent discharge. The disconnection of the sinus hair follicle from the surrounding skin caused a marked reduction in the directional response but no qualitative change in the directional sensitivity of St I units. Isolation of the sinus bodies almost invariably caused, in units of the St II(A) and (B) group, a high spontaneous discharge, or, if a resting activity existed before, an increase of the firing rate. This basic discharge from the isolated sinus hair follicle was only slightly affected by bending the hair in any

direction. All slowly adapting units could be excited by touching the upper half of the exposed sinus body capsule with a fine probe. While the side of the most sensitive spot of the sinus body capsule was variable for St II(A) and (B) units, for the St I responses it was always found to be situated on the side opposite the most effective bending direction. Extraction of the sinus hair from the follicle often abolished the mechanical excitability of St I units, but never led to a sustained discharge activity as was the case in many St II units. On the other hand, opening of the follicle capsule did not affect the excitability of St I units but stopped or diminished the response from St II receptors. Externally applied pressure on the sinus body increased the discharge of St II units while it had little effect on the activity of the St I units. These various results suggest that the St I receptors and the nerve endings which give rise to both kinds of St II responses are located at different places in the sinus hair follicle.

Stimulation of the facial nerve.

In a few instances the branch of the facial nerve innervating the muscles attached to the sinus hair follicles were freed for electrical stimulation. At tetanizing stimulus frequencies (40 - 45 Hz), the sinus hairs rose and spread out, standing perpendicularly to the surface of the skin. Neither the St I units nor the St II units responded with a sustained discharge to the change of the hair position caused by the tetanizing stimulation, nor was their excitability substantially affected. Single

shocks at low frequencies caused short twitch movements of the sinus hairs which were accompanied by short bursts of impulses if the quick movement caused the hair shaft, due to its inertia, to bend in the sensitive direction of the recorded unit.

B. Rapidly adapting units.

Thirty per cent (41 units) of the afferent responses from sinus hair follicles were rapidly adapting. Two kinds of units could be distinguished: The "high velocity threshold" rapidly adapting (HVRA) (32 units) units discharged only a few impulses when the hair was flicked briskly. Slow hair movements, sufficient to activate slowly adapting units, often failed to excite HVRA units and they therefore escaped notice unless high velocity stimuli were used. The most effective stimulus for this kind of receptor was vibration of the hair, as achieved by scratching the hair shaft with the serrated side of dissecting forceps or touching it with a vibrating tuning fork (440 Hz). A given HVRA unit was activated with small displacements of only one sinus hair. Responses could be obtained from surrounding structures only by using very much higher stimulus intensities. Isolation of the sinus bodies by dissection established that the HVRA receptors were located inside the follicle.

Fig.21 illustrates the response of a HVRA unit to sinusoidal stimulation at 200, 500, 800 and 1000 Hz with 1:1 following at the highest frequency. From some units the range of the frequency response was much narrower

and it depended not only upon the response characteristics of the individual unit but apparently was also determined by resonance properties of the hair shaft. Fig.22A shows the tuning curve of a HVRA unit at the 2:1 response level (1 impulse coupled consistently to two sine wave cycles). The width of the U-shaped response curve is much narrower than it is in another unit shown in Fig.22B, for which three 1:1 tuning curves were obtained at distances of the probe of the electromechanical stimulator from the skin of 3 mm, 14 mm and 24 mm. The threshold at a given frequency varied with the contact point of the oscillating probe on the hair shaft and in different units the lowest threshold point could lie at different parts of the hair. The direction of the vibratory stimulus had no effect on the discharge characteristics of HVRA units.

The "low velocity threshold" rapidly adapting (LVRA) units were relatively uncommon (9 units) (6·5% of the sample). Each unit was excited from only one sinus hair follicle unless much higher stimulus intensities were applied to surrounding tissue. The origin of LVRA units from inside the hair follicle was established for four units by isolating the sinus body. The rapidly adapting response could in each case be elicited by touching the sinus body capsule or by moving the tip of a needle inside the hair follicle. The response always disappeared after the sinus nerve was cut. The LVRA units were excited only by the dynamic part of a hair displacement. Both the number of impulses and their frequency increased with rising

movement velocities (Fig.23) and amplitudes. The velocity thresholds lay one or two orders of magnitude below the velocity threshold of HVRA units. A single LVRA unit responded to movement of the hair in all directions but the sensitivity was normally greatest in one particular direction. LVRA units were alsc distinguished from HVRA units by their responsiveness to vibration of the hairs. Only one LVRA unit was able to follow the vibration frequency of the tuning fork (440 Hz) and then only briefly. In the other eight units the frequencies at which 1:1 following of the stimulus was possible ranged from 5-200 Hz.

Conduction velocities.

For 41 afferent units of all types of response the conduction time of an impulse travelling from the hair follicle to the proximal recording electrodes never exceeded 1 msec. The calculated conduction velocities established that the afferent impulses from facial sinus hair receptors are transmitted via large myelinated fibres ($\rangle$ 6 μm diameter). Electrical stimulation of the whole nerve emerging from the sinus body revealed no slowly conducted component in a multifibre response.

The various kinds of units differed only slightly in their conduction velocities, the fastest unit being a HVRA unit. The mean values for the various units are given in Table I.

DISCUSSION

The response characteristics of both types of slowly adapting units have been shown to be essentially the same as those recorded in the skin of mammals and reptiles (Iggo,1968; Iggo & Muir, 1969; Chambers et al., 1972; Merzenich & Harrington, 1969; Kenton, Kruger & Woo, 1971). The generally higher dynamic sensitivity of the St I units and their relatively fast and irregular adaptation suggests that precise information from these units may be confined to the dynamic and early static phases of the response, a conclusion drawn by Harrington and Merzenich,(1970) for the cutaneous SA I response. The dynamic sensitivity of St II units is considerable but their persistent, regular response to sustained sinus hair displacement suggests a role in providing information relevant to the displacement to the central nervous system. The response ranges of the two slowly adapting unit types show a similar complementary relationship with very high frequency vibrations detected only by the HVRA unit and low velocity movements by the LVRA units.

On the basis of the distinctive properties of both types of slowly adapting units it is proposed that the St I and St II receptors are located at different places in the sinus hair follicle and that the receptor structures involved have been described anatomically by Andres (1966). As in the cutaneous SA I the generation of the St I response is attributed to Merkel cells, in this case

lying on the inner surface of the glassy membrane. The two types of lanceolate ending described by Andres (1966), straight and branched, are put forward as the source of the St II(A) and St II(B) response respectively. Although these lanceolate endings are similar in appearance and position to the rapidly adapting palisade endings in pelage hair follicles (Cauna, 1969), the latter are much smaller and simpler in structure and in particular lack the axoplasmic processes which contact the collagenous matrix surrounding the sinus body lanceolate endings. The suspension of the lanceolate endings in this matrix suggests that tension in the surrounding collagen fibres is transmitted to the axoplasmic processes, the deformation of which is part of the spike generation process. A similar sequence of events leading to the generation of action potentials has been proposed for the morphologically similar SA II terminal in the skin (Chambers et al., 1972). Physiologically it is possible to distinguish two variants of the St II discharge and this distinction appears to have a parallel in the morphologically distinct straight and branched lanceolate endings of Andres (1966). If the assumption is made that multiple generator zones in a nerve terminal produce a less regular discharge than a single generator zone then there is a clear case for attributing the highly regular St II(A) discharge to the straight lanceolate endings, and less regular St II(B) discharge to the branched lanceolate endings and the highly irregular St I discharge to the Merkel cells. Whilst this may seem a reasonable assumption there has not,

as yet, been a theoretical treatment of this problem as it applies to receptor terminals. Work on the origins of central cell variability (Poggio & Vernstein, 1964; Braitenberg, 1965; Moore, Perkel & Segundo, 1966) suggests that this may be the case. In this series of experiments investigations were made into the serial-order statistics of the slowly adapting discharges and substantial differences were found between the two main types of unit. The St I discharge (attributed to the multi-terminal Merkel cell complex) may be described as a renewal process (Cox, 1962) where each spike is generated independently of all other spikes, subject to an overall long-term increase in interspike interval as adaptation takes place. The St II(A) discharge in particular was found to be a highly non-random, non-renewal process with evidence of periodic fluctuations in the dependence of one interspike interval on others, i.e., the spike generating process is not 'cleared' after the generation of an action potential but remains under the influence of previous generating sequences. Serial dependence of this sort has been described in crayfish stretch receptor (Firth, 1966) which is known to possess a single spike generating zone at some distance from the parent axon (Edwards & Ottoson, 1958; Ringham, 1971) and a discharge pattern similar to the stretch sensitive cutaneous SA II mechanoreceptor and the sinus body St II(A) unit. The St II(B) discharge shows signs of periodic serial dependence but unlike the St II(A) discharge this never attains significant proportions. The irregular influence of

other branches of the branched lanceolate terminal may interrupt the pattern of serial dependence established by a single active terminal, a pattern which may become obvious under certain stimulus conditions (see Fig.5 C,D and page 10).

These results present a clear case for the division of the slowly adapting responses into two main groups on the basis of reliable statistical criteria. In section III an attempt is made to establish two categories of slowly adapting responses in cells of the trigeminal sensory complex which meet these criteria, and so identify the source of excitation within the sinus hair follicle.

Directional sensitivity is shown only in the St I response. This type of sensitivity is probably due to the position of the receptor relative to the hair shaft and not an inherent feature of the generator mechanism. The St I receptors seem to respond to a movement applied perpendicularly to the array of Merkel cells, as is the case in the cutaneous SA I receptor (Iggo & Muir, 1969). Regarding the inner surface of the sinus hair follicle as an invagination of the epidermis the plane of this surface is now shifted through 90^{o} with respect to the surface of the skin. Tangential movement of the external hair shaft will cause perpendicular movement of the inner hair shaft in one direction only (the opposite) and this will excite a restricted field of Merkel cells producing the effect of directional sensitivity. Accordingly, St I receptors would be located on the side of the follicle opposite that of the most effective bending direction, an observation consistently made in isolated follicle preparations.

The slowly adapting discharges vary consistently with the intensity of an applied stimulus. Under the conditions of these experiments most units revealed the possibility of fine discrimination between different deflection amplitudes applied at different velocities. The number of discriminable levels of stimuli which may be coded by discrete responses is a measure of the information transmitting capacity of the afferent discharge. The extent to which a range of stimuli may be divided into a range of discrete responses is dependent on two factors, (1) the magnitude of the change in response to change in stimulus (indicated by the slope of the stimulus-response relationship) and (2) the variability of each response, which limits the number of distinguishable responses within a finite response range. An investigation of the information transmitting properties of these discharges requires a type of analysis of variance (Garner & Hake, 1951; Garner, 1956) and this has been carried out by several authors (Werner & Mountcastle, 1965; Kruger & Kenton, 1973) who agree that approximately 17 - 18 different levels of response can be distinguished in a single-unit primary afferent response. This corresponds to 4 - 5 bits of information transmitted. Although this type of analysis has not been carried out in this series of investigations the comparison of the stimulus-response relationships in first and second order cells which could be related to the same receptor structure would be a valuable step on towards finding the extent of information transmission and loss in a specific sensory

pathway. A preliminary investigation of this sort was made by Darian-Smith et al. (1968) who, however, did not identify the functional properties or origin of the primary afferent or secondary cell discharge.

The maxillary sinus hair follicles differ from those of carpal sinus hairs in possessing the HVRA response to high velocity movements. In carpal regions this response is attributed to the numerous Pacinian corpuscles which surround the sinus hair follicles (Nilsson, 1969). These follicles lack the lamellated nerve endings (Golgi-Mazzoni corpuscles) found in the maxillary sinus bodies to which the HVRA response must therefore be attributed (their lamellated structure implies sensitivity to vibratory stimuli, Pease & Quilliam, 1957; Munger, 1971). Very similar responses have been shown to derive from Golgi-Mazzoni corpuscles in the cat jaw periostæum (Sakada & Aida, 1971). These receptors were not activated by movement of the sinus hairs.

The low velocity rapidly adapting responses (LVRA) bear great similarities to the discharges of normal hair follicle receptors (Brown & Iggo, 1967) which are thought to derive from the palisade endings described by Yamamoto (1966) and Cauna (1969). These endings and the lanceolate endings may be of common embryological origin and such palisade-type structures may exist in the sinus hair follicles without the characteristic axoplasmic processes of the lanceolate cells. Another possibility, suggested by Gottschaldt et al. (1972) is that the free nerve endings found in the follicle (Andres, 1966;

Nilsson, 1969) are the source of these responses. The observation that the St II discharge could be rendered rapidly adapting by puncturing the blood sinus might suggest that the former proposition is the case and that the tension exerted on the collagen fibres within the follicle by the blood-filled sinus has been removed, effectively converting these receptors to the palisade type without axoplasmic processes.

The sinus hair follicle receptors therefore provide a great deal of information relating to the velocity and amplitude of deflections of the sinus hairs. These responses cover a wide range of stimuli from low amplitude vibrations of up to 1000 Hz to large sustained deflections of the hair in all directions. Because of the probe-like structure of the sinus hair receptors activated by movement of these hairs will discharge before those of the surrounding hair and skin, if indeed these are activated at all in normal searching behaviour. The organisation of these sinus body responses at the level of the trigeminal nucleus may preserve the distinctive properties of the primary afferent discharge and reveal the functional significance of this highly organised primary afferent outflow.

The experiments described in this section were performed in collaboration with Professor A. Iggo and Dr. K.M. Gottschaldt and the results have since been published in the Journal of Physiology.

Fig. 1. Drawing of the cat face with overlying skin removed to show the arrangement of the upper labial and maxillary sinus hairs and some of the sinus bodies, exposed by dissection.

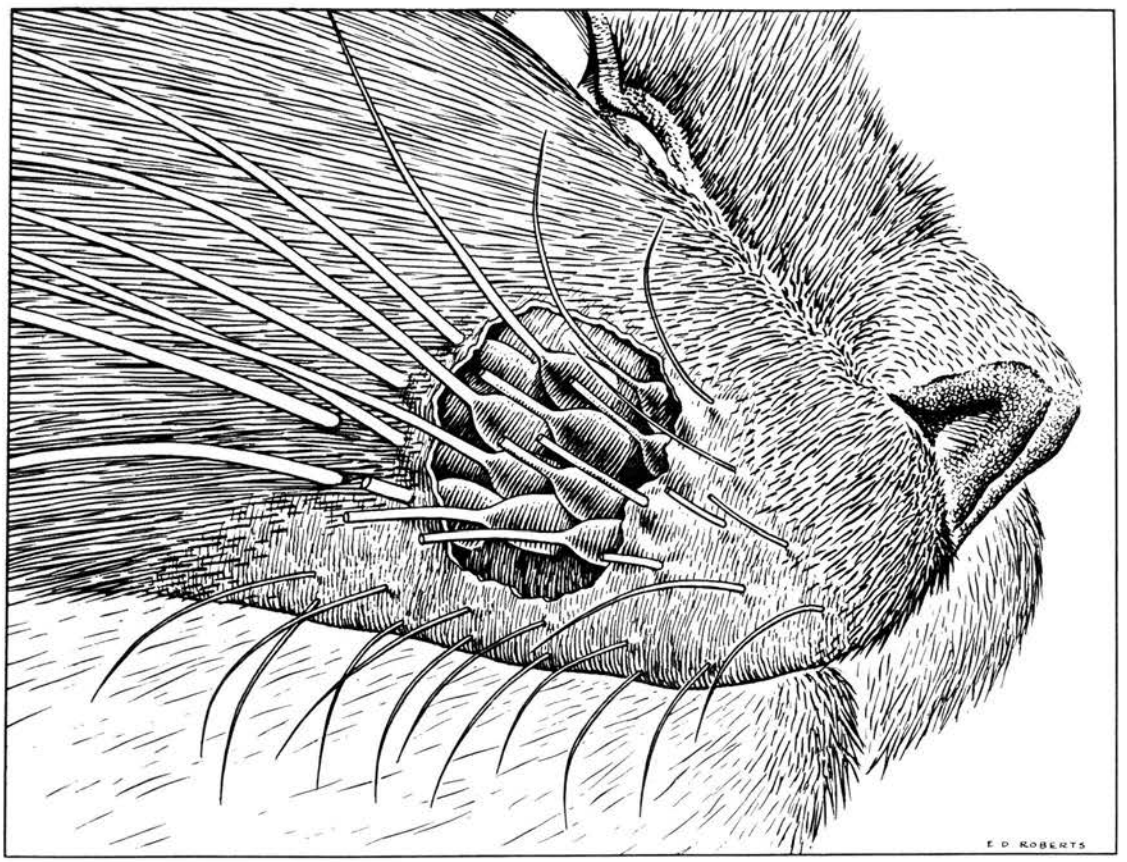
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Fig. 2. A diagrammatical long itudinal section of a sinus hair follicle in the cat (after K. H. Andres, 1966) indicating the general structure and the relative positions of the straight and branched lanceolate nerve endings (B,C), Merkel cells (A) and encapsulated nerve endings (E).

'Free' nerve endings are omitted from this diagram.

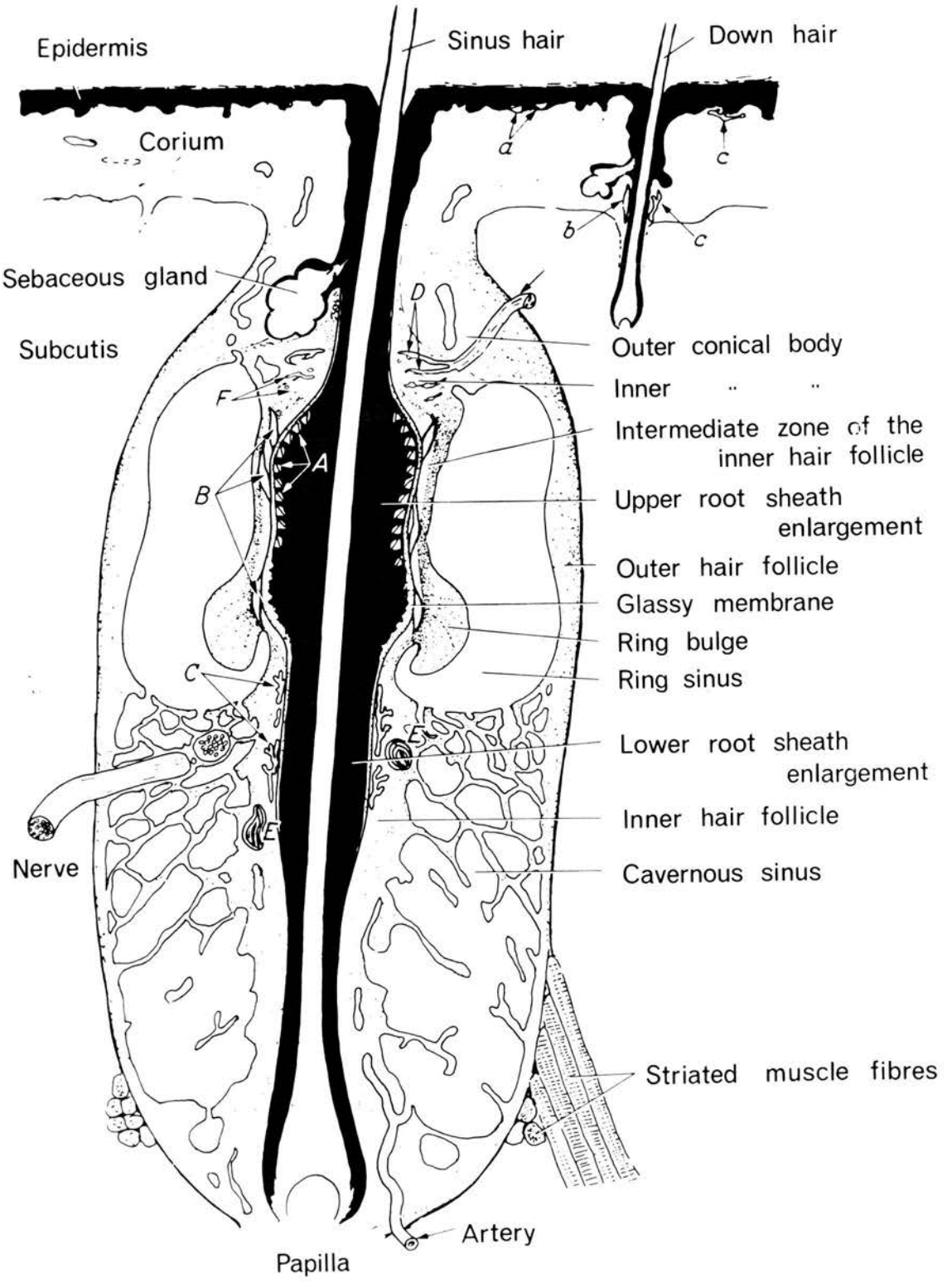

Epidermis
Sinus hair
Down hair
Corium
a
b
c
c
Sebaceous gland
Subcutis
D
F
A
B
C
E
E
Nerve
Papilla
Artery
Outer conical body
Inner " "
Intermediate zone of the inner hair follicle
Upper root sheath enlargement
Outer hair follicle
Glassy membrane
Ring bulge
Ring sinus
Lower root sheath enlargement
Inner hair follicle
Cavernous sinus
Striated muscle fibres

Fig. 3. The "Angle Stimulator". Rotation around the vertical axis (A) produced movement in the horizontal plane; around the horizontal axis (B) a movement in the vertical plane; around the longditudinal axis (C) an axial rotation of the hair. The head of the stimulator consisted of a spindle (D) with a central eye through which the hair was passed. The spindle rotated freely in a yoke (E) which itself could rotate perpendicularly to the spindle axis. This arrangement ensured that the orientation of the spindle eye remained at right angles to the axis of the hair shaft, thus minimising friction and buckling of the hair during application of a movement.

Movement around any given axis could be prevented independently by the locking devices F, G & H. The angular movement in any plane was measured on the appropriate scales. The electromechanical stimulator could be attached at any point on the horizontal arm, and was used to apply controlled movements, within a maximum amplitude of $\pm$ 8°.

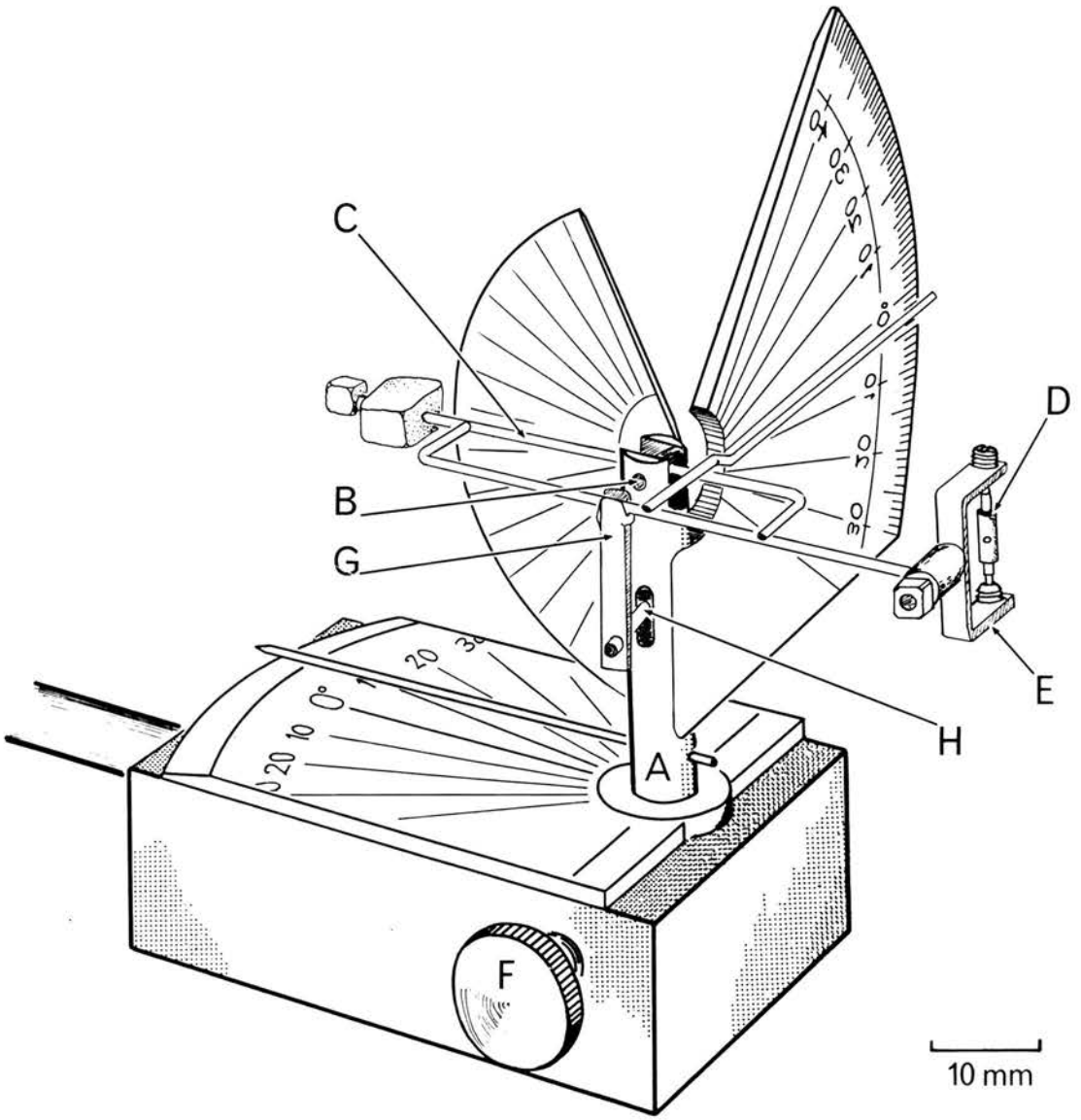

C
B
G
D
E
H
A
F
10 mm

Fig. 4. The discharge patterns of slowly adapting sinus hair follicle receptors (St I, St II(A), St II (B)) during sustained discharge. The three graphs to the left show the lengths of 250 successive intervals with each interval length, in ms, represented by the height of the point above the baseline. The three graphs to the right are the interval distributions of the same data where the abscissae show the interval lengths and the ordinates the numbers of intervals within each bin (bin width 1 ms.). The mean frequency of discharge of the three units is nearly the same but the distribution of intervals is substantially different. The upper pair of graphs is that of an St I unit (S1101) showing the typically irregular discharge with interval lengths of between 2 and 250 ms. (mean = 47·7 $\pm$ 34·9 ms.). The centre pair of graphs shows the discharge characteristic of the St II(B) unit (251017) with a modal interval around which both long and short intervals are scattered (mean = 48·9 $\pm$ 19·3 ms.). The third pair of graphs is that of an St II(A) unit (17811) showing a periodic discharge of low variability (mean = 49·5 $\pm$ 2·3 ms.).

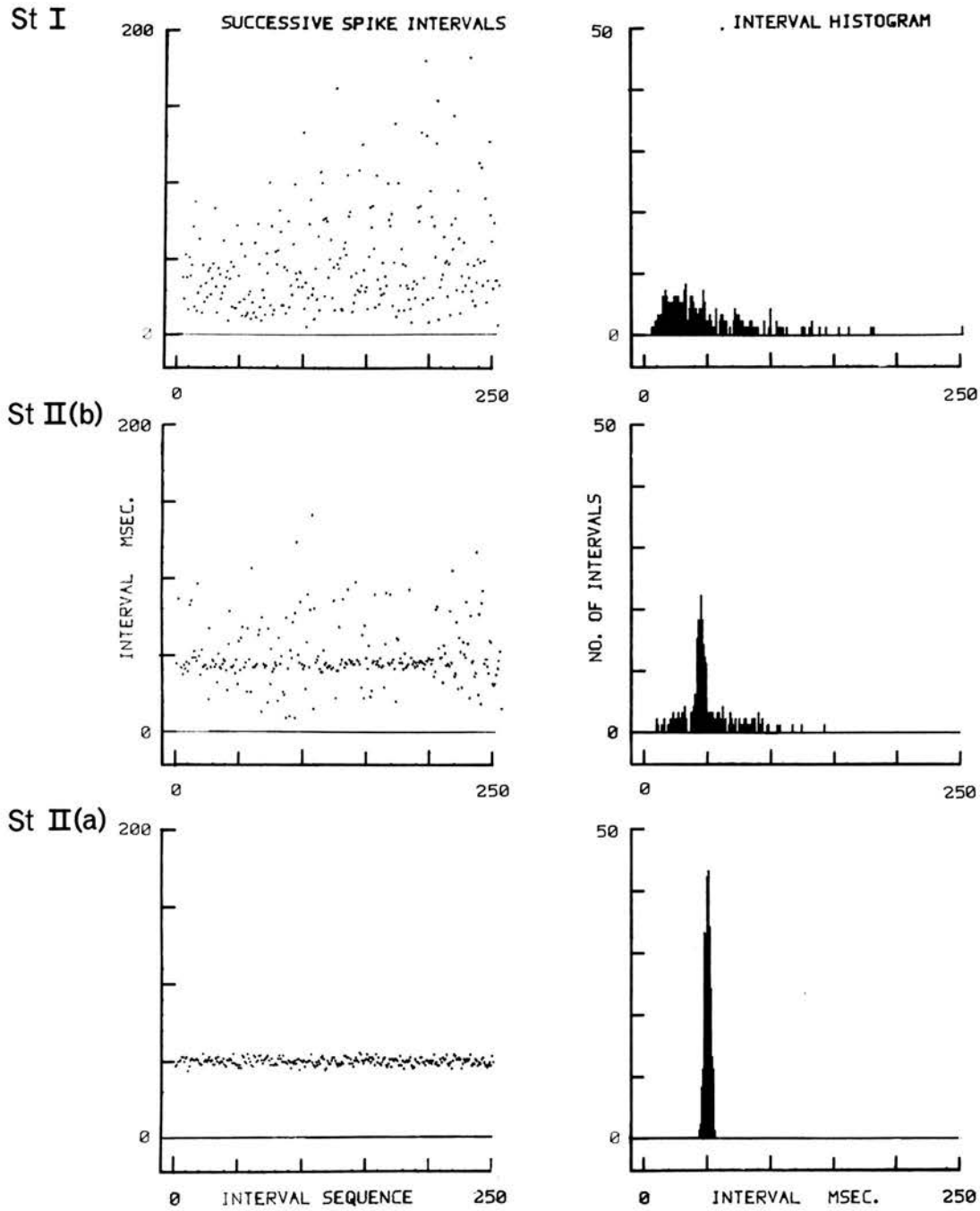

St I
SUCCESSIVE SPIKE INTERVALS
INTERVAL HISTOGRAM
200
50
0
0
250
St II(b)
INTERVAL MSEC.
NO. OF INTERVALS
St II(a)
INTERVAL SEQUENCE
INTERVAL MSEC.

Fig. 5. Theoretical and observed distribution of intervals between impulses in the discharge of the slowly adapting sinus hair follicle receptors. Each graph shows an observed interspike interval histogram with a theoretical distribution of the same parameters superimposed upon it (●), and the goodness of fit has in each case been tested by chi-square.

(A) The observed histogram of an St I unit (8111) fits an exponential relation ($f(t) = \lambda e^{-\lambda t}$ where λ is the rate parameter and t the interspike interval) for interval lengths between 14 and 100 msec.

(B) The interval distribution histogram of an St II(A) unit (1541-1) collected over approximately 240 sec (mean = 22.0 ± 1.4, N = 11879). The theoretical normal distribution $f(x) = \frac{1}{S.D.\ 2} \cdot \exp\ {}^{1}/_{2}\ \frac{X-\bar{X}}{S.D.}^{2}$ fits the observed distribution to a high degree of accuracy ($p = 0.01$).

(C) The interval histogram of the St II(B) unit (25101-1) fits neither Poisson or normal distributions of the same parameters, suggesting that the population of intervals is not homogeneous.

(D) The interval histogram of the St II(B) unit shown in (C) with the hair deflected in the opposite direction and held. The distribution of intervals is normal ($p = 0.01$).

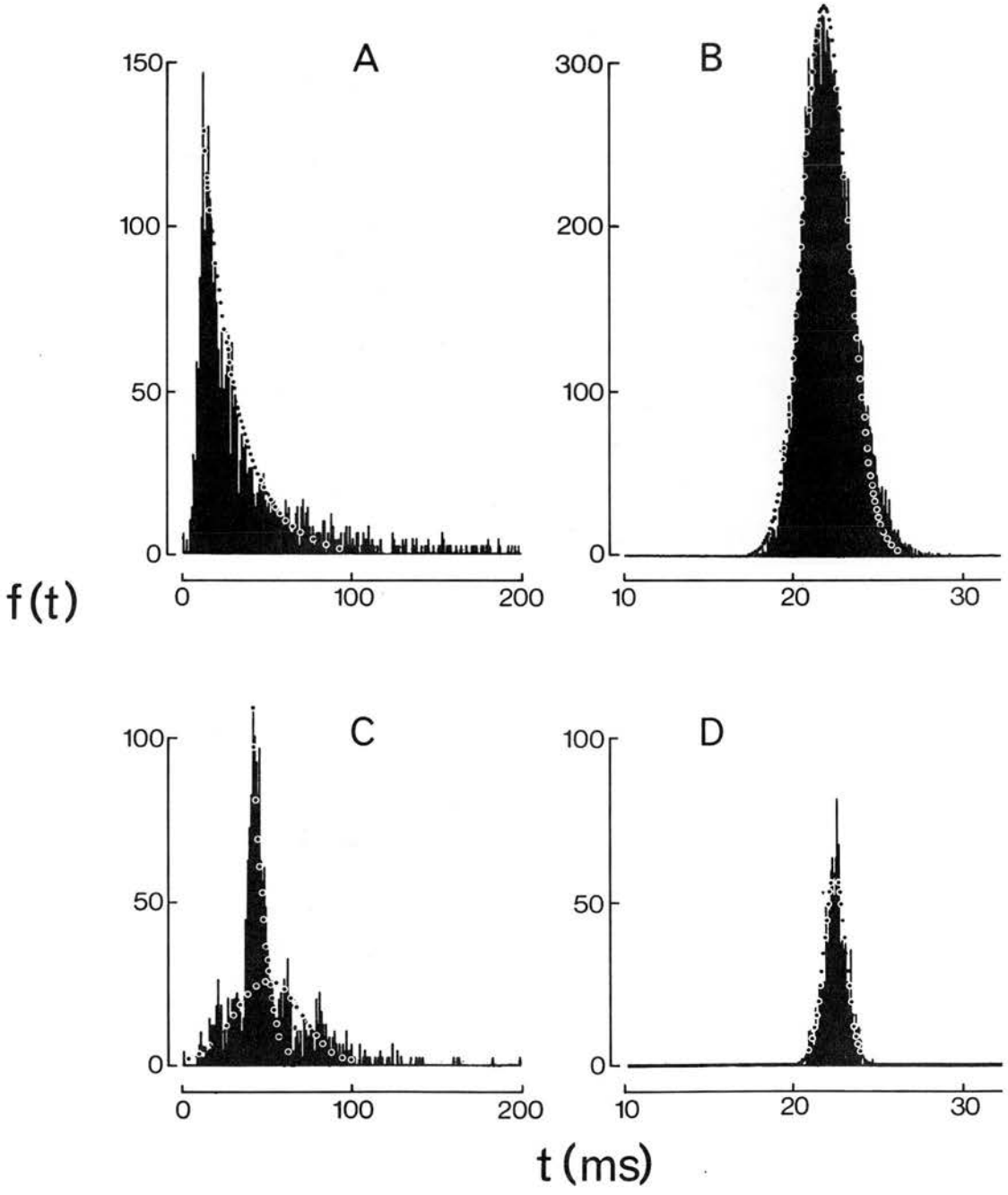

A
B
C
D
150
100
50
0
300
200
100
0
0
100
200
10
20
30
f(t)
t (ms)

Fig. 6. Interspike interval distributions of different sample sizes with theoretical probability density functions of the same parameters superimposed (solid line).

(A) The interval histogram of 4585 intervals of an St II($_A$) discharge does not fit the superimposed Gaussian distribution. Inset is another histogram of 3577 intervals of the same unit which fits the theoretical distribution of the same parameters closely ($p = 0{\cdot}05$).

(B) The interval histogram of 1577 intervals of an St I discharge which fits the superimposed exponential accurately ($p = 0{\cdot}05$).

Above the histograms of both units are samples of the unit discharge recorded in axons of the infraorbital nerve and photographed from the oscilloscope screen. Compare the regularity of the St II(A) discharge with the irregularity of the St I discharge.

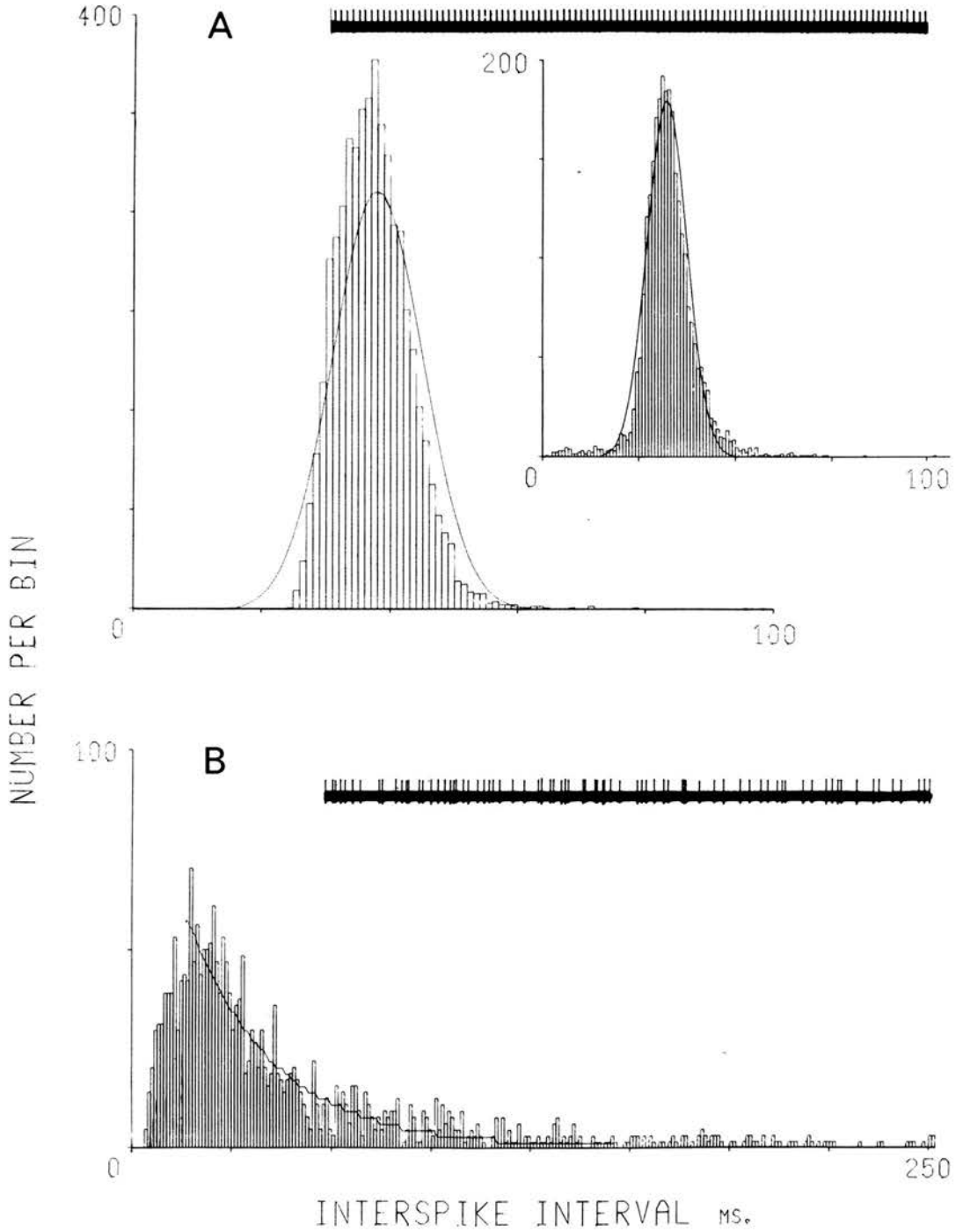

400
A
200
0
100
0
100
NUMBER PER BIN
100
B
0
250
INTERSPIKE INTERVAL MS.

Fig. 7. The relationship between the length of inter-spike interval and its position in the spike train.

The lengths of successive intervals in slowly adapting discharges have been plotted against the serial order of the interval in the spike train and the best fitting regression line drawn through the data.

(A) St II(A) discharge showing very little variability around the regression line and no correlation between interval length and serial order. ($r = 0{\cdot}028$, S.E. $Y/X = 4{\cdot}73$).

(B) St II(B) discharge showing some variability around the line but no apparent trend in the data ($r = 0{\cdot}101$, S.E. $X/Y = 6{\cdot}2$).

(C) St I discharge with great variability and a slight increase in interval length with increasing order, which is not significant. ($r = 0{\cdot}153$, S.E. $Y/X = 8{\cdot}97$).

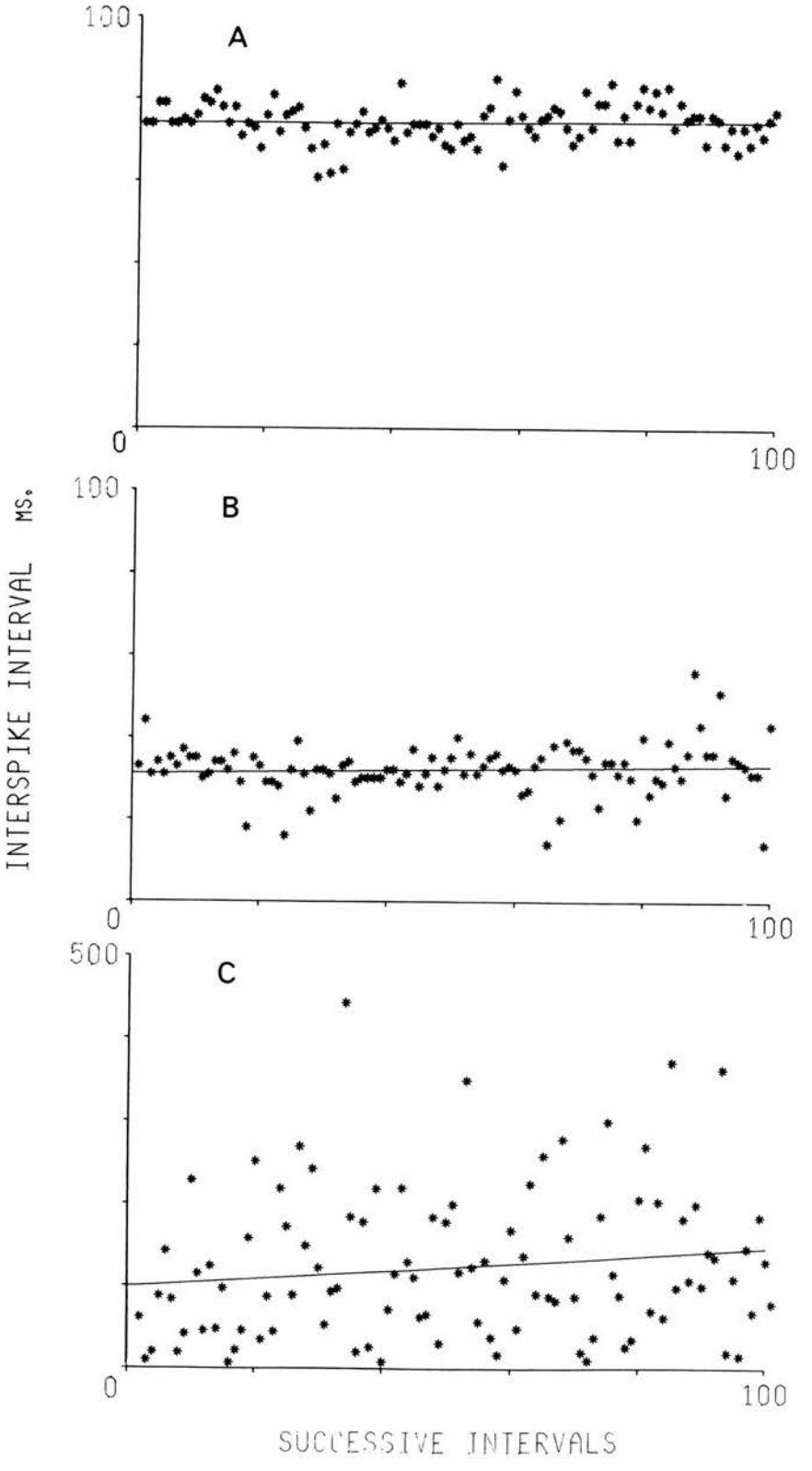

100
A
0
100
100
B
0
100
500
C
0
100
INTERSPIKE INTERVAL MS.
SUCCESSIVE INTERVALS

Fig. 8. The joint interval density (j.i.d.) of three slowly adapting units. Each interspike interval (T_1) is plotted against the succeeding interval in the spike train (T_{1+1}) in order to test for dependence between adjacent intervals. The case of no dependence produces a random scattering of points but whenever the length of one interval is dependent to some extent on the previous interval the j.i.d. scattergram will show a distinctive pattern.

(A) St I discharge displaying a random scattering of points over the full range of observed interspike intervals.

(B) St II(A) discharge producing a tight clustering of points and indicating a strictly non-random spike generating process.

(C) St II(B) discharge with a clustering of points similar to that of the St II(A) scattergram but showing some symmetrical scattering around the dense cluster.

Note the difference in scaling of the St I and St II graphs.

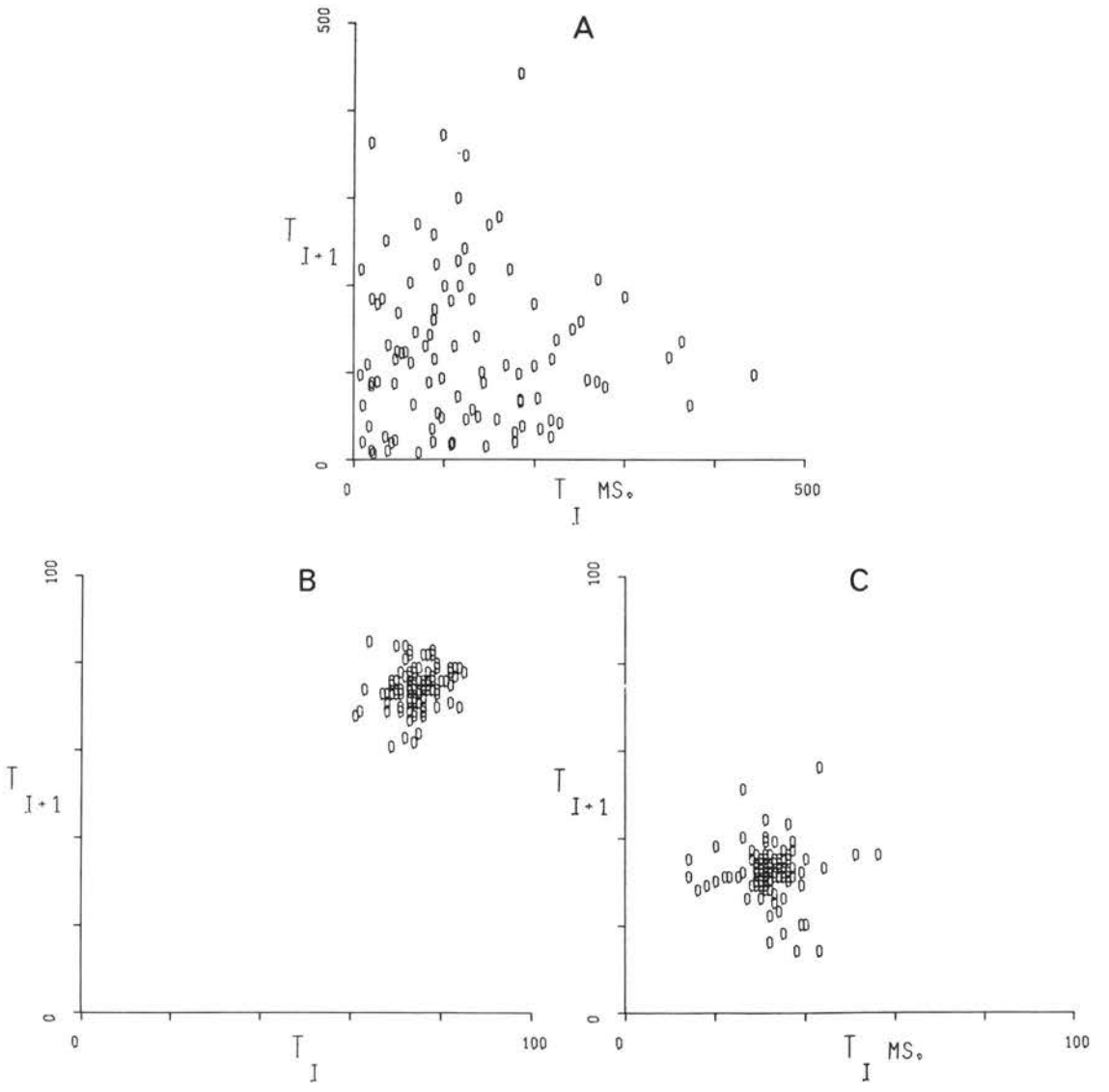

A
500
0
T I+1
0
T I
MS.
500
B
100
0
T I+1
0
T I
100
C
100
0
T I+1
0
T I
MS.
100

Fig. 9. Serial correlograms of the three types of slowly adapting discharge. The serial correlation coefficient (ρ_j) for each serial order of lag(j) is found by the relationship:-

$$\rho_j = \frac{1}{n - j} \sum_{i=1}^{i=n-j} ((X_i - \bar{X})(X_{i+j} - \bar{X})) / V$$

where $\bar{X}$ and V are the sample mean and variance respectively for the sample of 200 intervals (X_i) where i ranges from 1 - 150 and j from 1 - 50. The 1·96 x S.E. bands are drawn onto each curve marking the 5% level of significance of departure of ρ_j from zero.

(A) The serial correlogram of St I intervals shows only random fluctuations from zero.

(B) There are signs of significant periodic fluctuations of the St II(B) correlogram from zero.

(C) The St II(A) correlogram has very high positive serial correlation coefficients at low orders and periodic fluctuations at higher orders suggesting a highly non-random process.

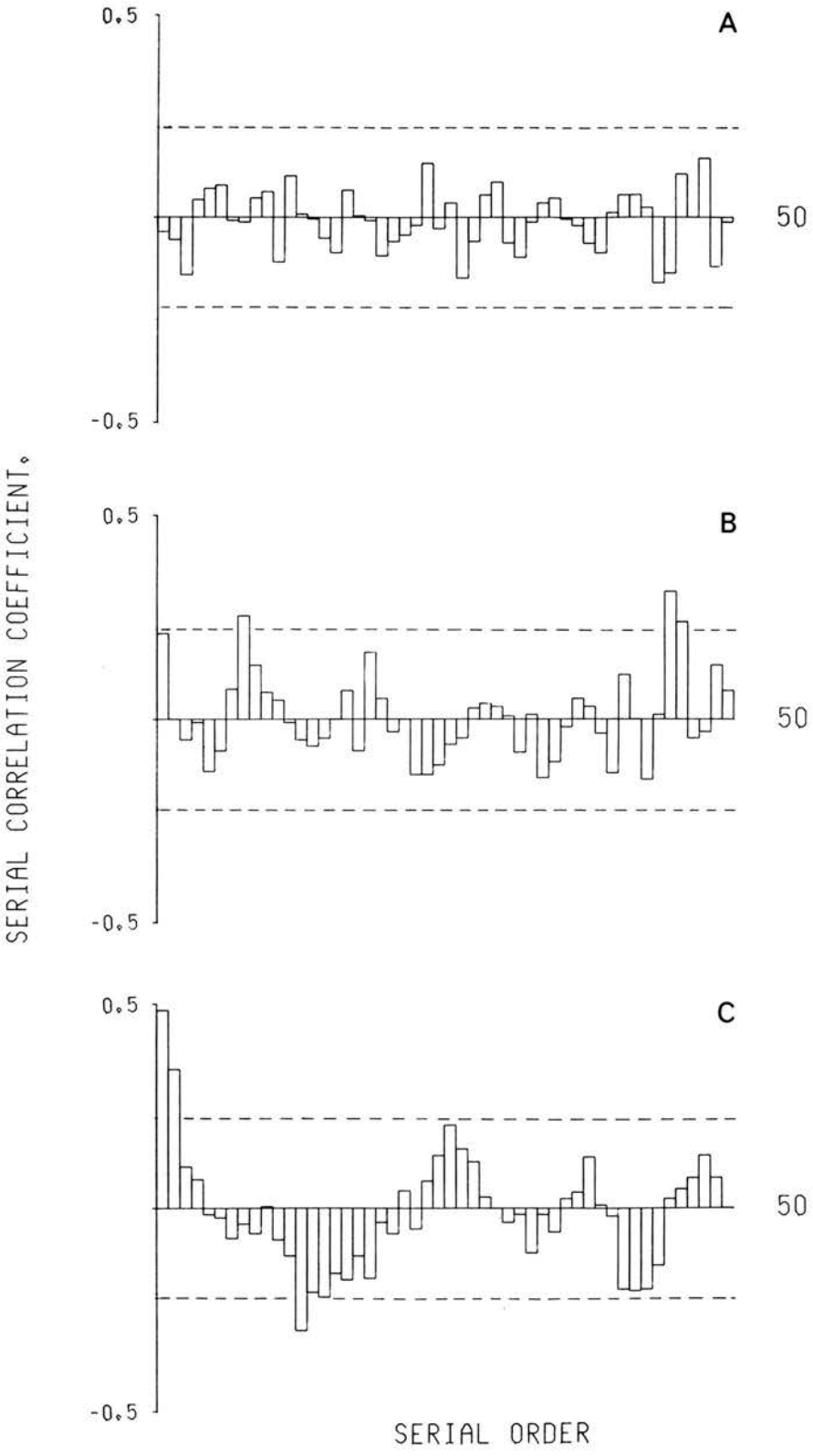

0.5
A
50
-0.5
0.5
B
50
-0.5
0.5
C
50
-0.5
SERIAL CORRELATION COEFFICIENT.
SERIAL ORDER

Fig. 10. Relationship between mean interval length and standard deviation in discharges of (A) St I, (B) St II(A) and (C) St II(B) units immediately following movement of the hair to a new fixed position. For each 50 intervals of a continuous number of successive intervals, plotted on the abscissa, each point represents the mean interval length (•) or the standard deviation (o) of the appropriate 50 intervals. The graph of the St I unit is plotted on semilogarithmic axes, the graphs of the St II units on linear axes.

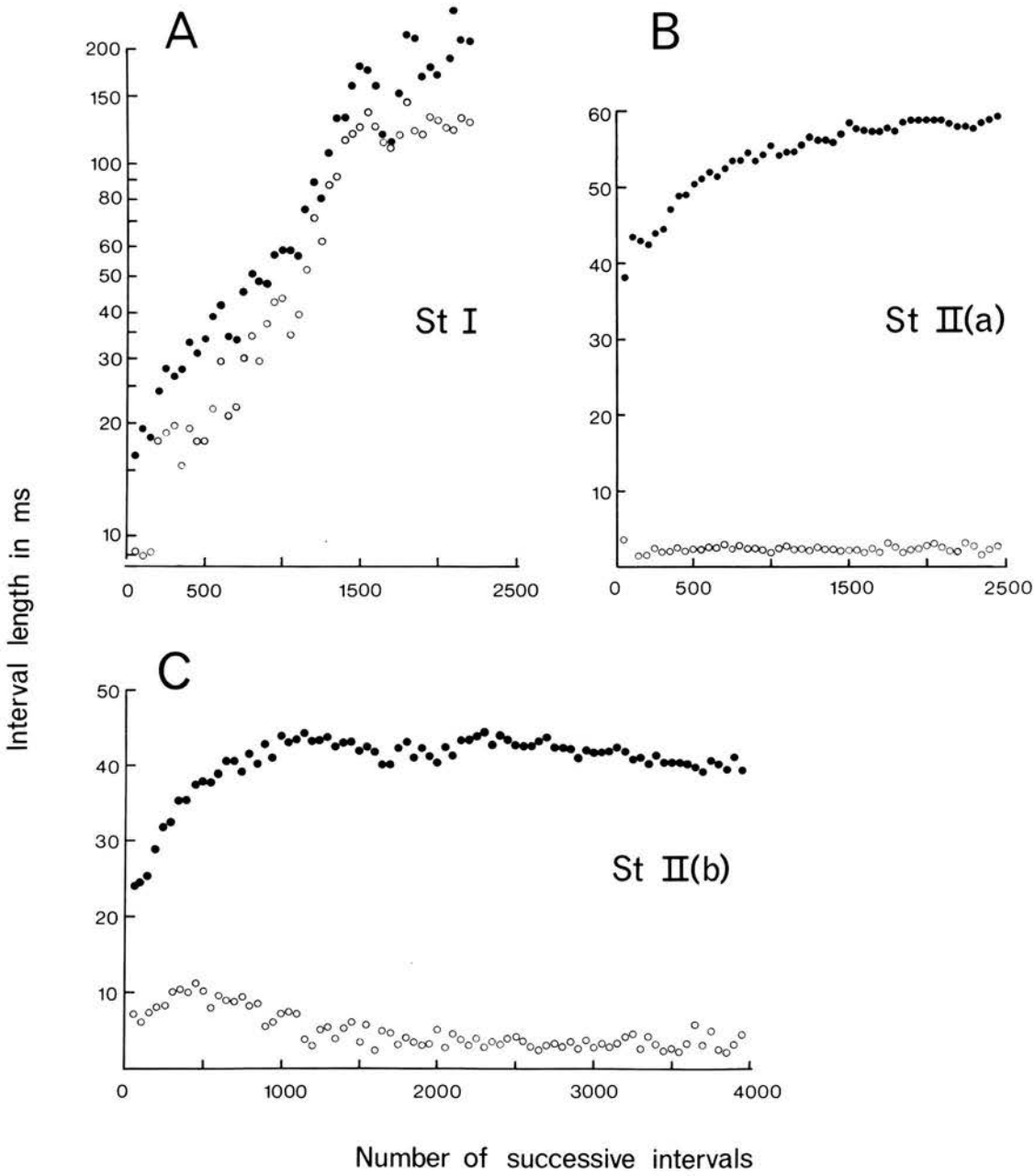

A
B
C
St I
St II(a)
St II(b)
Interval length in ms
Number of successive intervals
200
150
100
80
60
50
40
30
20
10
0
500
1500
2500
1000
2000
3000
4000

Fig. 11. The adaptation of slowly adapting responses. The frequency of sustained discharge in impulses per second is plotted against the time from stimulus onset. The frequency has been calculated by finding the average interspike interval in successive 500 millisecond time periods, and taking the reciprocal. Each graph therefore consists of 500 measurements of the firing frequency over a period of 250 seconds. The time of onset of the sustained hair deflection is marked by a vertical arrow.

(A) Ten seconds of spontaneous activity are shown (left hand side of graph) followed by a high frequency dynamic response and a slow steady decline to an approximately steady mean rate with very little variation. St II(A) unit.

(B) The initial burst of activity of an St II(B) response slows to a steady rate with some fluctuation of the instantaneous frequency.

(C) The St I discharge shows a sharp decline from the dynamic response frequency to a very low rate of discharge with considerable fluctuations in frequency over 500 ms. periods.

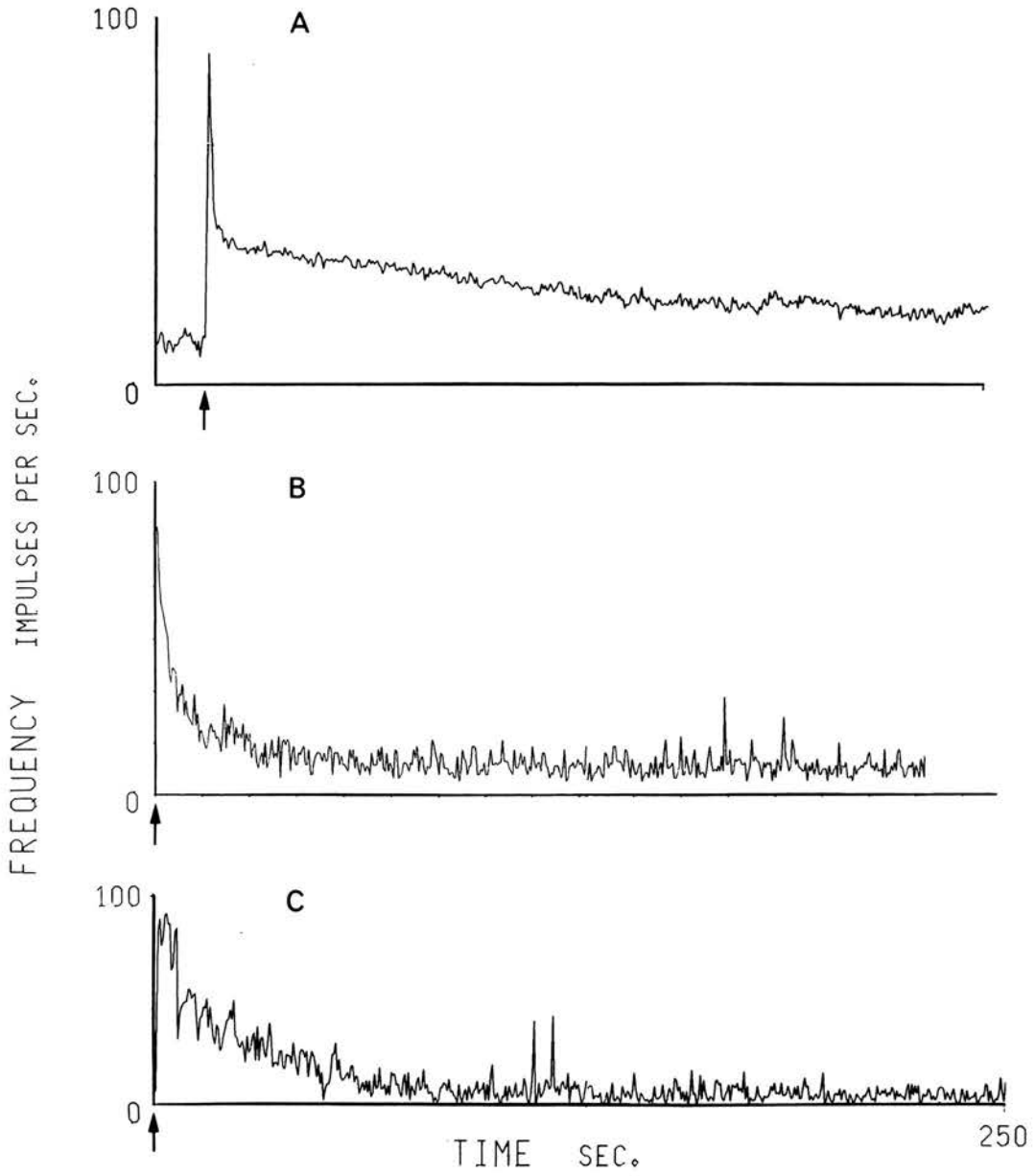

100
A
0
100
B
0
100
C
0
FREQUENCY IMPULSES PER SEC.
TIME SEC.
250

Fig. 12. The time course of the adaptation of the discharge of slowly adapting sinus hair receptors (St I = •-•, St II = o-o). The points represent average frequencies calculated over periods of 10 seconds and are plotted on semi-logarithmic axes. Straight lines were fitted by the method of least squares using the relation $\log_e f = \log_e f_o - t/\tau$ where f is the firing frequency, f_o the initial frequency at stimulus onset, t the time after stimulus onset and τ the time-constant of the exponential decline. Both type I and type II units show two time-constants in the period of recording from 10 to 180 seconds. The inset shows a replot of the early portions of both the St I and St II curves after subtracting the extrapolated lines of the late portions of the curves. This procedure enables the early time-constant τ_3 to be calculated accurately.

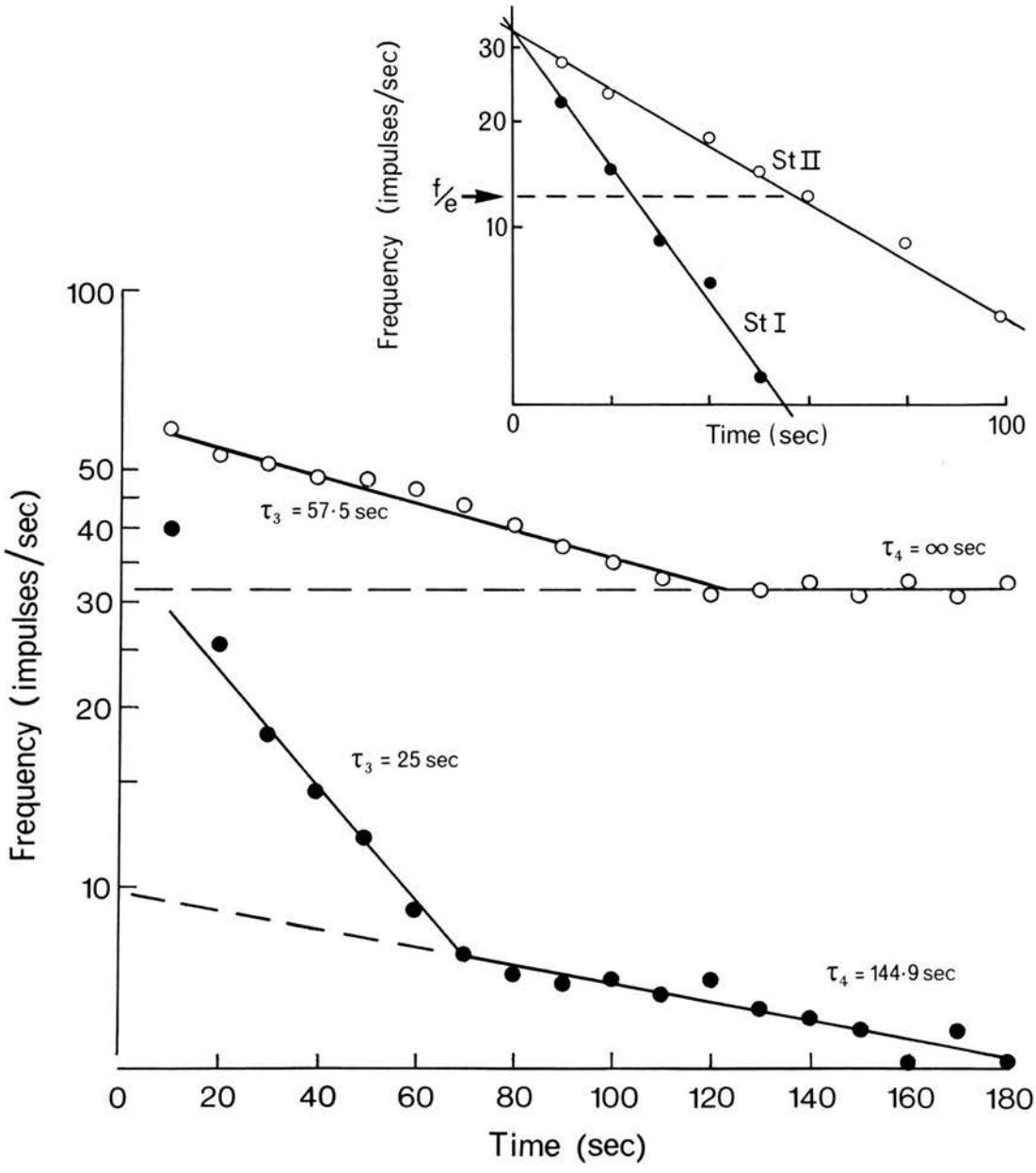

Frequency (impulses/sec)
30
20
10
f/e
St II
St I
0
Time (sec)
100
100
50
40
30
20
10
τ3 = 57·5 sec
τ4 = ∞ sec
τ3 = 25 sec
τ4 = 144·9 sec
0
20
40
60
80
100
120
140
160
180
Time (sec)

Fig. 13. Impulses recorded from a strand of the infra-orbital nerve and illustrating the responses of slowly adapting type I and type II(A) units to an angular movement of the sinus hair. The negative going slope represents a 4^{o} movement in one direction, the positive-going slope the return movement to the zero position at the same velocity. A, shows the high frequency St I response which ceases almost immediately after the direction of movement has reversed, whereas in B, the St II(A) response to the same stimulus shows the persistence of the discharge at a declining frequency for 500 msec after reversal of the movement.

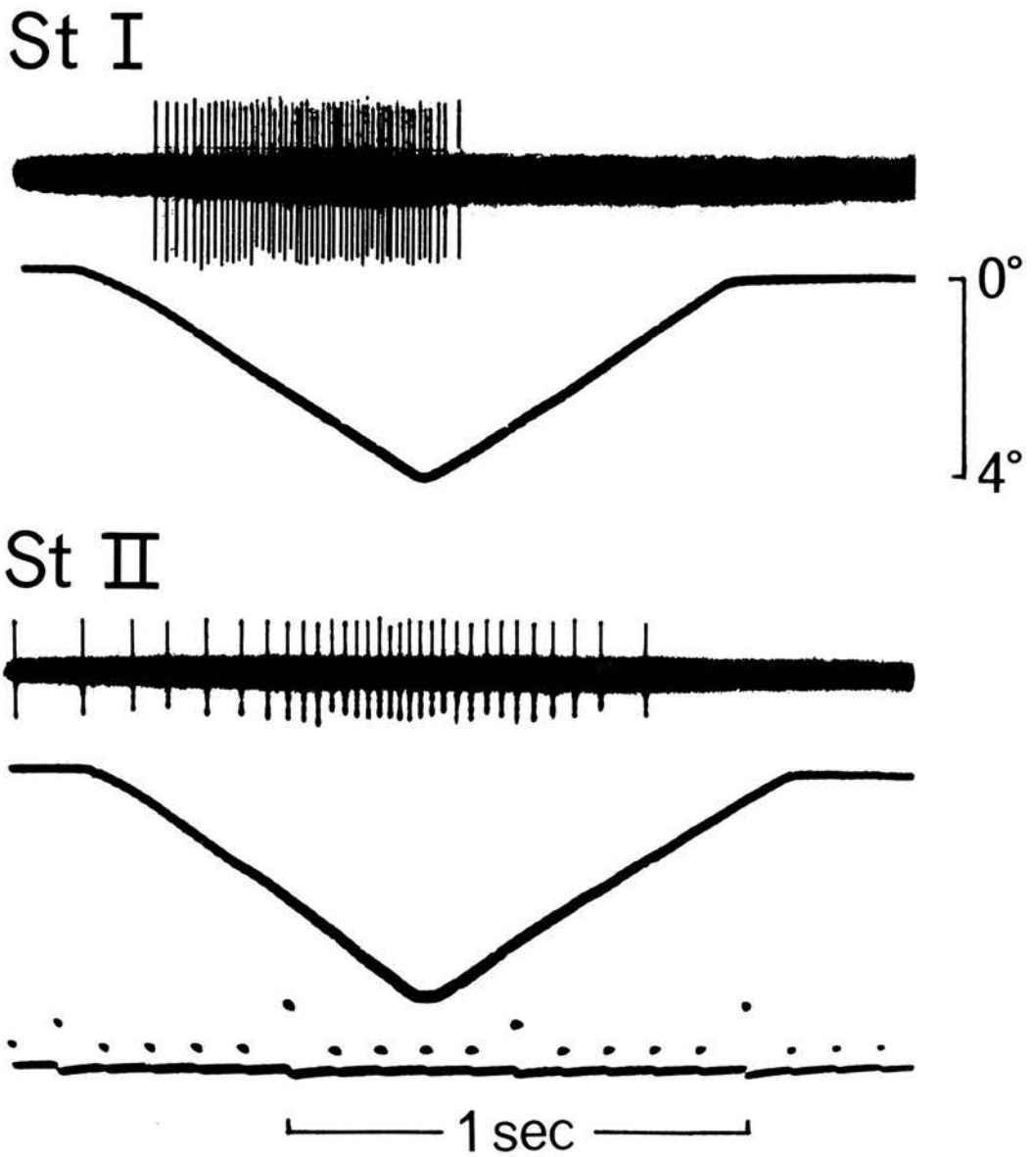

St I
0°
4°
St II
1 sec

Fig. 14. The directional sensitivity of slowly adapting units. Movements in the four cardinal directions (Rostral, Caudal, Dorsal, Ventral) result in responses which are plotted in impulses per second against direction. The hair in the resting position would extend vertically from the page at the origin of the two axes.

(A,B) St I units displaying high sensitivity to movements in one direction only, in opposite directions for the two units shown.

(C,D) St II(A) units with approximately uniform sensitivity to deflections in all directions.

(E) An St II(B) unit with very uniform directional sensitivity.

All graphs are drawn to the same scale which is displayed in the bottom left of the figure.

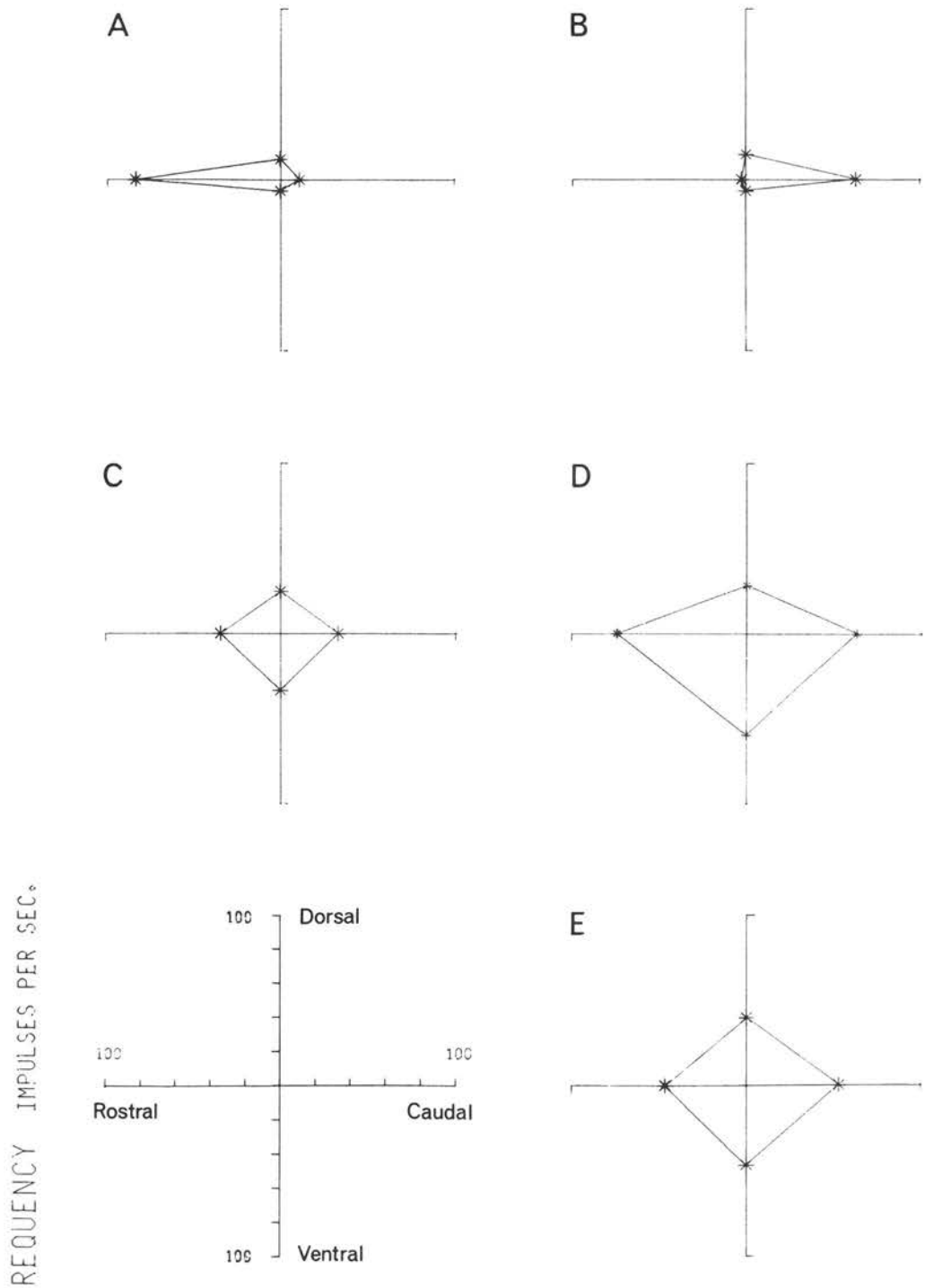

A
B
C
D
E
100
Dorsal
100
100
Rostral
Caudal
100
Ventral
FREQUENCY IMPULSES PER SEC.
FREQUENCY IMPULSES PER SEC.

Fig. 15. The relationship between the velocity of angular movement of the hair (to a fixed angular deflection of 4^{o}) and the threshold amplitude (expressed as a % of the final amplitude) for the first spike in St I, =▲ , St II(A) = ● , and St II(B) = o, units measured at rise times from 50 to 500 msec. Each point is the mean response amplitude for four different units. When the hair is moved through a constant angle at different rise times the 'critical amplitude' is defined as that percentage of the total angular movement at which the first spike occurs.

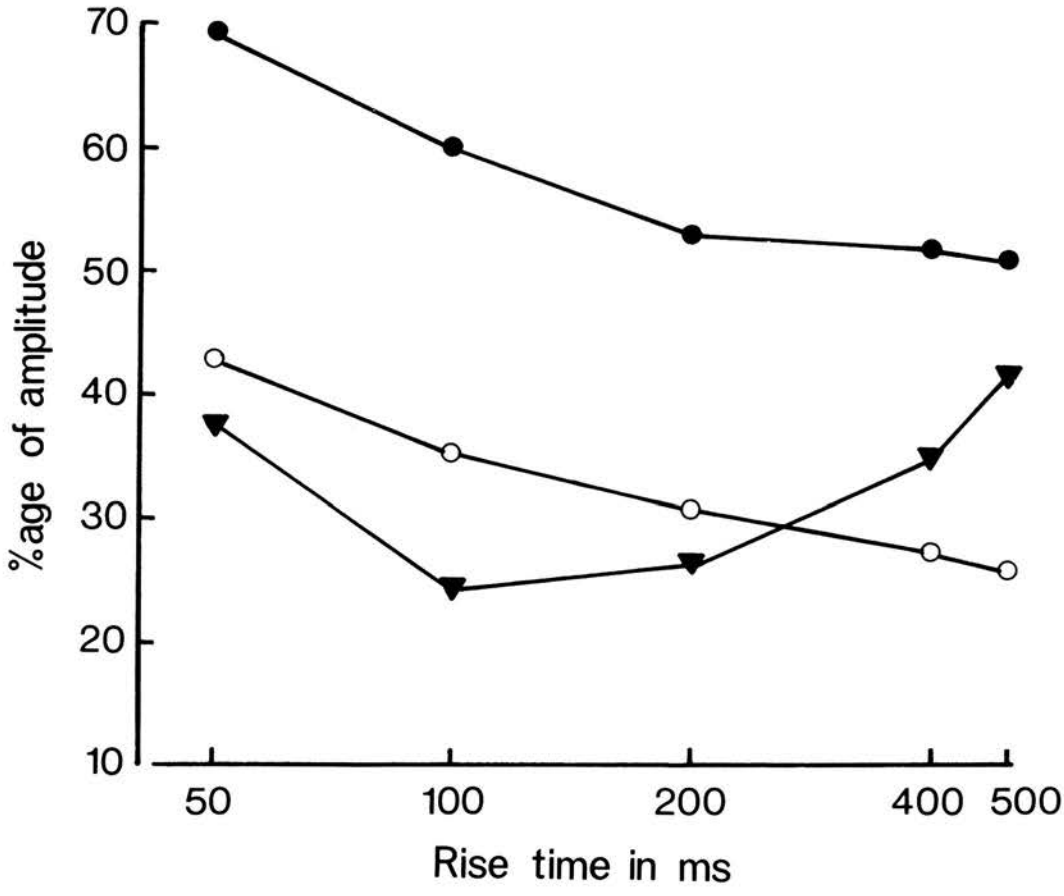
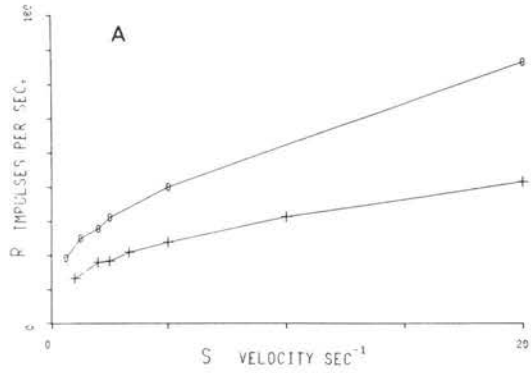

70
60
50
40
30
20
10
%age of amplitude
50
100
200
400
500
Rise time in ms

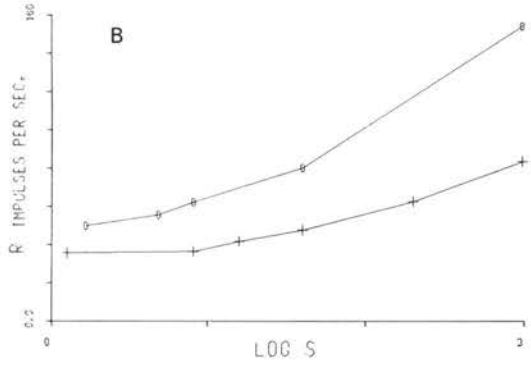
Fig. 16. The responses of a type I(X) and a type II (A)(O) sinus hair unit to a moving stimulus measured as the reciprocal of the rise-time to a fixed deflection angle. Each point represents the mean firing frequency during the last 10% of the movement calculated over 20 stimulus presentations from the post-stimulus time raster. The same data was subjected to several different transformations and plotted on linear axes in order to estimate the goodness of fit to various functions.

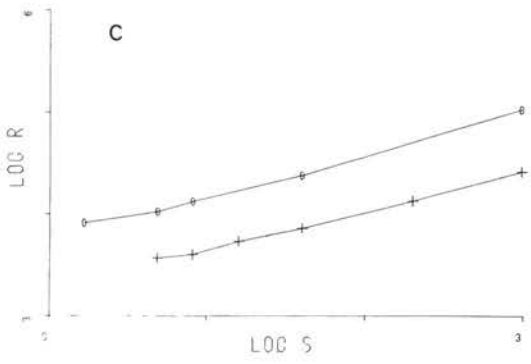
These functions and their representative transformations to linear co-ordinates are:-

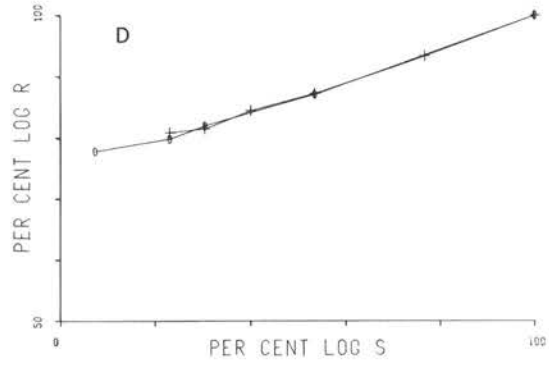
(A)	Linear	$R = b.\ S + A.$
(B)	Logarithmic	$R = b.\ \log S + A.$
(C)	Power	$\text{Log } R = b.\ \log S + \log A.$
(D)	'normalised' power	$\%\log R = b.\ \%\log S + \%\log A.$
(E)	power 'normalised'	$\text{Log}\% R = b.\ \log\% S + \log\% A.$

Full descriptions of these curves are given in the text.


A
R IMPULSES PER SEC.
0
20
S VELOCITY SEC^{-1}


B
R IMPULSES PER SEC.
0
3
LOG S


C
LOG R
0
3
LOG S


D
PER CENT LOG R
50
100
0
100
PER CENT LOG S

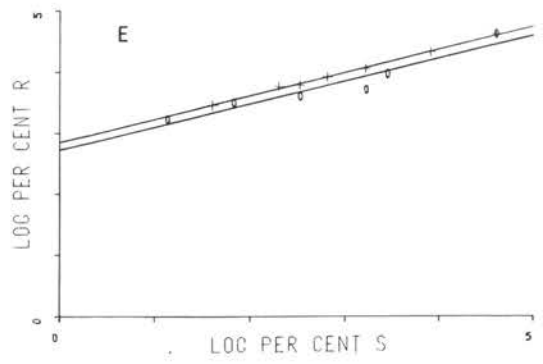

E
LOG PER CENT R
0
5
0
5
LOG PER CENT S

Fig. 17. The responses of several slowly adapting units to varying degrees of hair deflection, or in the case of St II(A) unit (●) the degree of rotation around the hair axis. Each point is the mean response of two stimulus presentations over the first 50 impulse following the application of a sustained stimulus, recorded after the initial dynamic response had subsided.
(A) The response of each unit plotted as linear co-ordinates.
(B,C) The responses of several units plotted against the logarithm of the stimulus, a straight line relationship representing a logarithmic function. The lines have been separated into two groups for clarity only.
The lines drawn through the data points are the best fitting regression lines.
The symbol codes are:-

St I(+), St II(A)(●,*), St II(B)(O).

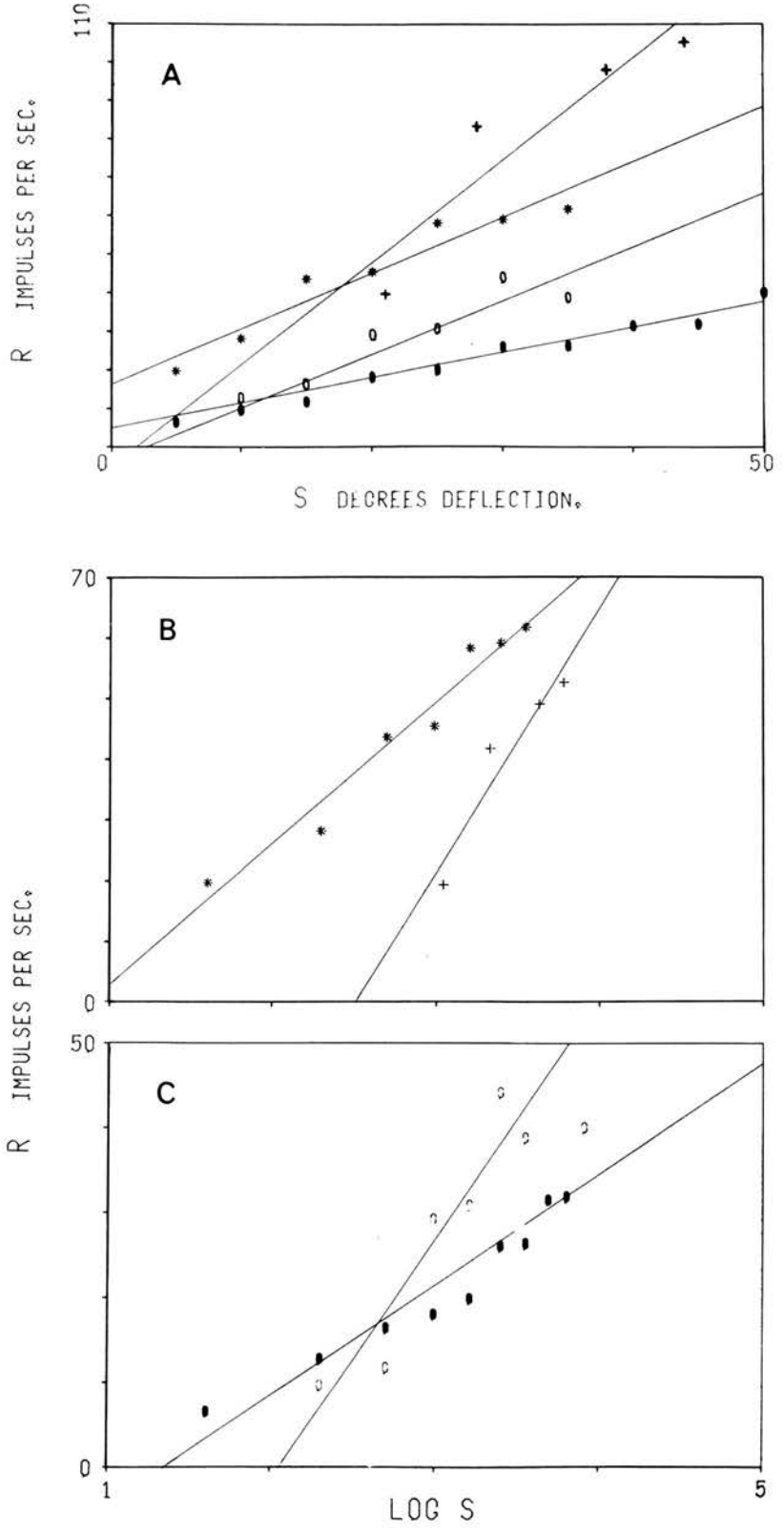

A
110
0
50
R IMPULSES PER SEC.
S DEGREES DEFLECTION.
B
70
0
C
50
0
1
5
LOG S

Fig. 18. The same responses as those of Figure 17 subjected to three further transformations.

(A) The log of the stimulus plotted against the log of the responses (power function).
(B) The percentage of the responses to the maximal stimulus against the percentage of the log of the maximal stimulus (normalised logarithmic function).
(C) The log of the percentage of the response to maximal stimulation against the log of the percentage of the maximal stimulus (power normalised function).

The symbol codes are:-

St I(+), St II(A) (●,*), St II(B)(O).

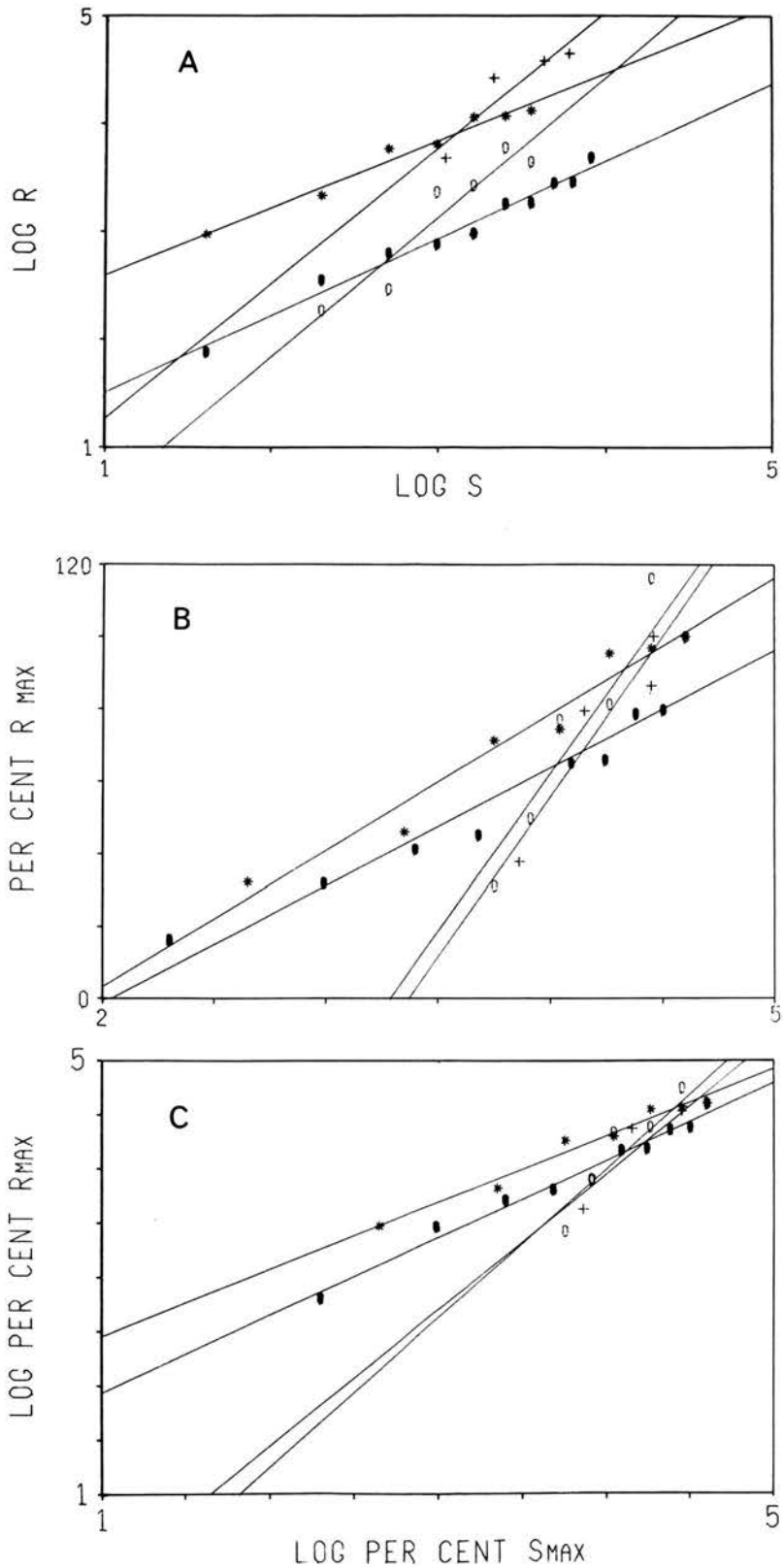

A
LOG R
5
1
1
5
LOG S
B
PER CENT R MAX
120
0
2
5
C
LOG PER CENT RMAX
5
1
1
5
LOG PER CENT SMAX

Fig. 19. The responses of 7 slowly adapting units to sustained, graded deflections of the sinus hair plotted as logarithms, corresponding to the power function $R = A. S^{b}$.

(A) Two different St I units (+,x).

(B) Five different St II units of both types, St II(A)(+,x,*) and St II(B)(O,●)

The best fitting regression lines are drawn through the points.

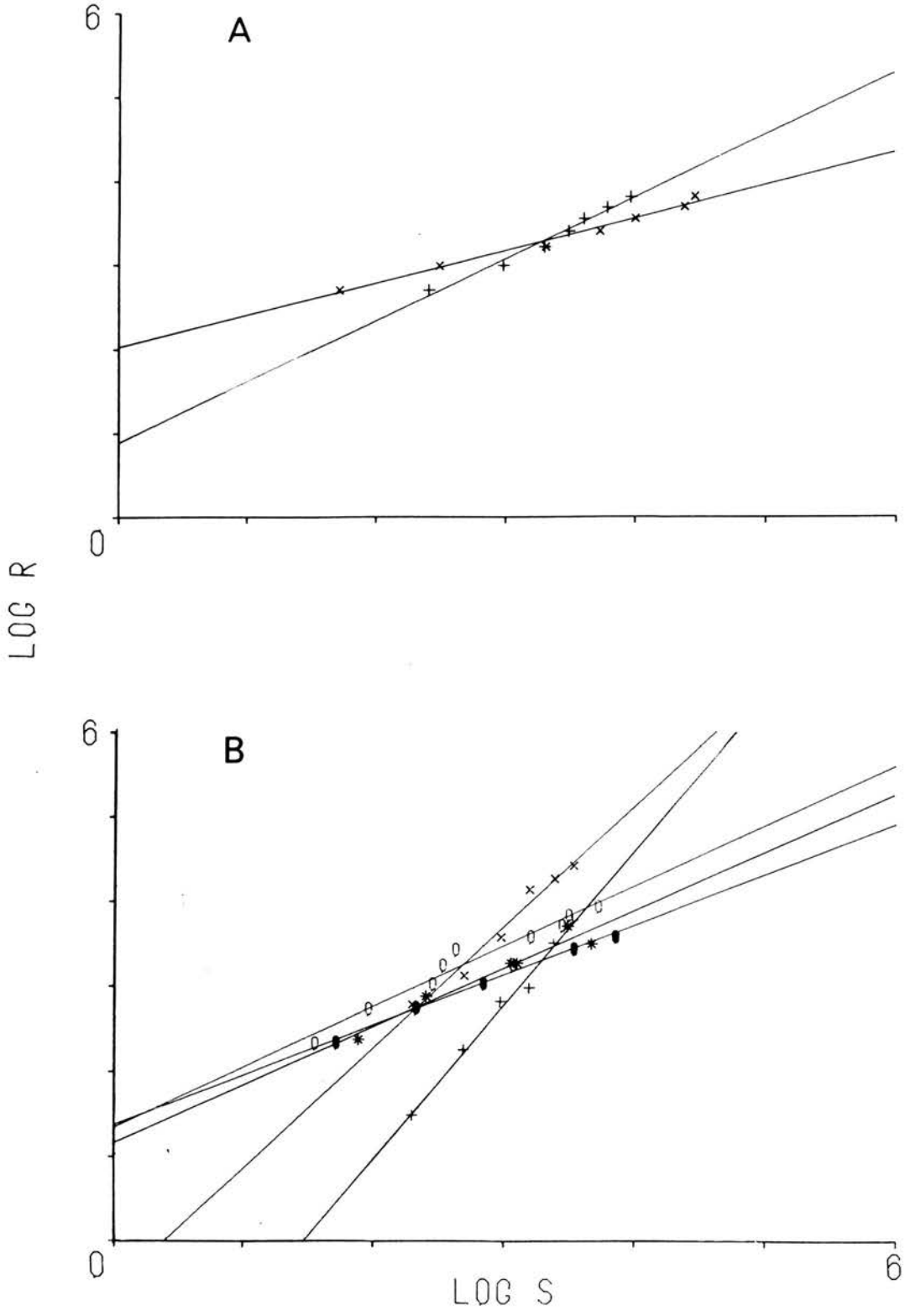

A
6
0
B
6
0
6
LOG R
LOG S

Fig. 20. The responses of 9 slowly adapting units to dynamic stimuli plotted as logarithms corresponding to the function $R = A. S^b$.

(A) All types of unit.
St I(*,●), St II(A)(○,+), St II(B)(x).

(B) All types of unit.
St I(+,○), St II(A)(●), St II(B)(×).

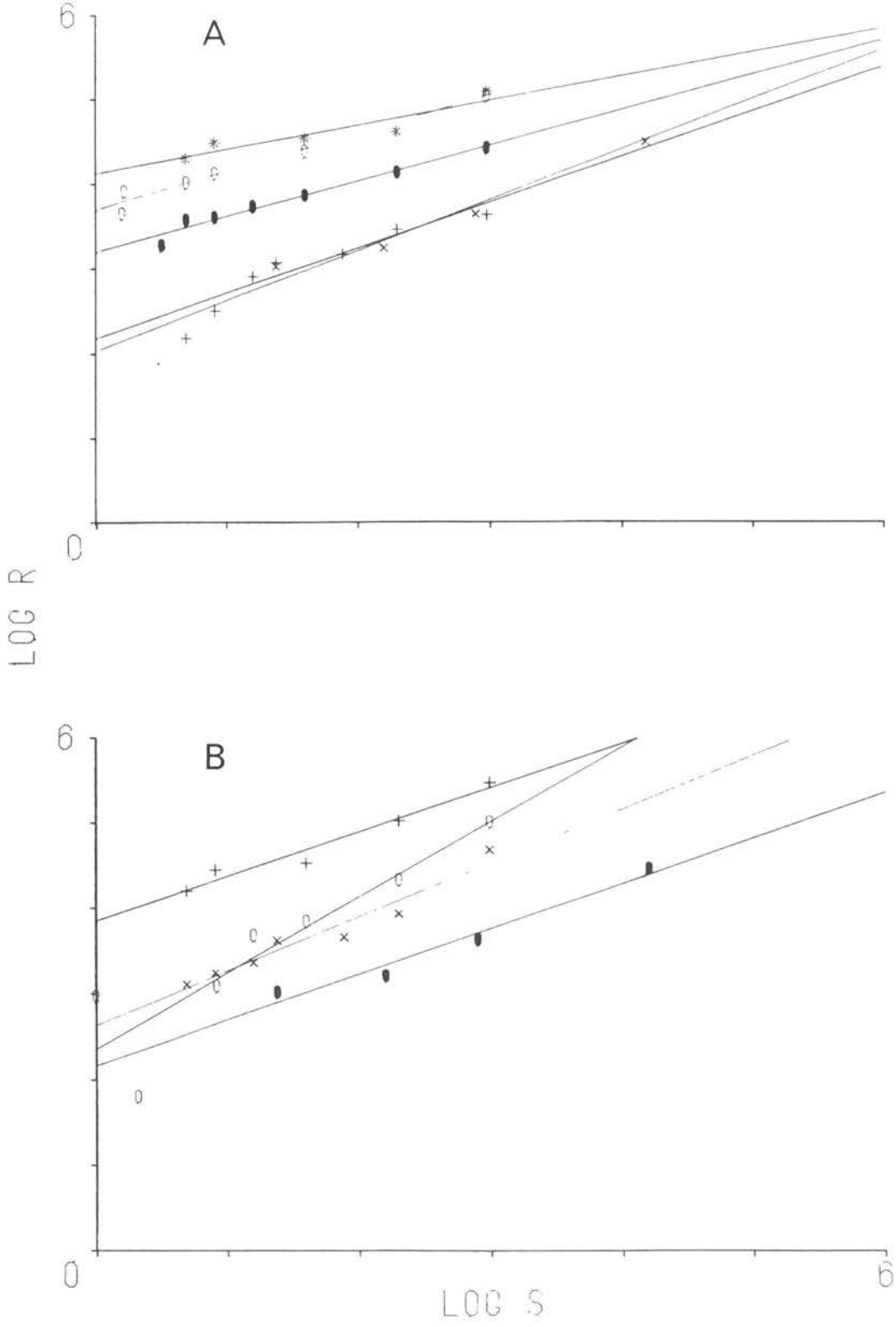

A
B
6
0
LOG R
LOG S

Fig. 21 The discharge of high velocity threshold rapidly adapting unit (1251-1) at the indicated frequencies of stimulation. For each record the upper tracing is the spike train recorded from a strand dissected from the infraorbital nerve, the lower trace is the output from the sinewave generator used to drive the electro-mechanical transducer. The records show a response of 1 spike per movement cycle over the range of frequencies from 800 to 1,000 HZ, whereas at the lower frequencies several spikes were initiated during each cycle.

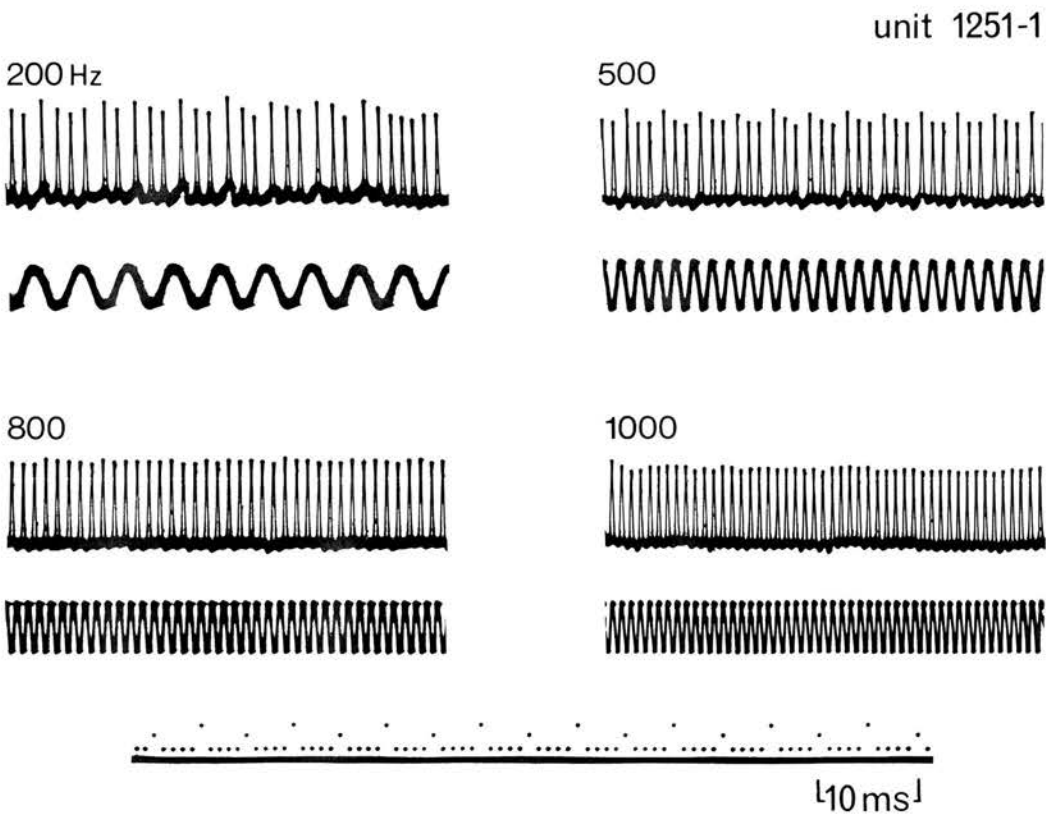

unit 1251-1
200 Hz
500
800
1000
10 ms

Fig. 22 A, B. Tuning curves of high velocity threshold rapidly adapting units. A, HVRA-unit (19101-4) response to sinusoidal movement over the range 500 - 700 HZ. The ordinate is the amplitude of the movement of the hair represented as the voltage driving the mechanical stimulator, adjusted so that 1 spike was evoked with every second cycle of movement. The unit is maximally sensitive to vibration at approximately 600 HZ.
B, HVRA-unit (8101-6) plotted as A, but for three attachment points of the probe to the hair (●-● = 3 mm, o-o = 14 mm and ▲-▲ = 24 mm from base of hair), 1:1 responses.

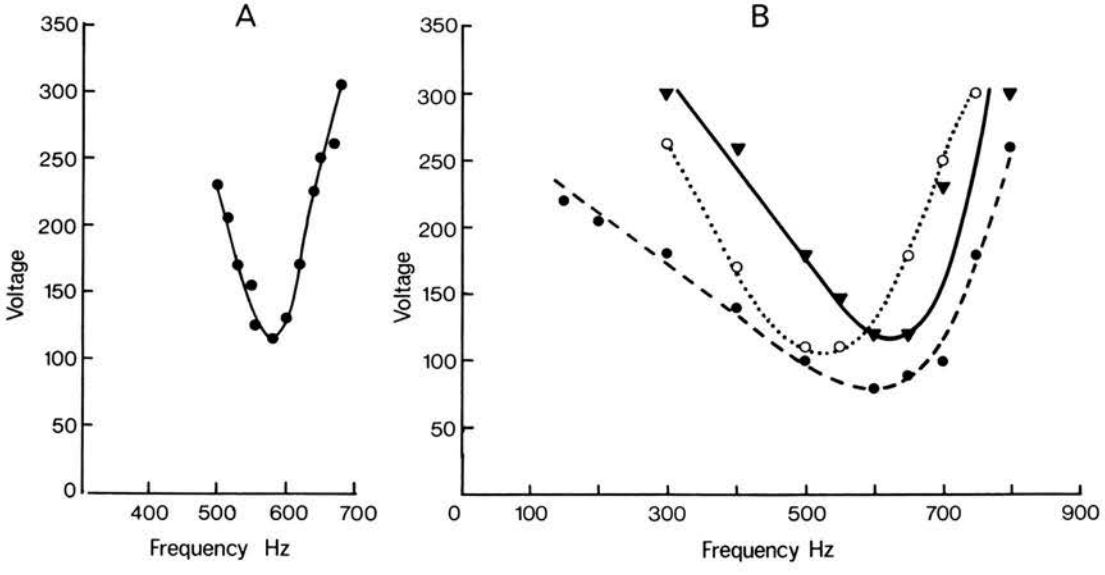

A
B
Voltage
Frequency Hz
350
300
250
200
150
100
50
0
400
500
600
700
100
300
900

Fig. 23. **Impulses recorded from a single filament of the infraorbital nerve showing the low velocity threshold rapidly adapting response (LVRA). A, B, C, D a series of hair deflections to a constant angle of 8 degrees at the velocities indicated. The number of impulses per response (upper traces) increases with increasing velocity (lower traces).**
E, F, G. A series of hair deflections to different angles, as indicated.
Lower trace represents the actual movement of the stimulator as measured by a mechano-electric transducer

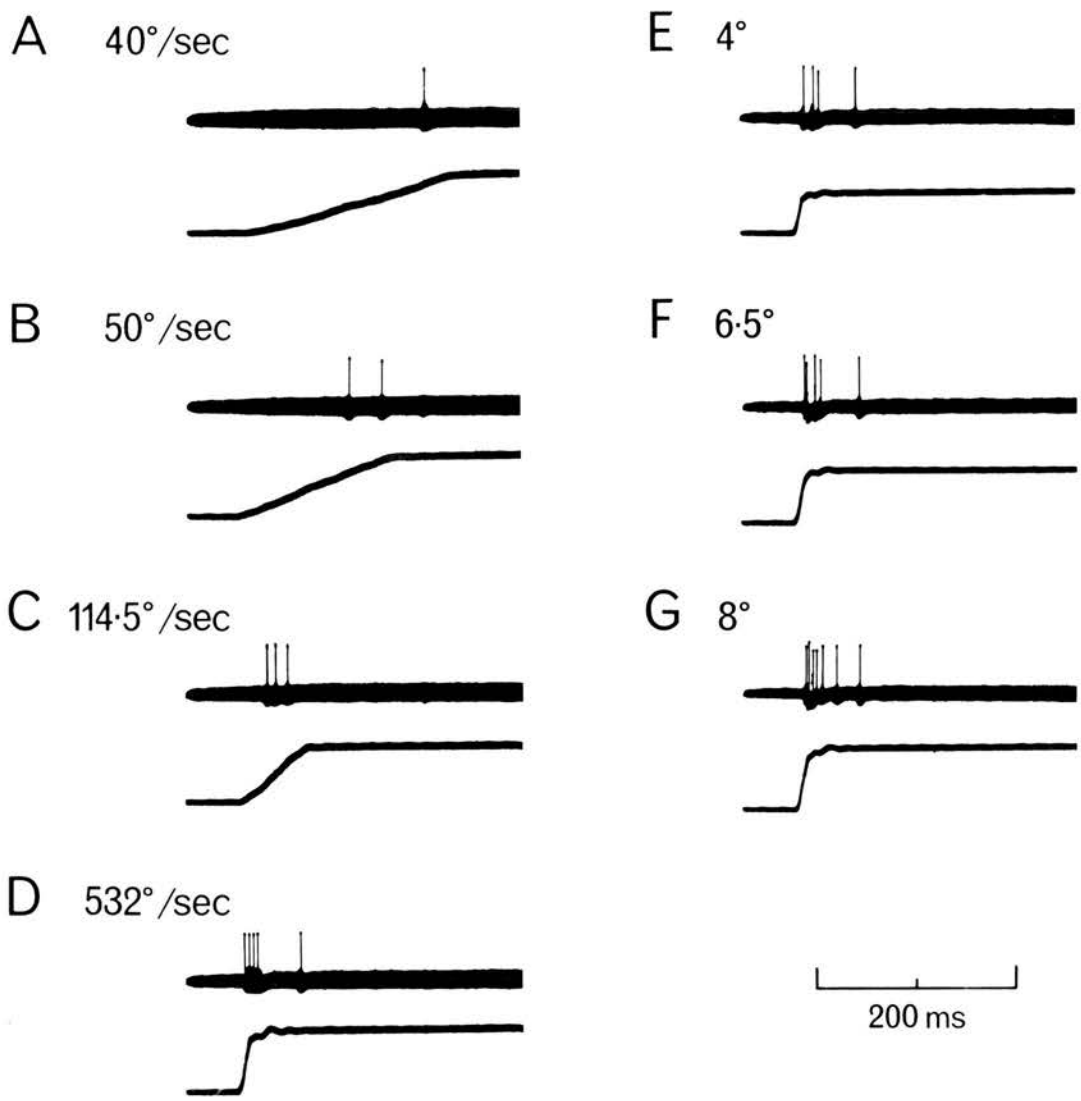

A
40°/sec
B
50°/sec
C
114·5°/sec
D
532°/sec
E
4°
F
6·5°
G
8°
200 ms

TABLE I

Conduction velocities in sinus afferent units.

Type of unit.	Number of units.	Conduction velocity (m/sec) mean (±S.D.)	range
		Slowly adapting	
SAI	9	53·9±10·2	33-77
SAII(a)	10	56·4±14·9	44-72
SAII(b)	3	57·5	47-68
SAII	13	56·6±13·5	44-72
		Rapidly adapting	
HVRA	13	61·3±24·2	34-104
LVRA	4	58·4±17·1	42·5-82·5

Table II.

The statistical parameters of stimulus - response relationships described by figures 16 - 18.

r = Correlation coefficient.

S.E. Y/X = Standard error of the estimate of the regression Y upon X.

S.E. b = Standard error of the estimate of b.

TABLE II

Fig.	Unit	Transformation	Function	r	S.E. Y/X	S.E. b
A	St I 2132 StII 0222	Linear	R=2.8S + 29.9	0.9802	4.2	0.25
			R=5.6S + 42.87	0.9829	6.9	0.52
B	St I 2132 StII 0222	Logarithmic	R=17.6 log S + 20.7	0.9804	3.42	1.69
			R=37·2 log S + 33·5	0·9716	10·78	4·01
16 C	St I 2132 StII 0222	Power	$R=3·2\ S^{0·37}$	0·9987	0·023	0·0087
			$R=3·7\ S^{0·43}$	0·9932	0·071	0·025
D	St I 2132 StII 0222	Normalised Power	$\%R=74·1\ \%S^{0·25}$	0·9976	0·57	0·0087
			$\%R=74·6\ \%S^{0·24}$	0·9953	1·0	0·013
E	St I 2132 StII 0222	Power Normalised	$\%R=2·84\ \%S^{0·37}$	0·9983	0·024	0·0097
			$\%R=2·71\ \%S^{0·37}$	0·9569	0·156	0·056
● 17C	StII(A) 3032	Logarithmic	R=13·03 log S-17·6	0·9478	3·4	1·55
● 18A		Power	$R=0·78\ S^{0·71}$	0·9897	0·08	0·036
✱ 17B	StII(A) 3032	Logarithmic	R=23·2 log S-20·64	0·9557	3·56	2·12
✱ 18A		Power	$R=1·97\ S^{0·62}$	0·9857	0·079	0·047
+ 17B	St I 2142	Logarithmic	R=86·9 log S - 218	0·9567	10·6	18·7
+ 18A		Power	$R=0.08\ S^{1.2}$	0.9855	0.20	0.08
O 17C	StII(B) 0452	Logarithmic	R=28.3 log S-58.3	0.9427	5.22	5.0
O 18A		Power	$R=-0.75S^{1.29}$	0.9502	0.22	0.21

Table III.

The statistical parameters of the power functions $R = A\ S^{b}$ described by figures 19 and 20.

TABLE III.

Unit	A	b	r	S.E. Y/X	S.E. b	Static Response
St I 2142-1	0·88	0·72	0·9898	0·061	0·046	+ 19A
St I 2142-2	2·02	0·38	0·9920	0·054	0·027	X 19A
StII(A) 8112-3	-2·6	1·79	0·9950	0·093	0·089	+ 19B
StII(A) 1111-2	-0·53	1·40	0·9862	0·12	0·11	X 19B
StII(A) 8112-4	1·12	0·53	0·7642	0·10	0·055	✳ 19B
StII(B) 1211-2	1·31	0·70	0·9811	0·111	0·052	O 19B
StII(B) 10121-2	1·32	0·58	0·9956	0·048	0·027	● 19B

Unit	A	b	r	S.E. Y/X	S.E. b	Dynamic Response
St I 2142-1	3·8	0·52	0·9784	0·12	0·063	+ 20B
St I 2142-2	2·35	0·88	0·9068	0·47	0·069	O 20B
StII(A) 1111-2	2·16	0·53	0·9853	0·14	0·069	● 20B
StII(B) 10121-2	2·63	0·63	0·9720	0·13	0·068	X 20B

StII(A) 1111-3	2.17	0.53	0.9860	0.13	0.063	+ 20A
StII(A) 0222	3.69	0.44	0.9408	0.19	0.094	O 20A
StII(B) 1211-2	2.03	0.58	0.9856	0.09	0.038	X 20A
St I 2132	3.19	0.41	0.9838	0.07	0.033	● 20A
St I 8111	4.11	0.28	0.9221	0.13	0.068	✳ 20A

SECTION III

The responses of cells in the trigeminal sensory complex to electrical and mechanical stimulation and the identification of a trigemino-cerebellar pathway.

INTRODUCTION

The experiments described in section II have shown that the movement of a single sinus hair may cause the activation of four main types of receptor located in the sinus body. A number of criteria were established by which each type of response may be clearly identified. The purpose of the experiments described in this section is to investigate the distribution of these afferent discharges over the trigeminal sensory complex by studying the discharge patterns of the second-order response. A specific functional pathway projecting to the cerebellar cortex is considered.

METHODS

Anaesthesia.

Twenty eight cats of both sexes weighing between 1·9 - 4·5 kg. were used for these experiments. In eight animals anaesthesia was induced with 44mg./kg Nembutal (Na.Pentobarbitone) administered intraperitoneally and maintained with subsequent doses of 10% w/v Nembutal intravenously. In nine experiments anaesthesia was induced with ethyl chloride and ether and maintained with chloralose 65mg./kg. i.v. In a further eleven experiments anaesthesia was induced with a mixture of nitrous oxide and oxygen (2:1) into which halothane 1·5 - 3·5% was slowly admitted and delivered to the animal via a face mask. These animals were maintained on subsequent doses of chloralose 65mg./kg. i.v.

Blood pressure was continuously monitored via a cannula inserted into the femoral artery and a pressure transducer (Bell & Howell) coupled to a calibrated voltmeter. Records were taken only when the mean blood pressure exceeded 90mm.Hg.

In early experiments when spontaneous breathing became inadequate and in all later experiments, the animal was artificially ventilated using a Palmer respiration pump connected to a tracheal cannula. In these later experiments expired air was sampled continuously using a Grubb-Parsons CO_2 meter and the respiratory rate and volume adjusted to maintain an end-tidal CO_2 concentration of 4%. Rectal temperature was monitored and held at 38·5^{o}C by a thermostatically regulated electric blanket.

Surgical preparation of the animal.

The trachea, femoral artery and saphenous vein were cannulated for the maintenance procedures described above. The dorsal surface of the cranium and the muscles overlying the upper cervical vertebrae were exposed by medial incision. The temporal and segmental muscles were carefully reflected using electrocautery where necessary. The dorsal surface of the cranium was scraped clean and the arch of the atlas bared.

The animal's head was held by ear, mouth and infra-orbital bars in a stereotaxic head holder which was fixed to the recording table. A tilt of 15 - 20 degrees around the ear bar axis was necessary to hold the brain-stem recording area in a horizontal plane. In some experiments, with smaller animals, a spinal clamp was placed on the

process of a high thoracic vertebra so lifting the spinal cord to the level of the medulla and eliminating distortion of the recording site. Under these conditions the effects of respiratory pulsation were not a problem. Starting at the rim of the occipital foramen and working anteriorly, the cranium was chipped away exposing the dura overlying the medulla oblongata and the posterior lobe of the cerebellum. This exposure was continued until the bony tentorium was reached anteriorly and the left pars intermedia exposed laterally. In most experiments the exposure was carried 1 - 2 mm. beyond the mid-line over the right hemisphere. This preparation left the fissura prima roughly in the centre of the field with the para-vermian vein to the left, lying in an anterior-posterior direction. In three experiments the arch of C1 was removed, allowing access to the caudal pole of the nucleus caudalis. It was obvious at an early stage that bleeding and air embolism would be a problem when removing the spongy bone at the lambdoidal ridge. This problem was solved by continuous irrigation of the surgical field with warm 0· 9% saline and frequent application of bone wax.

The free flaps of skin were sewn to a metal ring and the dura reflected from the bony tentorium to the arch of C1 or C2 using a sharp scalpel under a dissection microscope. Warm mineral oil was then poured into the pouch of skin to form a recording pool.

In two experiments the infraorbital nerve was exposed by the method described in section II and the whole nerve lifted in continuity onto bipolar silver electrodes for electrical stimulation.

Electrical and mechanical stimulation.

In all experiments, except two where the infraorbital nerve was exposed in the orbit, a pair of sharp steel electrodes was inserted into the skin at the base of the vibrissae. Square-pulses of 1 - 10 volts magnitude and 50 μs duration were delivered to the electrodes from a Grass stimulus isolation unit coupled to a Grass S4 stimulator.

Electrical stimulation of the cerebellar cortex was achieved using an electrode consisting of three lengths of electrolytically sharpened tungsten insulated to within 1 mm. of the tip. The central wire acted as the cathode, both outer wires as the anode. The electrode was inserted to a depth of 1 - 2 mm. and a square pulse of 1 - 2 volts amplitude and 50 μs duration passed from a second Grass stimulus isolation unit. Investigation of the current-passing characteristics of this electrode showed a capacitance effect with a time-constant of 3·5 μs. A 1 volt pulse applied met an initial impedence of 1·5 KOhm, passing 700 μA. At the end of the 50 μs pulse the impedence had risen to 3·3 KOhm, passing 300 μA.

The timing of stimulus pulses was under the control of a Devices Digitimer which provided trigger pulses for two Devices pulse generating units. These units were used to trigger the Grass stimulators and the recording equipment described below. In this way isolation of the stimulus pulse was assured, so reducing the size of the stimulus artifact. An early attempt at monopolar cathodal stimulation of the cerebellar cortex using the

animal earth as an indifferent electrode produced an intolerably large stimulus artifact. This was due in part to the loss of stimulus isolation and also to the close apposition of stimulating and recording electrodes.

Identification of trigeminal units.

A trigeminal unit was identified initially by its response to a supra-maximal stimulus applied to the facial electrode and/or its response to light manual brushing of the face with a camel hair brush. This procedure gave a bias to the selection of vibrissal units due to the position of the stimulating electrode. Once identified as a sinus hair unit the angle stimulator or the electromechanical stimulator was attached to the hair in the manner described in the previous section.

Two important limitations were imposed upon mechanical stimulation of the vibrissae in this series of experiments. Firstly, the amplitudes of deflection required for the activation of trigemino-cerebellar units was usually higher than that which could be achieved using the electromechanical stimulator. Secondly, the 'angle stimulator' described in section II could not readily be attached to the posterior hairs of the group due to the space limitations imposed by the stereotaxic head holder. The sine-wave generator was not available for this series of experiments and therefore high frequency stimulation of the vibrissae was not possible.

Recording electrodes.

Glass micropipettes were drawn with long shanks (1·5 - 2·0 cm) and the tips broken on fine tissue paper

to a diameter of 1-2 μm. The electrodes were filled either with 4 molar NaCl or a 1% solution of pontamine sky blue in acetic acid. The impedence of these electrodes varied from 2 MOhm to 12 MOhm and were suitable for recording unitary action potentials in the region of cell bodies within the trigeminal and adjacent nuclei. The electrode was fixed into a holder which moved on an adjustable arc positioned over the medulla oblongata. Axial movement of the electrode was achieved using an electronically controlled microdrive which advanced in variable numbers of 2 μm. steps and at variable speeds. The electrode was advanced in single steps in the region where facial responses were found.

Penetrations of the brain-stem were often trans-cerebellar (in rostral regions) and at an angle of 10° backwards to the vertical. This was necessary to avoid contact between the recording and stimulating electrodes. A computer programme was written which made the appropriate corrections when calculating the anterior-posterior position and depth of trigeminal cells.

A monopolar ball electrode was lowered onto the surface of the cerebellum in order to record gross cerebellar evoked potentials. This was done routinely in later experiments as a means of locating the best position for inserting the cerebellar stimulating electrode.

Recording and amplification.

A unity gain head - stage was clipped onto a silver wire which entered the micropipette through a hole in the electrode holder. Action potentials were amplified, and

displayed on a Tectronix 565 oscilloscope. The amplified signal was passed to the PDP-12 computer for interspike interval analysis as described in detail in the methods of section II. Slow waves recorded on the surface of the cerebellum were also passed to the PDP-12 for processing with a signal averaging programme. The averaged signal was stored on digital tape where it could be recalled for detailed inspection and plotted using the Complot incremental plotter.

Marking of recording sites.

When pontamine-sky-blue electrodes were used and after a trigeminal cell had been investigated, a direct current of 10μA was passed through the electrode (cathode) for 5 - 10 minutes. This caused the dye to be deposited in the recording site forming a blue spot which could be seen later on 40μm serial sections of the tissue. At the end of these experiments the whole of the brain-stem was removed and quickly transferred to a cryostat (Nuclear Instruments, Chicago) which froze the tissue within minutes. Serial sections were cut the following day and stained with either luxol fast blue alone or luxol fast blue counterstained with cresyl violet. This enabled the position of the pontamine blue spot to be determined accurately relative to the various brain-stem structures. For details of the histological techniques see Appendix II.

Positioning the microelectrode.

The medio-lateral position of the microelectrode was determined relative to the mid-line, as determined by aligning the electrode with touching ear bars before placing the animal in the head holder and recording the micromanipulator setting. It was a relatively simple matter to check this alignment against the mid-line of the dorsal surface of the medulla. The true anterior-posterior position was more difficult to determine accurately and an error of 0·5 mm. was expected. All A-P measurements were made relative to the obex. With the electrode in the vertical position the posterior lobe of the vermis was carefully deflected and, under the dissecting microscope, the tip of the electrode was moved until it was directly above the V-shaped cleft of the obex, usually 1 - 1·5 mm. rostral to the normal position of the caudal limit of the posterior lobe. Given these gross landmarks it was felt that positioning the electrode in this way offered no disadvantages over the use of stereotaxic co-ordinates.

The electrodes were driven at a high stepping speed through the arachnoid and more slowly thereafter to eliminate as much as possible the effects of surface dimpling. Penetrations were made in 0·5 mm. steps across the field of responses and a careful record kept of the vertical positions of responsive units, as measured by the stepping motor depth indicator.

RESULTS

The definition of a trigeminal unit.

Any cell of the medulla which responded consistently to electrical or mechanical stimulation of the face with a latency of less than 30 ms. was regarded as a cell of the trigeminal sensory complex. Occasionally, usually at the end of an electrode track, a cell could be activated consistently by electrical stimulation (with a long latency $>$ 50 ms.) but not by manual exploration of the face and the buccal cavity. These cells were assumed to be of a separate functional group activated by a tenous polysynaptic pathway. Histological examination of the recording areas was made in four experiments and cells which had been operationally defined as 'trigeminal' were found to lie within the spinal trigeminal nucleus or adjacent brain-stem structures which have immediate synaptic connections with the nucleus, as described by Brodal (1953), Carpenter & Hanna (1961) and Lund & Webster (1967).

No attempt was made to estimate the depth of these units relative to the usually obscured surface of the medulla. In this situation the position of a cell within the trigeminal sensory complex was determined relative to the point at which an electrically evoked response to facial stimulation could be heard on the audiomonitor, usually as the background noise of several distant units. The passage of an electrode through the overlying dorsal column nucleii was often a useful guide as to the imminence of a trigeminal response.

Unit records were usually biphasic and of somewhat variable latency to different levels of electrical stimulation, normally greater than 2 ms. The shape of the spike often changed as the electrode was advanced and units could be held for distances of over 100 μ. These factors suggest that primary afferent fibres were excluded from the records as might be expected with the relatively low impedence electrodes used.

The area of microelectrode penetrations.

Figure 24 is a map of the recording positions at the surface of the medulla relative to the mid-line and the obex. Within $\pm$ 3 mm. of the obex most penetrations were made within a 2·5 mm. strip from 2·5 to 5 mm. lateral of the mid-line. In more rostral locations penetrations were progressively more lateral, reflecting a widening of the brain-stem mass at this level and the lateral curving of the spinal trigeminal nucleus. Recordings were not made at positions more than 7 mm. rostral to the obex where the main sensory and motor nucleii of n.$\overline{V}$ reside. In view of the assertion of Darian-Smith and Phillips (1964) that 38% of all trigemino-cerebellar units lay within the region of the nucleus interpolaris at the obex, many more penetrations were made in this region in later experiments.

Unit activity of the trigeminal sensory complex.

Spontaneous activity.

Contrary to the results of Mosso & Kruger (1973) several cells were found, the activity of which could be modified by stimulation of the facial region, but which showed a spontaneous discharge in the absence of any stimulus. Figure 25 contains interspike interval histograms of six such units which form a representative sample. The rate of spontaneous activity varied from cell to cell and was affected by the depth of anaesthesia, often slowing considerably after the administration of a standard dose. In many units the appearance of spikes in time was random or near-random as illustrated in figure 25A where the histogram takes on the approximate shape of exponential distribution. The rate of discharge of some units could change abruptly over a few seconds, producing a multi-modal interval histogram such as that of 25B. The cell which yielded the interval histogram 25C responded to the movement of three vibrissae and the discharge had properties similar to the primary afferent St II discharge. A similar unit, but with a much larger receptive field covering most of the ipsilateral face, is shown in figure 25D. A commonly encountered feature was the discharge of impulses in a strict doublet sequence as in 25E. Typically, the interval histogram is bimodal with a very narrow distribution of the intra-doublet interval, close to the origin, and a much broader distribution of inter-doublet intervals over a wide range of interval lengths.

This unit was activated with a short latency (1·2ms.) from the cerebellum. Figure 25F shows the interval distribution of a cell firing at a high rate of discharge ($>$ 100 impulses/sec.) with very little variability. The rate of activity was increased by stroking the hairs of the upper lip, including the lower row of vibrissae.

Electrically evoked activity.

Cells of the trigeminal sensory complex showed a variety of response patterns to electrical stimulation of the face at the base of the vibrissae. The electrical stimuli employed were in the range 1·5 - 18 volts and the voltage chosen was that which produced the shortest latency and maximum number of evoked spikes in any unit discharge. At these intensities all sizes of afferent fibres could be excited but only those cells which responded to light tactile stimulation were investigated. Units receiving nociceptive and thermoreceptive input were therefore excluded from this sample of cells. Most commonly, a cell would fire with a burst of 3 - 6 impulses with a latency to the first impulse of 3 - 5 ms. and an interspike interval between successive impulses of 1 - 2 ms. Examples of this activity are shown in figures 26 A and B. Frequently the early burst was followed by a later burst of 1 - 3 spikes with a latency of 12 - 30 ms. as illustrated in figures 26 C,D,E. Some units responded only with a late repetetive discharge as shown in 26 F,G,H while a large group of cells discharged a single, short latency impulse as in 26I.

The post-stimulus-time histograms (PSTH) of figure 27

illustrate the distribution of impulses in time following electrical stimulation of the face. Alongside each histogram is a reconstruction of the type of spike discharge pattern which produced the histogram. Figure 27A is the PSTH of a unit which responded with a burst of 3 - 4 spikes at variable intervals. Figure 27B shows bimodal distribution where an early single spike was followed by a variable burst of 3 - 4 impulses at a latency of 12 - 15 ms. A second type of bimodal PSTH is illustrated in figure 27C corresponding to an early and late burst of spikes separated by an interval of 2 - 5 ms. These histograms were obtained from cells in the region of the nucleus interpolaris at varying depths.

The latency of the presynaptic volley at the level of the obex is 1·3 - 1·5 ms. (Darian-Smith et al., 1965) and therefore cells which discharge with a latency of 2 - 3 ms. are probably monosynaptically activated. Later bursts of activity in these cells and activity in cells with only a late discharge, must be the result of polysynaptic excitation.

<u>The latencies of unit responses to electrical stimulation of the face</u>.

The latencies of responses evoked in the trigeminal nucleus depends to a great extent upon the rostro-caudal position of the recording electrode. The descending spinal tract of the trigeminal nerve send collaterals into the spinal nucleus as it passes over it, terminating in the region of the nucleus interpolaris and the rostral pole of the nucleus caudalis. Conduction times increase

disproportionately towards more caudal regions as the conduction velocity decreases with the tapering of the descending fibres (Wall & Taub, 1962; Darian-Smith et al., 1965). The pooling of measurements for the purpose of finding an average latency must therefore be carried out only on measurements made in approximately the same rostro-caudal position. Figure 28 shows the distribution of latencies to the first spike of cells of the nucleus interpolaris to electrical stimulation of the maxillary skin. The mean latency of these discharges is 5·14 ms. and the modal latency 3·5 ms. As this is a relatively small sample (N = 47) the mode is a more meaningful measure of the average latency and agrees closely with the mean latencies of far larger samples ($>$500) collected by Darian-Smith, Phillips & Ryan (1963), Wall & Taub (1962) and Mosso & Kruger (1973).

<u>The receptive fields of trigeminal cells to manual stimulation of the face</u>.

A general survey of the receptive field characteristics of several trigeminal units was carried out. The stimulus was light brushing of the face with a fine camel hair brush. No attempt was made to differentiate between the various type of hairs and stimulate them separately (Brown & Iggo, 1967). Figures 29, 30 and 31 are track reconstructions from 3 experiments which represent the receptive fields of several cells recorded in successive lateral penetrations 500 µm. apart in three different transverse planes on the rostro-caudal axis recorded under chloralose anaesthesia. Figure 29 shows the pattern of receptive fields recorded in the region of the nucleus oralis 3 mm. rostral to the obex. A

mediolaterally reversed, inverted somatotopy was generally observed. On making a penetration at the medial border of the nucleus the first cells encountered generally had mandibular or maxillary receptive fields. The first cells of a more lateral penetration tended to have receptive fields in the chin/nose region and these fields moved progressively to maxillary and supraorbital/ear regions at deeper levels within the nucleus. There were many exceptions to this general sequence. Receptive field sizes varied considerably from cell to cell with some units responding only to the movement of a few vibrissae and others to the whole of the ipsilateral face. Responses were obtained to sharp taps, sustained pressure or deflection of individual sinus hairs. Many cells responded to touching the sinus hair with a vibrating tuning fork (440 Hz). In common with the published results of Darian-Smith, Phillips & Ryan (1963) large receptive fields covering the whole of the ipsilateral or bilateral face were found deep within any penetration, although this is not stated explicitly in these papers. These cells tended to show a phasic response to brushing the face and often had receptive fields which included one or more fore or hind limb. The threshold for responses to facial stimulation were always lower than thresholds for stimulation of other parts of the body. All of the cells which could be activated from the anterior lobe of the cerebellum were of this type and are marked with filled symbols in figures 29, 30 and 31.

Three penetrations 1 mm. rostral to the obex at the level of the nucleus interpolaris produced the receptive field distribution presented in figure 30. The general appearance of this figure is similar to that of figure 29 at the level of the nucleus oralis. The deeper parts of these electrode tracks show several units with large receptive fields and four of these could be activated at short latency from the cerebellum. This region was of particular interest as Darian-Smith and Phillips (1964) found the majority of trigemino-cerebellar cells in or near the nucleus interpolaris. Cells with very large bilateral receptive fields were generally found beyond the second millimeter of track from the point at which trigeminal responses were first obtained.

The transverse plane of figure 31 lies 2 mm. caudal to the obex and passes through the nucleus caudalis. The generally observed pattern of somatopic projection is present with a single deep cell having a large receptive field and a cerebellar projection. These results do not suggest a longitudinal organisation of receptive fields as postulated by Wall & Taub (1962) or Darian-Smith, Proctor & Ryan (1963). The contention of Kruger et al. (1961) and Kruger & Michel (1962) that there is a similar somatotopic projection to all transverse planes of the anterior-posterior axis is upheld.

The responses of trigeminal neurones to controlled mechanical stimulation.

Having established several criteria for the discrimination of four main types of discharge originating in the sinus hair follicle an attempt was made to classify second-order cells on the basis of these criteria. In this section the responses of other types of skin and hair activated discharge were not investigated.

Of 132 units which were activated by movement of the sinus hairs only 24 (18%) could readily be described as slowly adapting, (using the criteria discussed in section II), this compared with 66% slowly adapting responses in the primary afferent sinus body nerve. These second order slowly adapting responses were designated '<u>trigeminal sinus type I or II</u>'/ (14 & 10 units respectively) on the basis of the properties of the unit discharge.

A. The discharge characteristics of trigeminal slowly adapting neurones.

Figure 32A is a successive interval plot of the discharge of a unit which showed the characteristic St I response to a sustained 10^{o} deflection of a maxillary sinus hair. The best-fitting regression line is drawn through the points. At any instant in time both long and short intervals appear in the discharge although there is a progressive increase in the mean interspike interval, as revealed by the slope of the regression line. In 5 similar units there was a tendency for this adaptation to be apparent even in relatively small samples of data (100 intervals). The discharge of another unit plotted

in a similar fashion (32B) displays the characteristically level successive interval histogram of the St II discharge with interspike intervals deviating only slightly from the mean over 100 intervals. In six other units which displayed similar properties no distinction could be made between St II(A) and St II(B) discharges at the second order level.

Interval histograms of the same unit discharges are shown in figures 32 C,D. The distribution of intervals between spikes in a cell responding to a sustained 10^{o} rostral deflection of a single vibrissa has a shape which suggests a Poisson distribution of spikes in time with a dead-time of 2 - 3 ms. (32C). The portion of this distribution between 3 and 150 ms. does not, however, fit an exponential with any degree of accuracy as might have been expected. The distribution of times between events in a random process with no dead-time should be exponential. In practice the irregular primary afferent St I discharge showed a functional refractory period of 10 - 20 ms. during which spikes were relatively rare. This period of relative inactivity is probably due to a limitation on the spike generating process imposed at the receptor terminal. A convergence of different, asynchronous inputs of various distributions onto a second-order cell could produce a random excitation of this cell with a dead-time closer to the absolute refractory period of the cell membrane, in the order of 1 ms.. Movement of a single sinus hair probably activates more than 50 slowly adapting receptors,

the convergence of which could produce such a discharge. This is equally true of the convergence of axons from several sinus hairs onto a single cell, a situation which was frequently observed. The mean frequency of discharge of the unit shown in 32C was $48 \cdot 54^{\pm}$ 83·67 with a coefficient of variation of 1·72.

In contrast to figure 32C, the interspike interval distribution of figure 32D is Gaussian in appearance although deviations from a fitted normal probability density function of the same parameters are just significant at the 5% level. The mean interspike interval of this discharge is $54 \cdot 67^{\pm}$ 8·12, with a coefficient of variation of 0·177. Cells with this discharge pattern responded in the same way to movements of other hairs, but the effects of moving several hairs simultaneously have not been studied systematically. It may be possible to regard this discharge as relatively 'pure' input from the primary afferent St II receptors when other criteria are considered.

The range of coefficients of variation in the trigeminal St I units tested (5) was from 0·92 - 1·71 and for six trigeminal St II units 0·177 - 0·76, in both cases much larger than the equivalent values for the primary afferent discharges.

<u>The adaptation of trigeminal slowly adapting responses</u>.

The adaptation behaviour of the second-order slowly adapting responses was investigated and distinctions could be drawn between the two types of discharge. Figure 33A is a graph relating the frequency of discharge of a trigeminal St I unit to time after stimulus onset.

No tests were made to show an exponential decay of firing frequency, as described in section II, and the time-constants of adaptation were not accurately measured. An overall impression was gained, however, that the rate of adaptation of the second-order discharges, especially those classified by other criteria as trigeminal St I units, was significantly faster than that of the primary afferent slowly adapting units.

The effects of anaesthetic on the rate of spontaneous discharge has been discussed previously and its effects on the rate of adaptation of second-order discharges is probably related. These effects were not tested experimentally.

Directional sensitivity

There was no difference observed between the extent and patterns of directional sensitivity in the primary and second-order slowly adapting responses when chloralose anaesthesia was employed. Two clear groups were found which showed the St I and St II patterns of directional sensitivity. The trigeminal St I group responded to deflections on one or two cardinal directions (fig. 34 A,B) and the St II group to movements in three or four cardinal directions as illustrated graphically in figures 34 C,D,E. With such a small sample of second-order slowly adapting units it is impossible to tell whether

there is any significant grouping of cells with a preferential directional sensitivity.

Order-dependent properties of the slowly adapting discharge.

As in section II a more detailed investigation of the slowly adapting responses was made by consideration of the order-dependent properties of the discharge under chloralose anaesthesia. Figure 35 A,C are the joint interval density plots of trigeminal St I and St II discharges previously identified on the basis of discharge pattern. Figure 35A demonstrates the typically tight clustering of the St II discharge about the common mean of both axes. This particular discharge was the most regular of trigeminal St II responses recorded and shows a degree of scattering which one would associate with the primary afferent St II(B) discharge (see section II fig. 8B,C). It was not assumed that the less common St II(B) discharge excited all the second-order St II cells observed. The j.i.d. plot of figure 35C shows the typically random scattering of points associated with the St I discharge where no prediction can be made as to the length of any interval (T_{I+1}) based upon knowledge of the previous interval (T_I).

Figure 35B is the serial correlogram of 200 intervals of the St II discharge plotted in the adjacent j.i.d. diagram. The changing serial correlation coefficients of 50 orders of lag show some evidence of cyclical activity as in the St II correlograms of section II, figures 9B,C. However, in this case there was no significant serial correlation coefficient, even at the first order of lag -

a measure of the 'cohesion' of the j.i.d. diagram which is quite noticeable. The trigeminal St I serial correlogram of figure 35D shows no significant deviations from zero and no sign of increasingly positive serial correlations at high orders which would occur if adaptation were having a significant effect on the data (Perkel etal., 1967a). These results were typical of 7 other units tested in this way.

Quantitative investigations of the slowly adapting responses to controlled movements of the vibrissae.

Controlled mechanical displacements were applied to sinus hairs which activated 7 slowly adapting cells of the trigeminal sensory complex. A limited range of controlled movement amplitudes was available ($0-4^{o}$) but considerable variation was possible in delivering movements at different velocities from rise times of less than 50 ms. to more than a second.

Figure 36A illustrates the stimulus-response relationships of 4 units to manual deflections of the hair through varying angles from $5 - 30^{o}$. The response was measured over the first 20 intervals of the static phase and plotted as a logarithm against the logarithm of the stimulus, corresponding to the power function $R = A. S^{b}$. Comparing this figure with figure 19 of section II a great similarity can be seen between the two sets of lines. Power function exponents vary from 0·16 - 0·89, within the ranges of exponents observed in the primary afferent responses. No distinction can be drawn between the two St I and two St II units plotted in this figure (see legend).

The dynamic responses of 5 units are plotted in figure 35B and may be described by power functions with exponents in the range 0·26 - 0·41, again, within the range observed for the primary afferent dynamic responses. The statistical parameters of these lines are given in table IV.

B. Rapidly adapting units.

Most (108) second-order trigeminal units which were activated by movements of the maxillary sinus hairs gave only a rapidly adapting response. Of these about 45% could be readily entrained by touching the hair with a vibrating tuning fork (A; 440 Hz). These were assumed to be units activated by the HVRA units of the maxillary sinus hair follicle, as no other primary afferent units showed this response. No further investigations were made of these units as the sinewave generator was no longer available to drive the electromechanical stimulator at high frequencies. It was not possible to identify cells of the trigeminal sensory complex which were specifically activated by the LVRA units of the sinus hair follicle. Phasic responses to low velocity movements were common but could have arisen from activation of the second-order cell by any of the four main types of sinus body afferent unit with a subsequent attenuation of the response by pre or post-synaptic inhibitory processes within the neucleus. The receptive fields of such units were not noticeably different from either the slowly adapting units or those rapidly adapting units which could be entrained by vibratory stimuli.

The special case of those rapidly adapting cells with a projection to the anterior lobe of the cerebellum is considered below.

Cells of the trigeminal sensory complex which could be activated from the anterior lobe of the cerebellum.

The records shown in figure 37 are photographs of spike responses to electrical stimulation of the cerebellar cortex. Of 42 units which could be activated from the anterior lobe 31 were activated with a latency of less than 2 ms. and were taken to have a direct projection to the anterior lobe.+ In the region of nucleus interpolaris 22 trigemino-cerebellar units were found, 16 of which could be directly activated. This ratio is probably not representative of the true distribution of trigemino-cerebellar cells along the rostro-caudal axis of the brain-stem trigeminal sensory complex. There was a bias in the sample weighted around the obex in the region of the nucleus interpolaris.

The most commonly encountered discharge evoked by stimulation of the cerebellum is illustrated in figure 37A where a single spike followed the stimulus with a latency of 0·75 - 2·0 ms. (13 cells). Figure 37B shows the discharge pattern evoked in 6 cells where a burst of activity with a latency to the first spike of 1 - 2 ms. and an intra-burst frequency of 500 - 800 impulses/sec. Many units were activated with latencies of longer than 2 ms. (figs. 37 C,D) and, in agreement with Darian-Smith & Phillips (1964), these cells were judged to be trans-synaptically activated. Those cells which could be

+ This simple criterion is used in accordance with Darian-Smith & Phillips(1964) who used collision to verify antidromic activation and found cells which discharged with latencies of less than 2 ms. to be so activated.

antidromically activated from the cerebellum with a latency of less than 2 ms. were regarded as forming part of a direct cerebellar projection from the trigeminal sensory complex.

The distribution of latencies of activation of these cells from both the ipsilateral face and the anterior lobe of the cerebellum are plotted as histograms in figure 38 for those responses recorded in the region of the nucleus interpolaris. For comparison, the latencies of those cells in the same region which DID NOT have a cerebellar projection are plotted in figure 38A with a mean latency of 5·08 ms. and a mode of 3·5 ms. The mean latency of directly activated trigemino-cerebellar cells to facial stimulation was 5·47 ms. (fig. 38B), the mode 3·5 - 4·0 ms. Although there is no statistical difference between these values no trigemino-cerebellar cell was observed with a latency from the ipsilateral facial skin of less than 2·5 ms., whereas several non-trigemino-cerebellar cells had latencies of 2 ms. or less. The latencies of all trigemino-cerebellar cells in the region of the obex to electrical stimulation of the anterior lobe of the cerebellum is presented as a histogram in figure 38C. The mean latency of activation is 1·6 ms. with a mode of 1·3 ms. The five cells in this histogram which have latencies of longer than 2 ms. are regarded as having been trans-synaptically activated. At high intensities of stimulation other cells could be activated at very long latencies ($>$30 ms), these were regarded as functionally unrelated and have been ommitted from the results.

The functional properties of trigemino-cerebellar cells.

Referring back to figures 29, 30 and 31 all units marked with a filled symbol on the diagrams are those which had a direct projection to the anterior lobe of the cerebellum. As previously stated these cells had receptive fields which were very large; always covering the whole of the ipsilateral face, in all but one instance the whole of the contralateral face as well and in all but two cases one or more limb and parts of the trunk. The afferent information projecting to these cells is therefore highly convergent but it was observed that the thresholds of responses to mechanical stimulation of the face were invariably lower than to stimulation of other parts of the body.

All of the 31 trigemino-cerebellar cells investigated gave phasic responses of 1 - 3 spikes to either sharp tap or slow, sustained deflections of the sinus hairs. In 4 cells which carried a spontaneous discharge the effect of mechanical stimulation was a phasic burst of increased activity involving 1 - 3 spikes. No inhibitory effects were observed following mechanical or electrical stimulation of the face but one cell had an inhibitory field on the contralateral hind limb. There was a noticeable latency shift when mechanical rather than electrical stimulation was employed. In 12 cells where measurements were taken no mechanically excited response showed a latency of less than 4 ms. This suggests that the summation of excitatory input requires a finite period of time, probably in the order of 1 - 2 ms.

The location of trigemino-cerebellar cells.

As can be seen in figures 29, 30 and 31 the locations of trigemino-cerebellar cells were always in ventral regions of electrode tracks passing through the spinal trigeminal nucleus. On four occasions the absolute locations of these cells were determined by the electrophoretic deposition of pontamine-sky-blue into the recording area and locating the mark on subsequent histological preparations. An example of such a preparation is given in figure 47A which is a photomicrograph of a stained transverse section through the region of nucleus interpolaris. In all cases the location of the recording site was found to be within the lateral reticular nucleus, ventromedial to the cell mass of the spinal trigeminal nucleus.

The highly convergent nature of the cutaneous responses of trigemino-cerebellar units and their long latency, suggests, as does the histological evidence, that these units are neurones of the lateral reticular nucleus and form part of a trigemino-reticulo-cerebellar pathway.

DISCUSSION

The most persistent argument in connection with the afferent input to the spinal trigeminal nucleus concerns the distribution of modalities along the rostro-caudal axis (for example, Darian-Smith, 1973; Mosso & Kruger, 1973). In the present series of experiments no systematic rostro-caudal variation of receptive field sizes was observed throughout the brain-stem trigeminal sensory complex and the distribution of responses to light mechanical stimulation was uniform along its longitudinal axis. A conclusion to be drawn from this is that the information necessary for fine touch discrimination is transmitted to the three morphologically distinct subnuclei; caudalis, interpolaris and oralis (Olszewski, 1950; Eisenman et al., 1963). Whether or not this information is utilised in fine touch discrimination or whether noxious and thermoreceptive afferents project to all regions of the spinal nucleus has yet to be established. The electrically and mechanically evoked discharges of this group of trigeminal cells have latencies which indicate that there are both mono and polysynaptically activated neurones which respond to low intensity mechanical stimulation of the face. No differences could be found between the latencies of cells with rapidly and slowly adapting responses.

The projections of mechanoreceptive afferents originating in the maxillary sinus bodies to the spinal trigeminal nucleus is also uniform on the rostro-caudal axis.

The great majority of these responses were rapidly adapting (108) and of these about 45% were able to follow high frequency vibratory stimuli (tuning fork), suggesting that they were excited by the HVRA units found in the infraorbital nerve and probably originating from the sinus hair follicle Golgi-Mazzoni corpuscles described by Andres (1966).

Although much reduced in number, the essential features of the primary afferent slowly adapting discharges were preserved in the responses of second-order cells of the trigeminal sensory complex. It was possible in these experiments to establish two categories of slowly adapting trigeminal discharges which showed substantially different interspike interval distributions, which displayed different types of directional sensitivities and which differed greatly in their adaptive properties. The possibility of a convergence of non-random input to a single second-order cell producing an irregular St I-like discharge has been considered. The generally shorter dead-times and greater variability of these discharges suggests that this may be the case. However, the directional sensitivities and adaptive properties of these second-order discharges present clear evidence to suggest that the cells involved receive excitation from the slowly adapting St I discharge of the sinus hair follicle. A close study of the distributions of interspike intervalsof these responses reveals that in both groups there is an overall increase in variability compared with the equivalent groups of primary slowly adapting responses. It is

probable, then, that even where there is a single source of excitation the synapse(s) between the primary axon and the second order cell introduces 'noise' into the transmission of impulses to higher levels of the nervous system. From the standpoint of information theory this noise could be the result of pre- or post-synaptic interaction between afferent input from different sources (Braitenberg, 1965) or a highly organised descending 'control' from other parts of the nervous system. The degradation of the afferent input is particularly evident in the trigeminal St II discharge which consistently displays a degree of variability 10 - 20% greater than the St II(A) discharge of the primary axon. Further evidence of this is revealed by investigations of serial correlation within the unit discharges. The trigeminal St II discharge does not display the strong interdependence between adjacent and non-adjacent interspike intervals always found in the primary St II(A) discharge. The cyclical sequence of dependency appears to be broken by the introduction of synaptic noise rather like the less common primary St II(B) discharge which displays a similar degree and type of irregularity and which may be due to a multiple or 'noisy' generator region, as discussed in section II. It is difficult to assess the effects of impinging noise upon the already irregular St I discharge although in this small sample of cells the variability of the discharge relative to the mean rate of activity was greater than in the primary St I discharge.

The time-constants of adaptation of these secondary St I discharges were also shorter, suggesting some tonic or recurrent inhibitory control of activity. The effects of anaesthetic, however, may be considerable.

It is important to consider these observations in relation to the ability of all slowly adapting receptors so far described to respond differentially to graded levels of stimulation (Werner & Mountcastle, 1965; Iggo & Muir, 1969; Chambers et al., 1972). The stimulus-response functions of the second order slowly adapting neurones investigated in this study do not differ significantly from those described for the primary afferent responses of section II. The increased variability of the second-order responses must mean, however, that the degree of discrimination between responses of a single cell is reduced and that the information content of the discharge is degraded in crossing the synapse(s). If this increase in variability is in the order of 20% (page 30) then the loss of information is probably of the same order, a fact tentatively established by other methods in cells of the trigeminal nucleus (Darian-Smith et al., 1968) and axons of DSCT (Walloe, 1970). Darian-Smith et al. (1968) proposed that this information loss was greatest in the nucleus caudalis and so excluded this nucleus from participation in the function of fine tactile discrimination. Among cells examined in this series of experiments the variability and responsiveness of cells appeared uniform over the whole of the spinal nucleus. This proposition must therefore be rejected,

at least for those slowly adapting responses which originate in the maxillary sinus bodies.

Gordon et al. (1961) found cells in nucleus caudalis with restricted receptive fields and low thresholds to mechanical stimulation which projected to the arcuate nucleii of the contralateral thalamus ('A' cells). Darian-Smith, Proctor & Ryan(1963) found similar cells in the nucleus oralis and the main sensory nucleus. In both cases the assumption was made that these cells formed part of a 'medial lemniscal' projection, ultimately to the somatosensory cortex. The participation of sinus hair follicle afferents in this pathway is possible, given that these responses project to all levels of the nucleus. The region of the nucleus interpolaris has been described as 'medial lemniscal' by Wall & Taub (1962) and as part of a trigemino-cerebellar pathway by Darian-Smith & Phillips (1964). The results of this section suggest that the former is possible, but no more likely than a 'medial lemniscal' projection from any other part of the nucleus. The results of Darian-Smith & Phillips (1964) may be explained by the dorsal upswing of the spinal trigeminal nucleus at the level of the nucleus oralis, away from the underlying lateral reticular nucleus which follows a horizontal course pointing deep into the pons along the main tracts of the reticular formation. At the level of the obex the nucleus interpolaris is immediately dorsal to the lateral reticular nucleus which tapers to a small cross-sectional area at the level of the nucleus caudalis (Brodal, 1953). The chance of an electrode

track passing through the lateral reticular nucleus will therefore be greatest if this track also passes through the nucleus interpolaris. In this series of experiments all trigemino-cerebellar cells were in the location of, and showed the properties of, cells of the lateral reticular nucleus.

The involvement of this nucleus in a spino-reticulo-cerebellar pathway has long been established (Blakeslee et al., 1938; Grant et al., 1966) and Brodal (1953) regarded this nucleus as an important relay in the transmission of tactile information to the cerebellum. The functional properties of the cells of this nucleus are dominated by the great convergence of excitatory and inhibitory imput from most parts of the body, from glabrous and hairy skin and from all types of mechano-receptors, in addition to group II and III muscle and joint afferents. There is no somatotopic organisation within the nucleus in the sense that receptive fields are extensive and overlapping. Input from the maxillary sinus hair follicles to the nucleus conforms to this general scheme except that no inhibitory effects were observed following facial stimulation. Rosen & Scheid (1973a,c) found evidence of tonic inhibitory action by cutaneous mechanoreceptors on the activity of cells of the lateral reticular nucleus. This has not been confirmed in this series of experiments. They point out, however, (Rosen & Scheid, 1973c) that tonic inhibition is rare in the anaesthetised preparation and much more evident in decerebrate animals. The absence of this response to

sustained deflection of the sinus hairs may be due to the compounded effects of descending inhibition and anaesthetic on the tonic activity driven by sinus body slowly adapting mechanoreceptors. Alternatively, the sinus hair follicle afferents may provide specifically phasic input to the nucleus.

Axons of cells within the lateral reticular nucleus have been identified as forming the bilateral ventral flexor reflex tract which projects as mossy fibres to the cerebellar cortex (Lundberg & Oscarsson, 1962; Oscarsson & Rosen, 1966; Rosen & Scheid, 1973b) where a late (7 - 30 ms. from forelimb) Purkinje cell response may be evoked (Eccles et al., 1972b). The lack of modality specificity and crude spatial organisation of discharges in this tract has suggested to Oscarsson (1967a) that information relating to the levels of excitation and inhibition of segmental interneurones are transmitted by this pathway. A powerful input from the maxillary vibrissae might indicate their role in postural and orientation reflexes associated with searching and feeding behaviour.

The nature of this sinus hair follicle projection to the cerebellar cortex is considered in section IV.

Fig. 24. A map of the area of penetrations on the dorsal surface of the medulla which produced a 'trigeminal' response. Distances are relative to the obex and the mid-line. Concentric circles at some locations represent penetrations at the same co-ordinates in different experiments. Pooled from 28 experiments. The approximate positions of the trigeminal subnucleii are:-

n Oralis;	2·5 - 7 mm. rostral
n Interpolaris;	1 mm. caudal - 2·5 mm. rostral
n Caudalis;	3 mm. caudal - 1 mm. caudal.

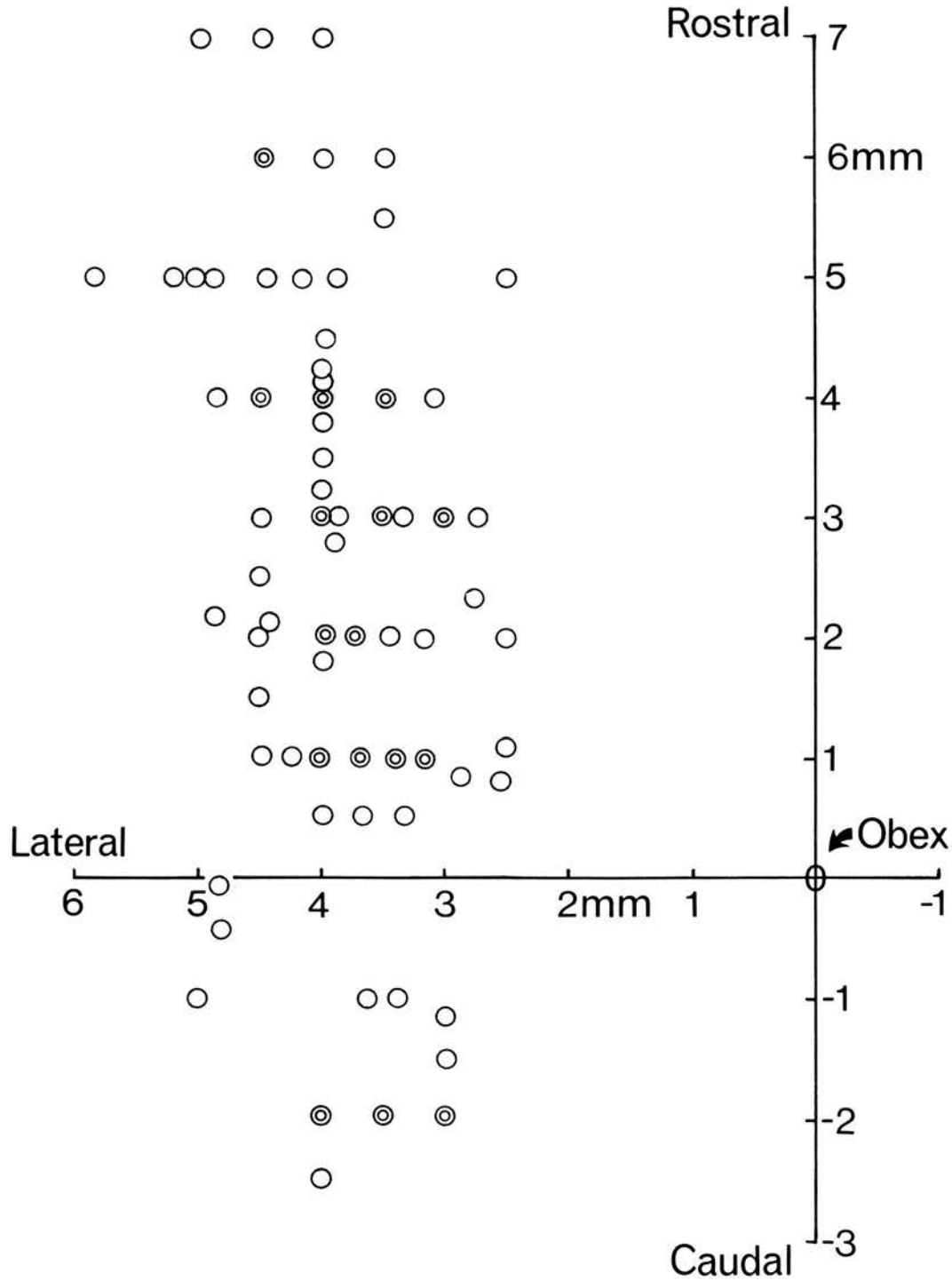

Rostral
7
6mm
5
4
3
2
1
Obex
-1
-2
-3
Caudal
Lateral
6
5
4
3
2mm
1
-1

Fig. 25. Spontaneous activity of cells of the trigeminal sensory complex. The distribution intervals over 250 ms. is represented by interval histograms with a bin width of 1 ms. Note the difference in scaling of the vertical axes in different units.

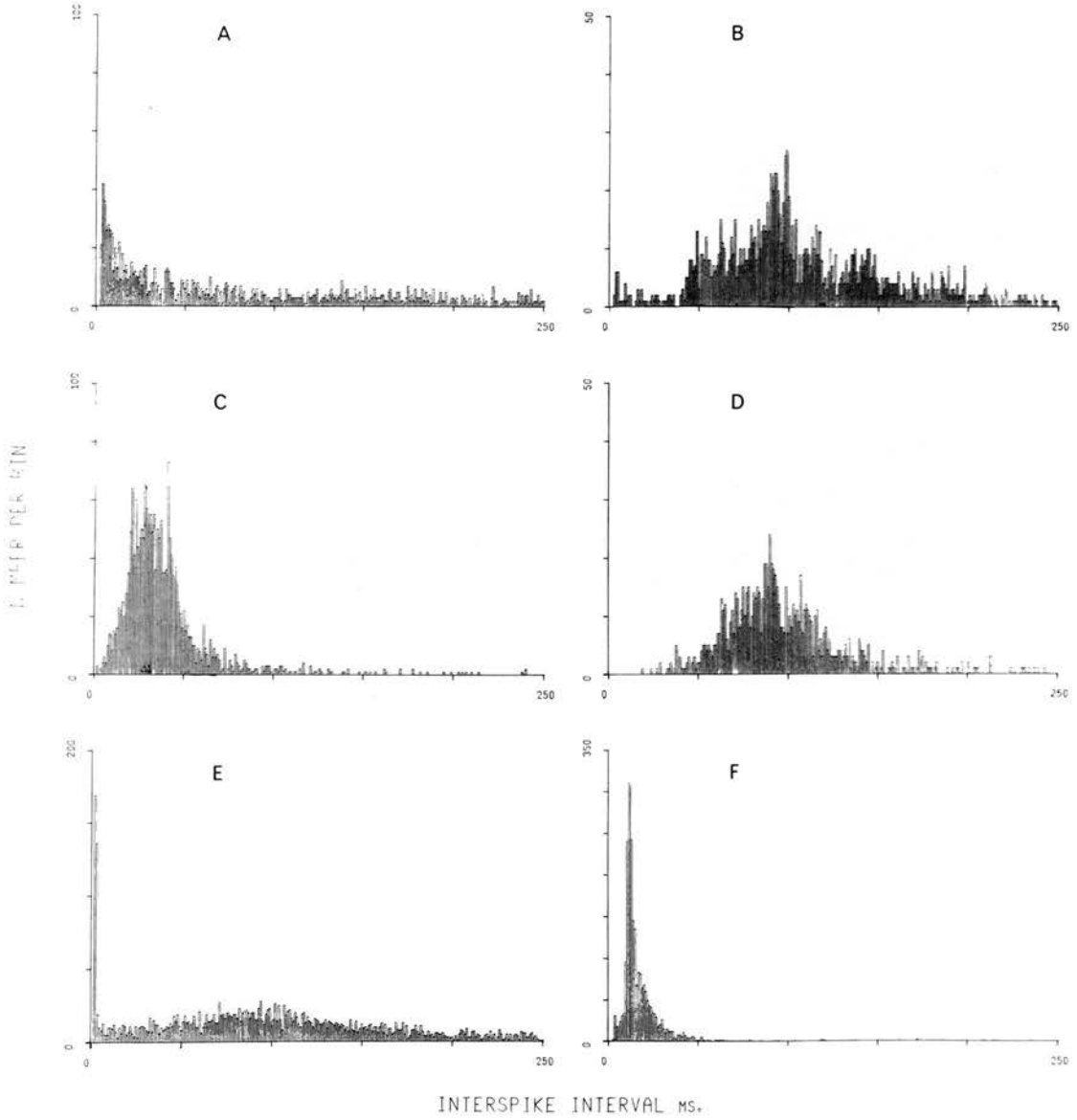

A
B
C
D
E
F
0
250
INTERSPIKE INTERVAL MS.

Fig. 26. Responses of trigeminal cells to electrical stimulation of the skin of the face at the base of the vibrissae. Microelectrode recordings, photographed from the oscilloscope screen at different amplifications. The time-base for each trace is given separately.

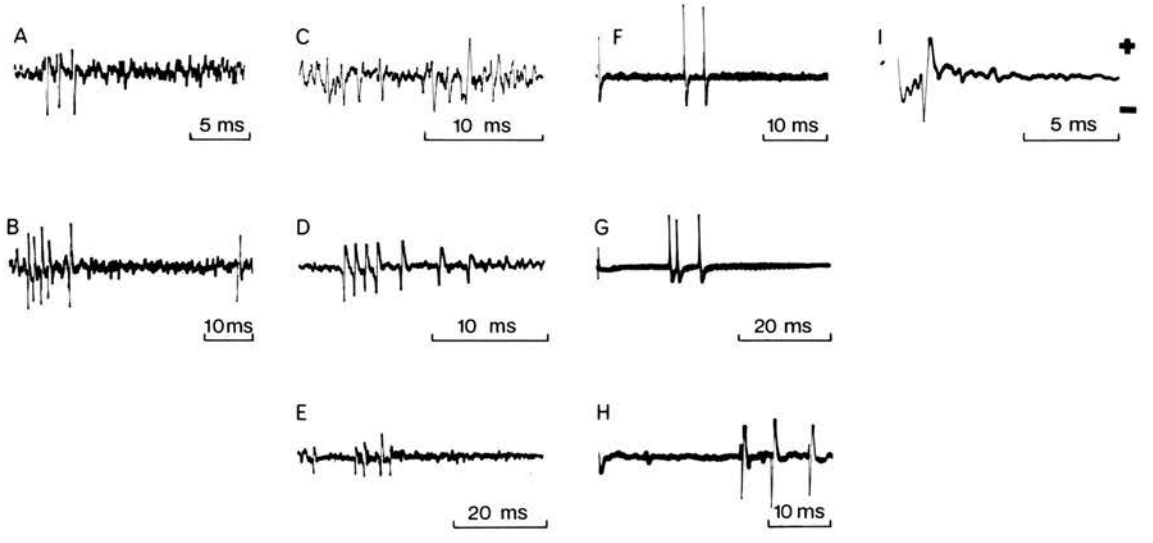

A
5 ms
C
10 ms
F
10 ms
I
5 ms
B
10ms
D
10 ms
G
20 ms
E
20 ms
H
10 ms

Fig. 27. The distribution of impulses in time following repetitive stimulation of the skin of the face. Post-stimulus-time histograms (PSTH) of three different units are given, showing commonly encountered patterns of repetitive discharge.

A A single burst of 3 - 4 impulses.

B A single spike followed by a burst of 3 - 4 impulses.

C Two bursts of 3 - 4 impulses.

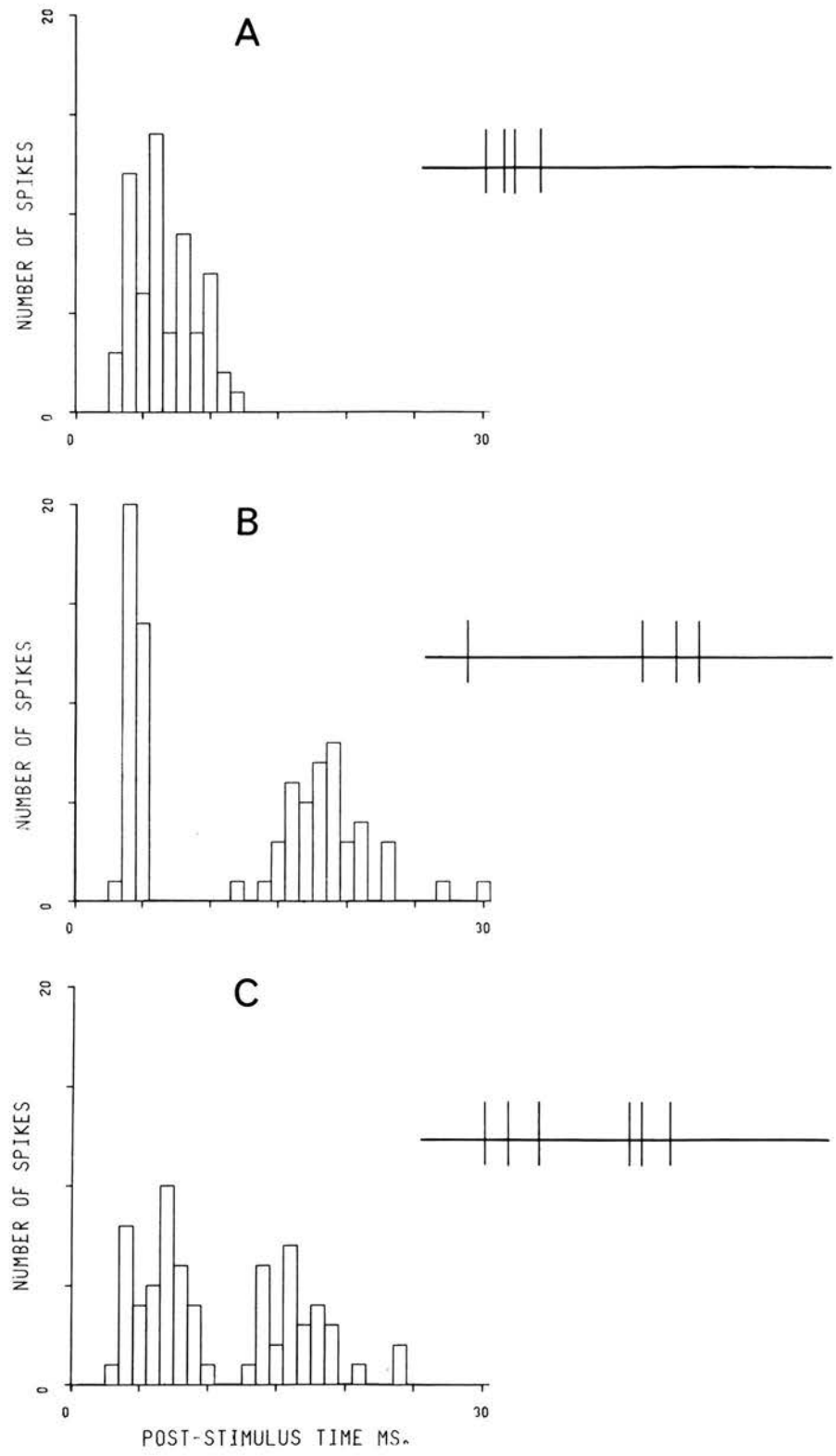

A
B
C
NUMBER OF SPIKES
20
0
30
POST-STIMULUS TIME MS.

Fig. 28. The distribution of the latencies to the first spike of trigeminal cell responses evoked by electrical stimulation of the skin of the face. All recordings were made at the level of the nucleus interpolaris near the obex.

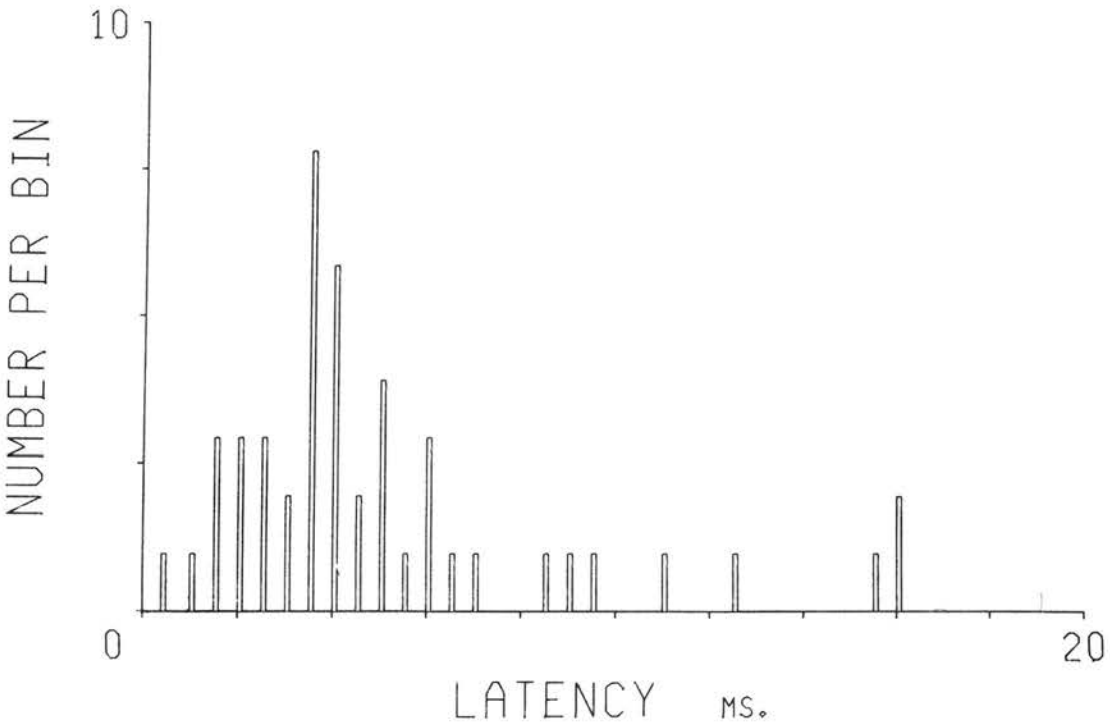

10
NUMBER PER BIN
0
20
LATENCY MS.

Fig. 29. The relative positions and receptive fields of neurones in a transverse plane passing through the nucleus oralis 3 mm. rostral to the obex. Vertical distances within any electrode track are measured relative to the point at which the first trigeminal response was obtained, not the dorsal surface of the brain stem. Overlapping portions of receptive fields appear as darker areas. Neurones marked with a filled symbol are those which could be activated at short latency from the anterior lobe of the cerebellum.

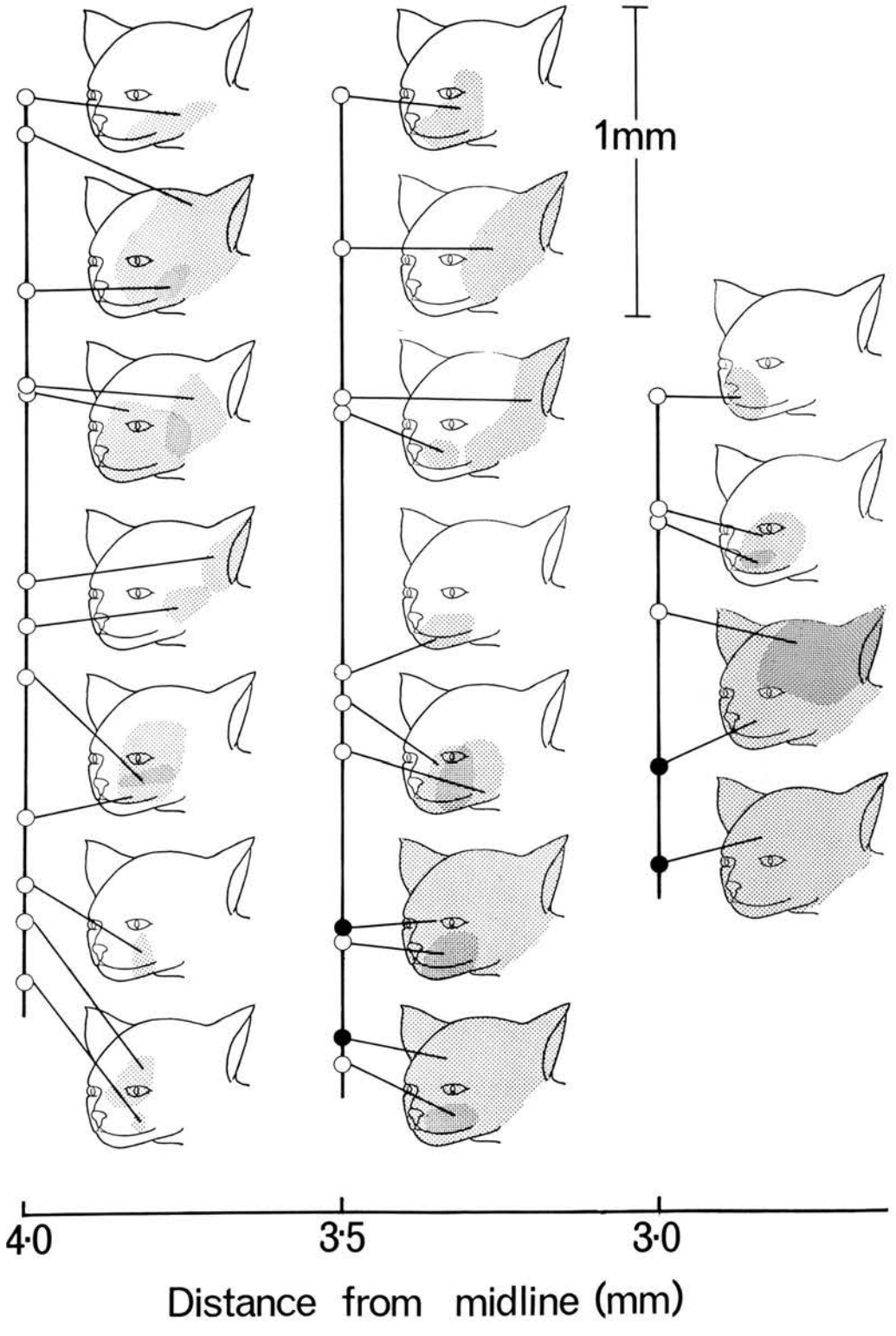

1mm
4·0
3·5
3·0
Distance from midline (mm)

Fig. 30. The relative positions and receptive fields of neurones in a transverse plane passing through the nucleus interpolaris 1 mm. rostral to the obex. Neurones marked with a filled symbol are trigemino-cerebellar cells.

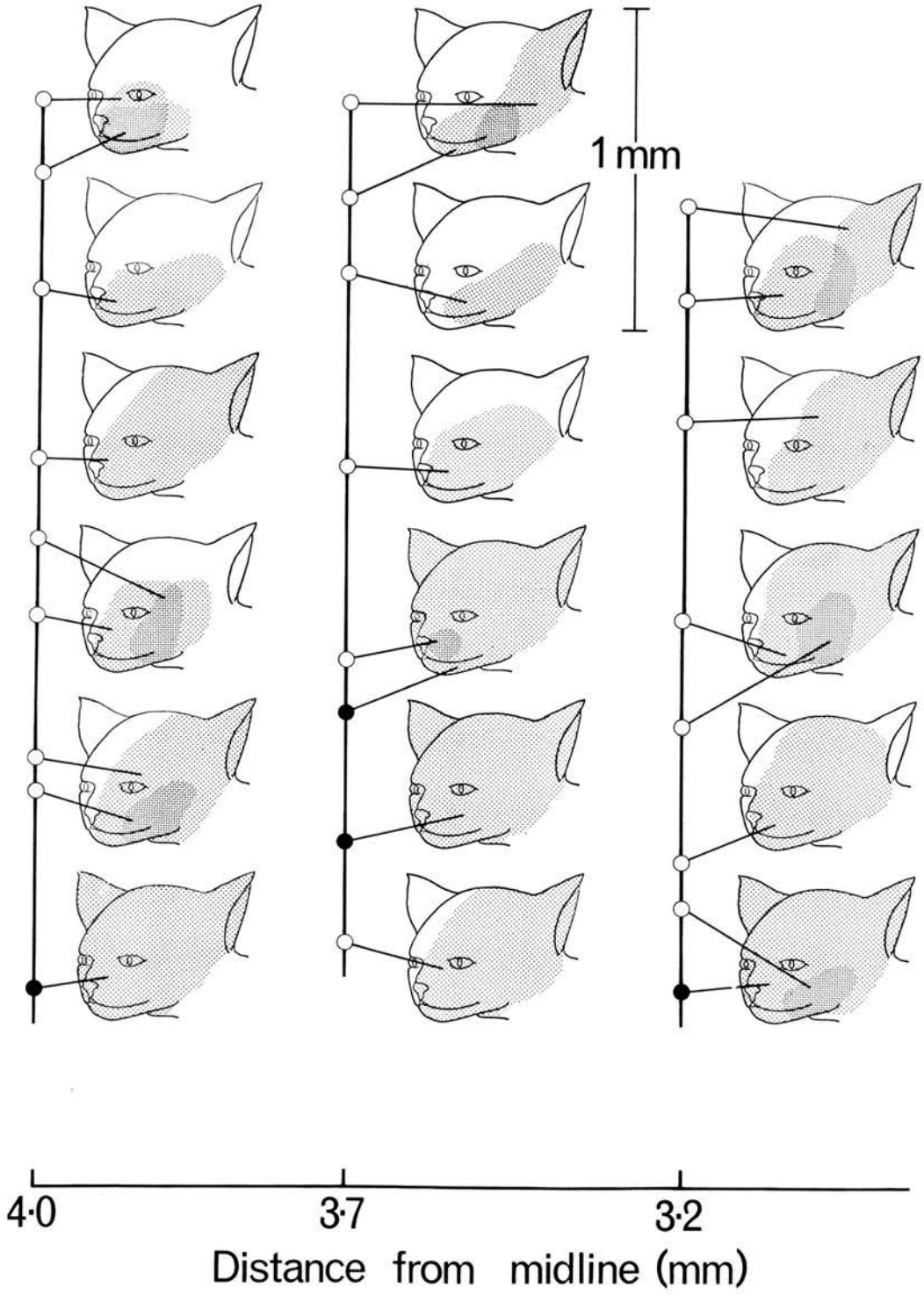

1 mm
4·0
3·7
3·2
Distance from midline (mm)

Fig. 31. The relative positions and receptive fields of neurones in a transverse plane passing through the nucleus caudalis 2 mm. caudal to the obex. The neurone marked with a filled symbol is a trigemino-cerebellar cell.

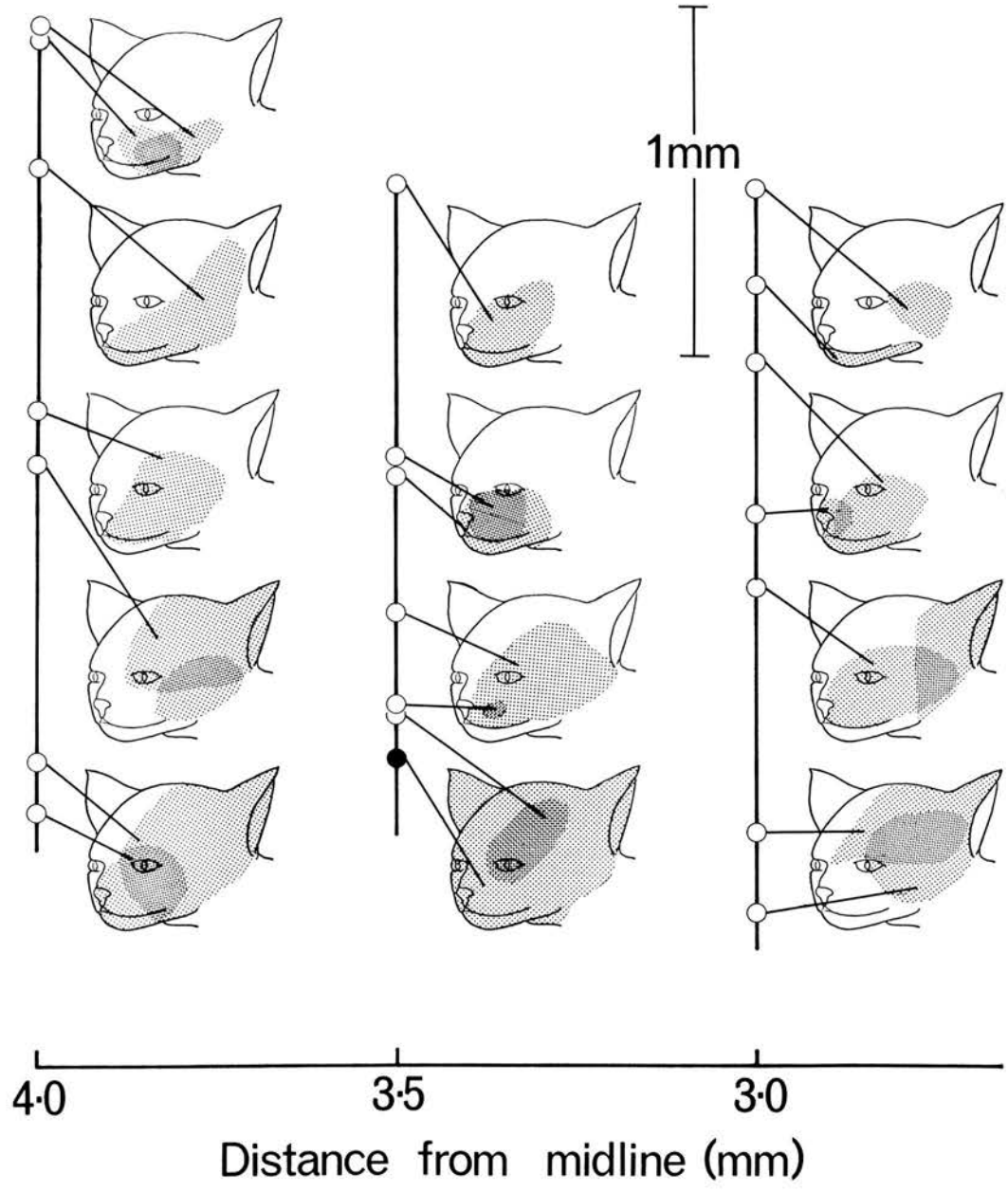

1mm
4·0
3·5
3·0
Distance from midline (mm)

Fig. 32. The discharge patterns of trigeminal slowly adapting cells.

A,B successive interval histograms where each interspike interval is plotted against the position of that interval in the spike train.
A, St I discharge. B, St II discharge.

C,D interspike interval histograms of bin width 1 ms. illustrating the distribution of interval lengths.
C, trigeminal St I discharge with a superimposed exponential function (solid line).
D, trigeminal St II discharge with a superimposed gaussian function of the same parameters.

Note that all graphs are plotted on different scales.

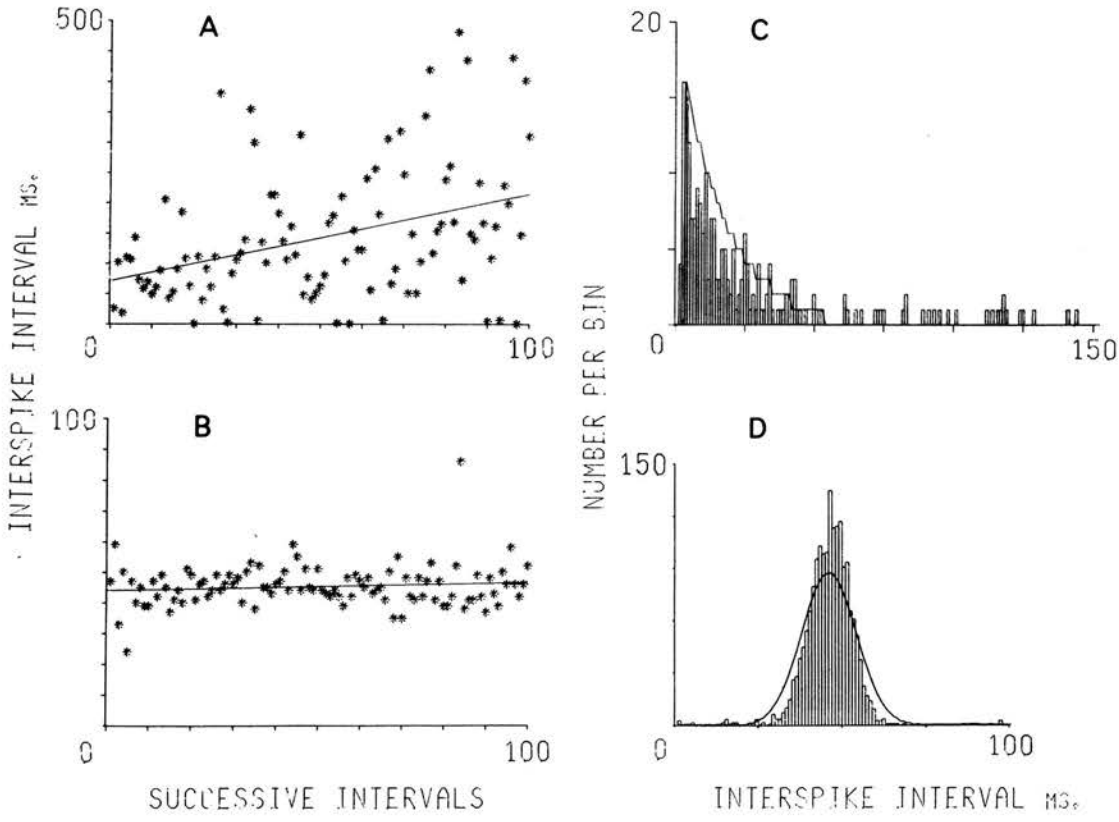

A
B
C
D
500
0
100
100
0
20
0
150
150
0
100
INTERSPIKE INTERVAL MS.
SUCCESSIVE INTERVALS
NUMBER PER BIN
INTERSPIKE INTERVAL MS.

Fig. 33. Adaptation of the trigeminal slowly adapting response. The frequency of discharge from the onset of a 20° sustained deflection (↑) of the sinus hair is measured over successive periods of 500 ms. and plotted in impulses per second against time.

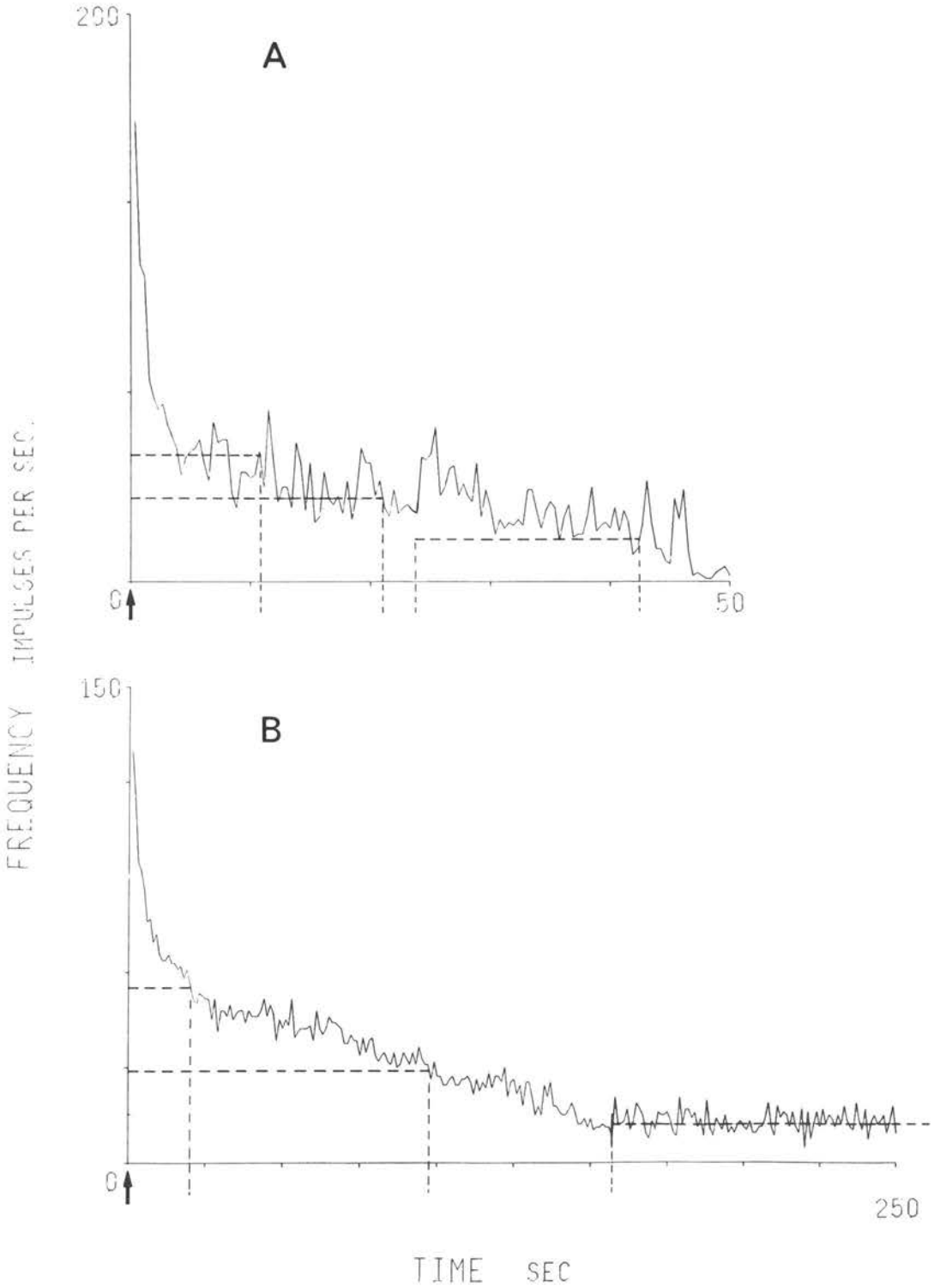

200
A
0
50
150
B
0
250
FREQUENCY IMPULSES PER SEC.
TIME SEC

Fig. 34. The directional sensitivity of trigeminal slowly adapting neurones. The frequency of discharge in response to movements of vibrissae in each of the four cardinal directions (Rostral, Caudal, Dorsal, Ventral) is plotted on axes representing these directions. The hair in the resting position would extend vertically from the page at the origin of each graph.

A,B trigeminal St I units.

C,D,E trigeminal St II units.

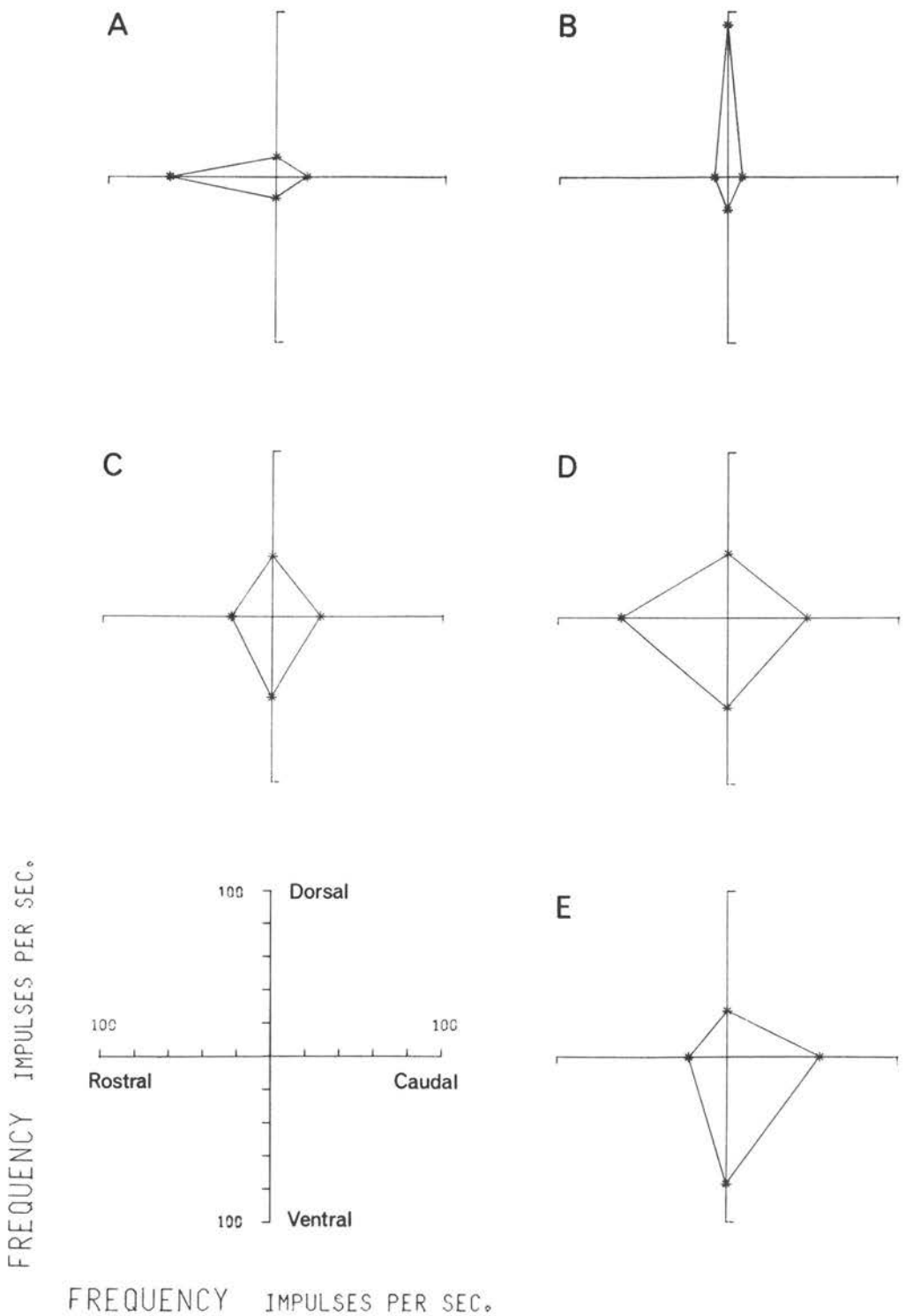

A
B
C
D
E
100
Dorsal
100
100
Rostral
Caudal
100
Ventral
FREQUENCY IMPULSES PER SEC.
FREQUENCY IMPULSES PER SEC.

Fig. 35. The order-dependent properties of the trigeminal slowly adapting discharge. A,C joint interval density diagrams of St II and St I discharges respectively. Each interspike interval (T_1) is plotted against the succeeding interval in the spike train (T_{1+1}). A random scattering of points as in C indicates no interaction between adjacent interspike intervals, where there is interaction a patterned distribution of points as in A is evident.
B,D serial corellograms of 50 orders of lag for St II and St I discharges respectively. The dashed lines mark the 5% significance limits of departures of the serial correlation coefficient from zero.

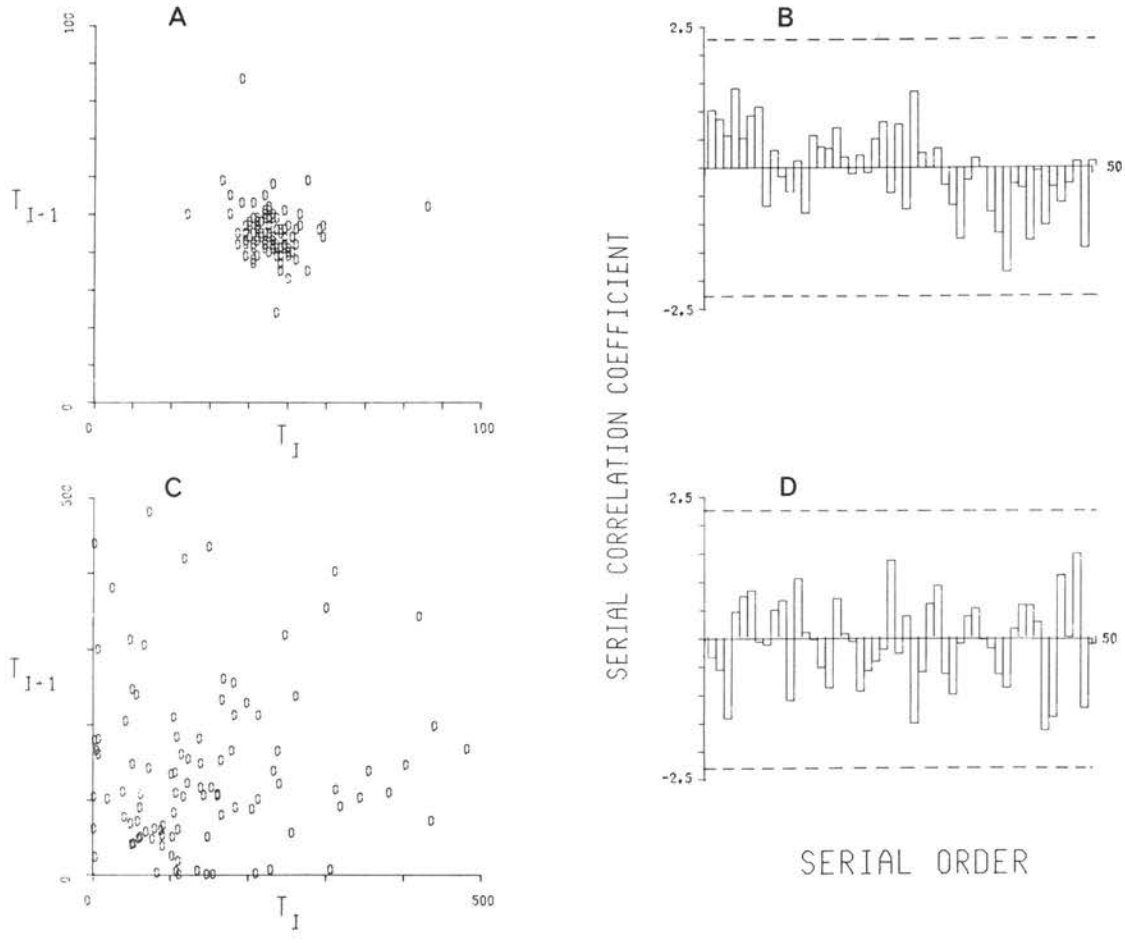

A
B
C
D
100
0
100
T_{I-1}
T_I
2.5
-2.5
50
500
SERIAL CORRELATION COEFFICIENT
SERIAL ORDER

Fig. 36. The responses of trigeminal slowly adapting units to controlled movements of the vibrissae.

A, the responses of four cells to manually applied deflections of the vibrissae from 5 - 30°. The response is measured in impulses per second over the first 20 interspike intervals following the completion of movement.

B, the responses of five cells to movements of 3° applied at different velocities from 30 - 360°/sec. In both A and B the values of stimulus and response are plotted as logarithms corresponding to the power function $R = A.S^b$ where the straight line relationship is log $R = \log A + b.\log S$.

The statistical parameters of these lines are given in table IV.

The symbol codes are:-

A; St I (●,+), St II(▼,O)

B; St I (O,●), St II(▼,+,X)

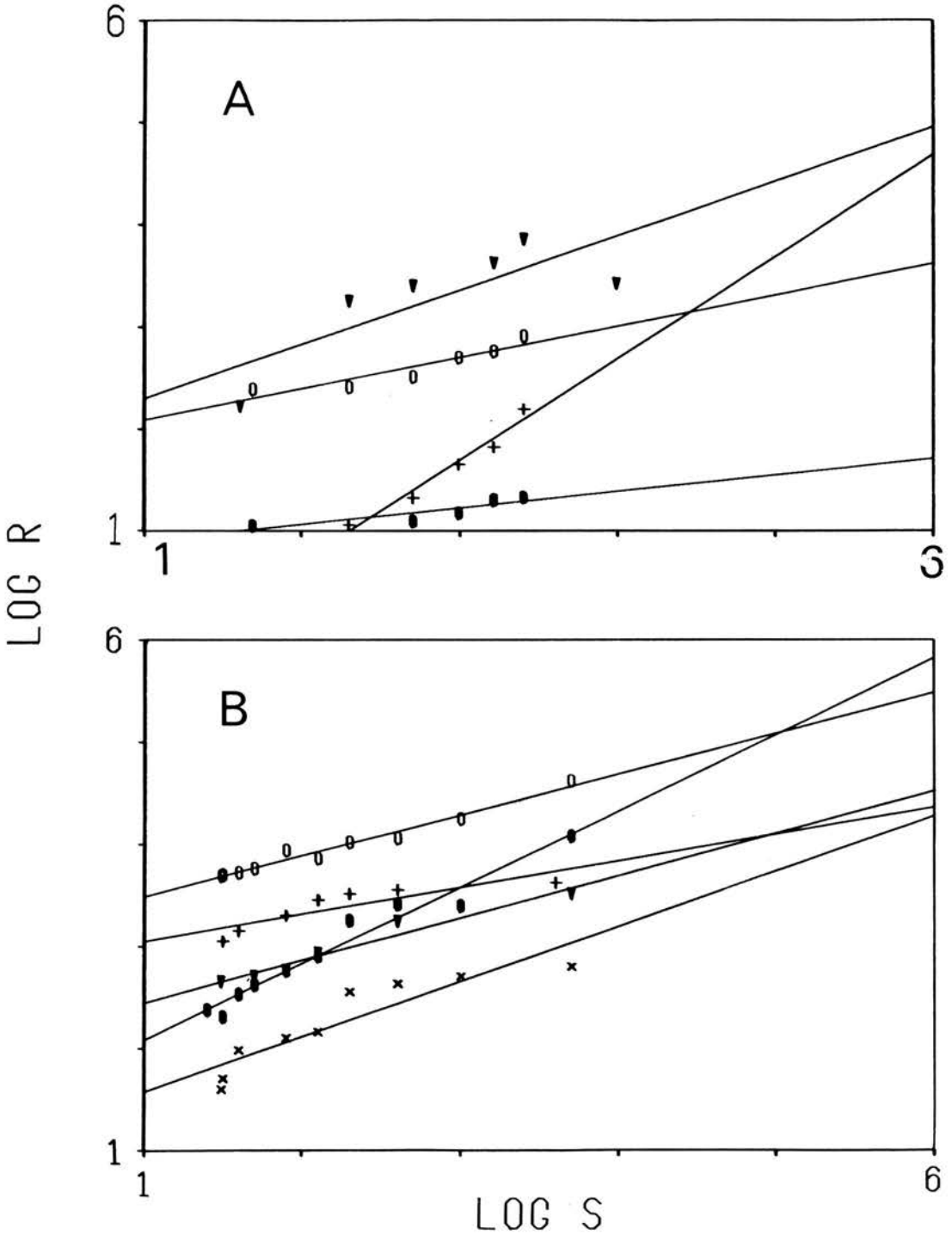

6
A
1
1
3
LOG R
6
B
1
1
6
LOG S

Fig. 37. The discharges of trigemino-cerebellar cells photographed from the oscilloscope screen. A,B discharges activated by electrical stimulation of the anterior lobe of the cerebellum with a latency of less than 2 ms. C,D discharges similarly evoked but with latencies greater than 2 ms. and probably trans-synaptically activated.

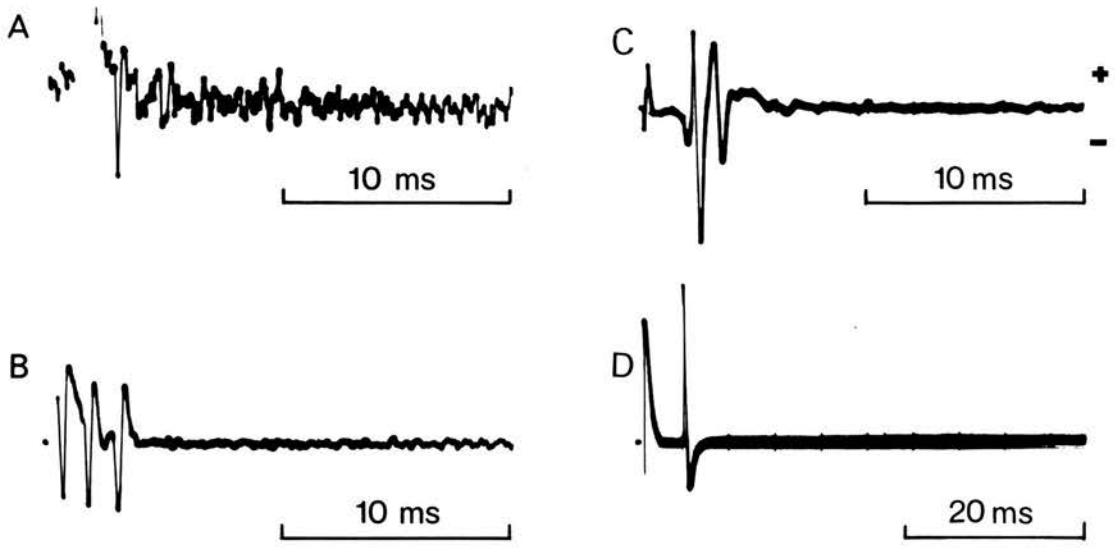

A
10 ms
B
10 ms
C
+
-
10 ms
D
20 ms

Fig. 38. The distribution of latencies of excitation of trigemino-cerebellar cells recorded at the level of the obex.

A, the distribution of latencies of cells without a cerebellar projection to electrical stimulation of the face.

B, the distribution of latencies of trigemino-cerebellar cells to the same stimulus.

C, the distribution of latencies of response to a stimulus applied at the anterior lobe of the cerebellum.

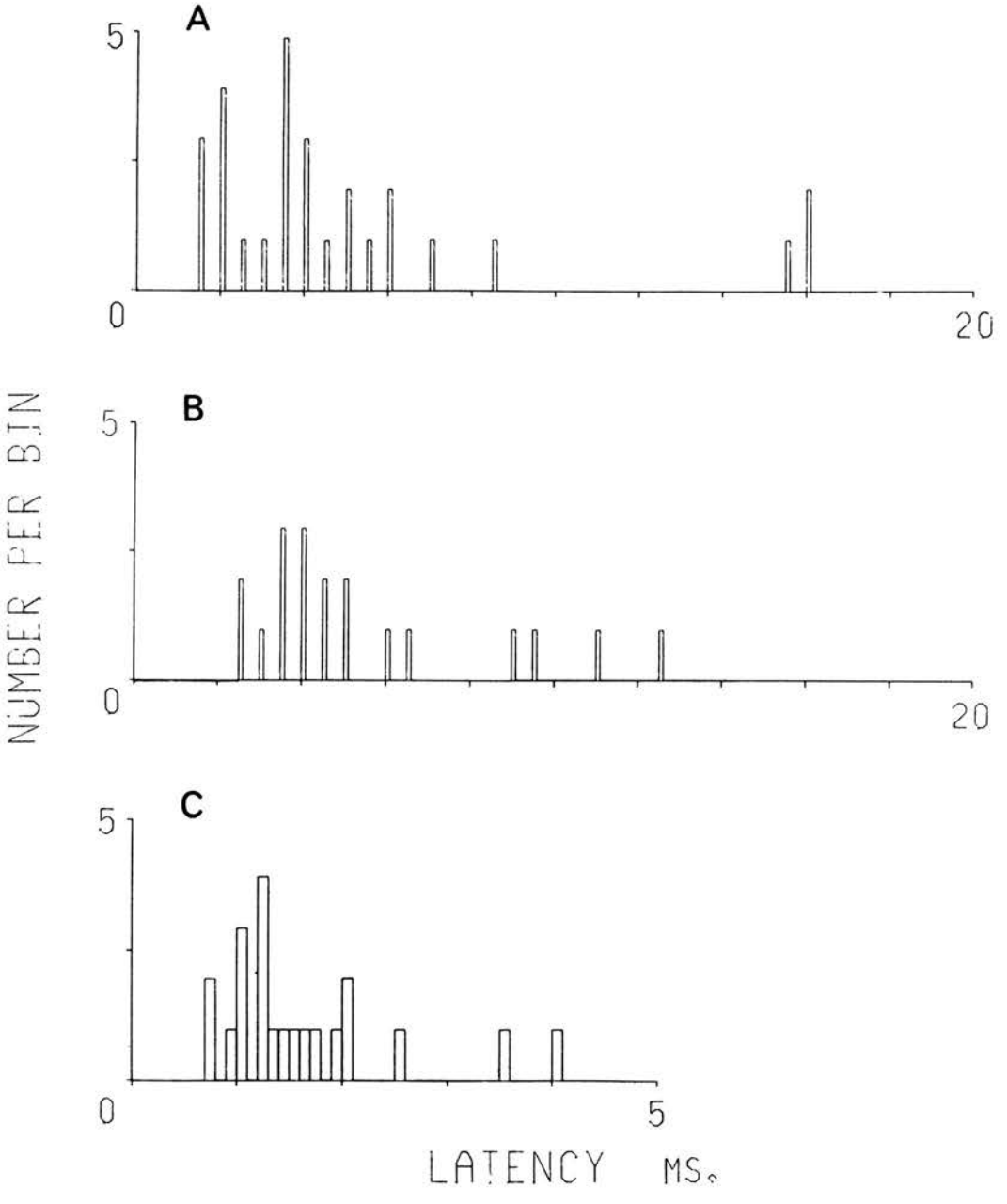

A
5
0
20
B
5
0
20
C
5
0
5
NUMBER PER BIN
LATENCY MS.

Table IV.

The statistical parameters of the power functions $R = A\ S^{b}$ described by figure 36.

TABLE IV

Unit	A	b	r	S.E. Y/X	S.E. b	Static Response
StII 0283-2	1·76	0·53	0·7965	0·38	0·20	▼ 36A
St I 2563-3	0·74	0·16	0·8897	0·064	0·047	● 36A
StII 0763-1	1·78	0·30	0·9257	0·088	0·062	O 36A
St I 3173-2	-1·3	0·99	0·9811	0·098	0·11	+ 36A

Unit	A	b	r	S.E. Y/X	S.E. b	Dynamic Response
St I 2123-4	1·33	0·74	0·9806	0·116	0·05	● 36B
St I 0283-1	3·08	0·39	0·9856	0·053	0·024	O 36B
StII 0953-1	1·03	0·54	0·9155	0·19	0·089	X 36B
StII 2773-1	2·79	0·26	0·8722	0·11	0·065	+ 36B
StII 0283-3	2·03	0·41	0·9778	0·08	0·044	▼ 36B

SECTION IV

Cerebellar responses to stimulation of the vibrissae.

Introduction.

In section III a trigemino-cerebellar pathway via the lateral reticular nucleus was described. The input to cells in this pathway were phasic and highly convergent, often with receptive fields including the fore and hind-limbs.

No direct projection to the cerebellum was observed in cells with slowly adapting responses or cells firmly established as second-order neurones of the spinal trigeminal nucleus. No investigation was made of the input to the principal sensory nucleus where such projections may exist. The experiments described in this section reveal some of the properties of the cerebellar responses to stimulation of the maxillary vibrissae and the possible pathways involved are discussed. The somatotopic and functional localisation of mossy and climbing fibre evoked responses within lobules V and VI, as described by Eccles et al. (1968a, 1972b) and Leicht et al. (1973a), was not investigated.

Methods.

In these experiments 17 male and female cats weighing between 1·8 and 4 kg. were used.

Preparation of the animals.

In four experiments anaesthesia was induced with injections of 10% w/v. Nembutal i.p. and maintained with supplementary doses of 44 mg./kg. Nembutal i.v.. In six experiments anaesthesia was induced with ethyl chloride/ether applied with a face mask and maintained with chloralose 65 mg./kg. i.v.. In a further seven

experiments induction was effected with a 2:1 mixture of $N_2O:O_2$ followed by 1·5 - 3% halothane delivered via a face mask and maintained with chloralose 65 mg./kg. i.v.. These seven experiments were carried out in conjunction with investigations of the trigeminal sensory complex and the surgical procedures were those described in section III. In the previous 10 experiments a separate investigation of the cerebellar cortex was carried out using a different preparation and a closed chamber recording technique. The trachea, femoral artery and saphenous vein were cannulated for the maintenance procedures as described in section III. The cranium was exposed by medial incision and the temporal muscles were reflected exposing the skull which was cleaned with a solution of hydrogen peroxide in water. A trephine was used to drill a hole ½" in diameter centred approximately 2 mm. to the left of the mid-line, extending rostrally to the tentorium cerebelli and caudally to the lambdoidal ridge. The dura was excised. The left paravermal vein was generally visible to the left of the opening and the fissura prima was visible as a deep mediolateral depression crossing the centre of the field. Larsell's lobules V and VI were clearly visible on either side of the fissura prima. A brass chamber was screwed into the trephine hole and the edges sealed with acrylic cement. Warm mineral oil was then poured into the chamber until it was completely full. An 'O' ring placed on top of the chamber formed a seal between the recording chamber and the flat base of the microelectrode stage. A nylon sleeve formed

a seal between the microelectrode and the base of the stage through which it was inserted. When this stage was lowered onto the 'O' ring a closed recording chamber was formed into which the micropipette could be advanced. The advantage of this arrangement was the elimination of respiratory movements in the chamber and the subsequent stable recording conditions. A disadvantage was that surface blood vessels were obscured by the recording stage and were punctured on occasions. The recording electrodes used and the methods of recording, amplifications and data collection were identical to those described in section III.

RESULTS

Cerebellar evoked potentials.

Slow surface potentials evoked by electrical stimulation of the face at the base of the vibrissae were recorded over a wide area of the vermis. The largest responses, however, were always located ipsilaterally in lobules V and VI bordering the fissura prima, in accordance with the results of Snider & Stowell (1944). The intensity of the stimulus was adjusted so that no increase in the size of the evoked potentials could be obtained by further increasing the voltage. Figure 39 illustrates a typical series of evoked potentials recorded at 8 different locations on lobules V and VI. The origins of these potentials may be established by reference to the field potential studies of Eccles et al. (1967, 1968a). The initial positive-negative wave at a latency of 12 - 14 ms. corresponds to the N_2-P_2 waves recorded in the granular layer. The positive component of this surface

wave is attributed to the depolarisation of granule cell dendrites by mossy fibres and the negative component the subsequent activation of Purkinje cells by parallel fibres (Körlin & Larson, 1970). The large positive potential which follows this mossy fibre (MF) evoked wave at a latency of 18 - 21 ms. originates in the Purkinje cell (P-cell) layer as a result of the powerful activation of these cells by climbing fibres (CF). The region of maximum response was often difficult to locate without resorting to averaging techniques. The records of figure 40 are the evoked potentials of a different experiment averaged over 10 stimulus presentations by the PDP 12 computer. In this case the area of maximum response was taken to be in the region of positions 2 and 3 of lobule V as indicated, rostral to the fissura prima and ipsilateral to the stimulating electrode. The climbing fibre response is particularly evident in these regions whereas the earlier MF response is generally very small. The significance of the relative sizes and latencies of these waves will be considered in the discussion of this section.

The evoked potential records of figures 39 and 40 were collected under chloralose anaesthesia (65 mg./kg.). Körlin & Larson (1970) and Gordon, Rubia & Strata (1972) have shown that even very small doses of barbiturate (4 - 8 mg./kg.) depress the MF responses by action at the Mossy fibre-granule cell synapse. This was commonly observed when the animal was anaesthetised with Nembutal, and the use of this anaesthetic was discontinued after four experiments. The use of chloralose does not affect

the mossy and climbing fibre responses differentially (Eccles, et al., 1972d).

Single-unit activity in the Purkinje cell layer.

Single-unit activity of the Purkinje cell layer reveals the dual nature of the afferent input and may be related to the sequence of electrical activity described in figures 39 and 40. The records of figure 41 were photographed from the oscilloscope screen and show the types of discharge which may be elicited by electrical stimulation of the face at the base of the vibrissae. In the chloralose anaesthetised preparation unit discharges recorded in the P-cell layer 50-300 μm. beneath the folial surface maintain a spontaneous activity of between 5 and 100 impulses per second. Figure 41A is the record of a spontaneously active unit showing the definitive features of P-cell discharge; the simple and complex spike patterns. Thatch (1967) and Eccles et al. (1971b) have attributed the simple response to the activation of a P-cell by the mossy fibre-granule cell-parallel fibre relay and the complex burst of 2 - 6 impulses (marked with a ●) to the powerful activation of the P-cell by a single climbing fibre. The simple spike discharge is therefore described as the MF response and the complex discharge the CF response. The alternating pattern of simple and complex discharges in figure 41A was not common, in general spontaneous activity was predominantly of the simple pattern. A spontaneously active unit with a simple pattern of discharge (41B) produced a single complex burst when several of the vibrissae were tapped individually, indicating that this must be a Purkinje cell and not one

of the stellate or basket cell interneurones also found at this depth (50 - 300μm.). The most commonly observed pattern of spontaneous activity is illustrated in figure 41C where a high frequency discharge of simple spikes (90 - 100 impulses/sec.) is interrupted occasionally ($\triangleq$1/sec.) by a burst of the complex pattern. This dual pattern of discharge was the main criterion used for identification of a unit as a Purkinje cell.

Examples of electrically driven unit activity are shown in figure 41, D-I and are typical of the responses obtained under light chloralose anaesthesia. The variability of the unit discharge is illustrated by consecutive records of the same cell in figure 41 D,E. The MF response of figure 41D is almost non-existent at a long latency of 21 ms. and may only represent sub-threshold depolarisations of the P-cell dendrites by a weak parallel fibre volley. A stimulus applied 1 second later caused the same cell to generate an MF response at a latency of 24 ms. (Fig.41E). In both cases the CF response at a latency of 34 ms. was a burst of 4 - 5 spikes, not clearly shown in these photographs. Another cell (Fig.41F) generated a much earlier MF response at a latency of 14 ms. and a CF response of shorter duration (2 - 3 spikes) after 34 ms. A typical CF response is clearly shown in figure 41G with a latency of 28 ms. but as in figure 41D the MF response appears to be sub-threshold and visible only as increased baseline noise at a latency of 20 ms. All these cells (Fig.41D-G) were recorded in the same electrode track and the rate

of spontaneous activity was low ($<$5 impulses/sec.).

The records of figure 41 H,I are shown on a longer time-base and show the responses of two P-cells over a period of 100 milliseconds. The cell of figure 41H responded with CF evoked bursts at intervals of 8 ms. and a latency to the first burst of 18 - 19 ms., with no MF responses at all. This type of response was observed occasionally but was not common. A more typical response pattern is shown in figure 41 I where both types of responses are evoked with the MF response preceeding the CF response by 3 - 6 ms..

Eccles, et al. (1971b) states that the short latency (7 - 15 ms.) MF responses to electrical stimulation of the forelimb are more reliable than CF responses, producing a burst of 3 - 4 simple spikes at a relatively invariant latency. The responses obtained under similar conditions to stimulation of the face have a longer latency than one might expect for such a short conduction distance (4 - 5 cm.). In addition, the CF evoked responses appear to be more reliable than the MF responses and generally have latencies within the expected range (18 - 30 ms.).

The random and often high frequency resting discharge of the Purkinje cell becomes a problem when attempting to recognise a pattern of responses over a long period of time. In many cases this is impossible to achieve by studying unit discharges on the oscilloscope screen. In a few experiments analogue averaging techniques were employed to make these patterns more evident. Figure 42A is an averaged waveform of focal activity in the vicinity

of a Purkinje cell. The recorded activity was filtered to remove both high (> 1 KHz) and low frequency (< 100 Hz) components of the signal and 10 responses were averaged over a period of 256 ms.. This method was not generally successful but the early MF response (17 ms.) can be seen followed by the CF response extended over the period from 29 - 35 ms.. This sequence is followed by a period of relative inactivity with an MF driven discharge appearing at approximately 160 ms.. The analogue record is complemented in figure 42B by the post-stimulus time histogram (PSTH) of the same cell recorded with wide bandwidth amplification and the real-time clock of the PDP 12 computer triggering on both the MF and CF evoked impulses. The pattern of excitation-inhibition-recovery can be seen very clearly, a pattern commonly observed following stimulation of cutaneous limb nerves (sural, superficial peroneal, superficial radial: Eccles et al., 1970).

<u>The responses of Purkinje cells to Mechanical stimulation of the vibrissae</u>.

Purkinje cell responses to mechanical stimulation of the vibrissae were usually well defined and occured over a wide area of lobules V and VI. Most recordings were made, however, in the region of maximum evoked response in any experiment, usually in the first or second folium of lobule V as illustrated in figures 39 and 40.

The receptive fields of CF evoked Purkinje cell responses were often confined to 5 or 6 vibrissae although some cells responsed to brushing the fore or hindlimbs as well. Mossy fibre evoked responses were much less specific generally responding to small movements of the

maxillary vibrissae and vigorous brushing of the limbs and trunk. Features of these responses are shown in figure 43. The commonly observed CF response of figures 43 A,B superimposed upon a slow (10/sec.) spontaneous discharge of simple spikes. The velocity threshold of the CF response was 50°/sec. (Fig.43B) below which no change in the spontaneous activity of the cell could be effected. The response of the same cell to a deflection of the hair through 3° at a velocity of 75°/sec. (Fig.43A) shows a small increase in latency within the normal range of variation. In general, once threshold had been reached no decrease in the latency of a CF evoked response could be obtained, suggesting that even at threshold there is a considerable barrage of afferent discharges onto the inferior olive where the climbing fibres originate. Mossy fibre evoked discharges were found to be highly variable in response to mechanical stimulation of any intensity. The cell shown in figure 43C produced a similar CF evoked discharge but with no preceding MF activity. A similar situation is seen in figure 43D where two CF responses were evoked separated by approximately 16 ms.. The response of figure 43E consisted of an early MF evoked spike followed by a CF burst and irregular MF discharge. This cell shows evidence of directional sensitivity, although it is possible that a post-stimulus inhibition of the P-cell prevents the response to movement in the return direction. With longer stimulus durations, however, this was also seen (e.g. Fig.43H) and in 9 cells where this was tested the arc of movement in which a response could be elicited was quite small, usually in one cardinal

direction only. Of these 9 cells 5 were excited by movements in the rostral-caudal direction. Unfortunately, with such a small sample of cells no evidence can be presented for a functional cluster of CF-evoked Purkinje cell responses related to directional sensitivity, as might be postulated from the results of Eccles et al. (1972c) and Leicht et al. (1973a). This remains, however, an interesting possibility of some significance. The prolonged effects of mechanical stimulation on Purkinje cell discharge may be seen more clearly in the longer time-base records of figure 43 F-H. The cell illustrated in figure 43F produced no CF response but at the time when this response might be expected there was a strong inhibition of the ongoing simple spike spontaneous activity. Figure 43G shows a double CF response superimposed on a high rate of spontaneous simple spike activity with a consistent 180 ms. between CF evoked bursts. An extreme case of inhibition is presented in figure 43H where an initial CF response is followed by 260 ms. of complete inhibition and an abrupt resumption of both simple and complex discharges at a higher rate than the resting rate.

Averaged responses.

The patterns of excitation and inhibition in a single P-cell response may extend over several hundred milliseconds and show considerable variation during repetitive stimulation. These patterns may be expressed more readily in the post-stimulus-time histogram (PSTH) of latencies to impulses in a response, and its integral, the cumulative frequency distribution (CFD). The CFD has been scaled down several

times for display and is therefore a highly 'smoothed' curve which enables long-term changes in the rate of spike activity to be seen clearly. An upward deflection of the curve represents an increase in the rate of discharge, a levelling off of the curve, a decrease in the rate of discharge. Horizontal portions of the curve represent no activity which indicates, in these spontaneously active cells, complete inhibition.+

The graphs of figure 44 present the patterns of responses in time following electrical stimulation of the face. The same general sequence may be seen in all four cases; a period of excitation with a latency of 15 - 45 ms. followed by varying degrees of inhibition up to 200 - 300 ms. and a return to normal or even increased activity up to 500 ms.. Two distinct peaks of excitation may be seen in figures 44 A,B with latencies to peak of 20 and 35 ms. corresponding to the MF and CF evoked discharges respectively (as illustrated in figure 42). A subsequent period of inhibition lasts for approximately 150 ms. and is followed by a return to a normal level of activity. An early MF response of latency 16 - 17 ms, is followed by a period of intense inhibition for 200 ms. without an intervening CF response in figure 44 C,D. The discharges presented in figures 44 E,F and 44 G,H are particularly interesting as they were recorded in the same electrode track. Within the period of 70 - 100 ms. following the stimulus an early (12 - 15 ms.) and a late (80 - 90 ms.) MF response is visible in figures 44 E,F with no CF response in between. This pattern of discharge appears to be complemented in the

+ All the units displayed in figures 44-46 were investigated under chloralose anaesthesia.

activity of the adjacent cell (44 G,H) where a complex CF activated burst appears at 35 ms. followed by total inhibition of simple spike activity for 75 ms. and a subsequent return to irregular simple discharge and repetitive CF bursts.

The averaged responses to mechanical stimulation were often similar in magnitude and pattern to those evoked by electrical stimulation. In all cells investigated it was possible to reveal changes in the base rate of activity with very few stimulus presentations (usually 10). This compares with the 128 repetitions normally used by the Eccles group to show modifications of the erratic spontaneous discharge of Purkinje cells by the influence of cutaneous mechanoreceptive input (Eccles et al., 1972 a-d). The CFD diagrams of figures 45 and 46 include an additional feature made possible by delaying the stimulus presentation relative to the start of data collection and the inclusion of spontaneous activity preceding the evoked response. On the CFD diagrams the portion of the curve corresponding to this activity is extrapolated over the whole duration of the curve (broken line) and the slope of this line represents the rate of spontaneous activity. Deviations from this rate may thus be seen very clearly and an evoked CFD curve parallel to this line indicated that the rate of activity has returned to normal.

All responses to mechanical stimulation recorded in this series of experiments were phasic in nature. That is, although the response, including inhibition, could last for several hundred milliseconds the only portion of the

stimulus which could be related to the response was the onset of movement. In contrast to Eccles et al. (1972 b,d) and Leicht et al. (1973a) no tonic excitatory or inhibitory actions mediated by mossy fibre pathways were observed.

Figure 45A shows the PSTH and CFD of a cell responding to a 3^{o} movement of a single vibrissa with an onset velocity of 300°/s and held for 100 ms. Accordingly, this movement should activate all types of mechanoreceptor found in the sinus hair follicle, as described in section II. The excitatory response of this cell was phasic and predominantly of the complex pattern, indicating activation via a climbing fibre pathway, relaying in the inferior olive. The initial CF response was followed by another two at latencies of approximately 150 ms and 300 ms. An identical stimulus applied to another unit (45C,D) evoked an early MF response at 25 ms. followed by a weak CF response at 44 ms.. In both of these cells there is no response associated with the 'off' movement of the hair. Changing the intensity of the stimulus had very little effect on the pattern of discharge evoked in the cerebellar Purkinje cell, although the range of amplitudes which could be provided was severely limited. Figures 45 E,F show the response of the same cell as 45 C,D stimulated with a 3^{o} movement of the hair at half the previous velocity (i.e. 150^{o}/sec.). In this situation the differences in latencies between the MF and CF evoked responses became less distinct due to an increase in variability of the response and it can be seen that the change in slope of the CFD curve is less abrupt than that of 45D

(N.B. 45F is on a larger scale). The duration of this stimulus was also much longer but no difference can be seen between the later parts of these responses. With a limited range of possible stimuli and the poor resolution between different response levels it was not possible to construct a meaningful stimulus-response relationship in these terms. With two distinct afferent systems operating to produce a highly structured response the coding of intensity is probably not modulated simply by the rate of discharge of cerebellar Purkinje cells. All the responses of figure 45 show an initial phase of excitation followed by a decline to the original rate of resting discharge with irregular mossy and climbing fibre evoked activity. This sequence was observed independently of the duration of the stimulus.

Two adjacent Purkinje cells which lay within 50 μm of each other gave vigorous responses to a 3^{o} movement of a single maxillary vibrissa at a velocity of 600^{o}/sec. and a duration of 250 ms. The response shown in figure 46 A,B was an initial complex burst of very high frequency ($\rangle$ 500 impulses/sec.) at a latency of 30 ms. followed by a slow decline of the simple spike activity over a period of 100 ms. and a late increase in the rate of activity compared with the spontaneous discharge. The second cell of 46 C,D also exhibits a high frequency burst of complex activity followed by an abrupt decrease in the rate of MF evoked discharge, returning to normal after a period of about 200 ms. These response patterns were observed in 16 other cells and are very similar to the electrically

evoked responses of figure 44 and of a similar magnitude.

The recording sites of these units were all within the Purkinje or ganglionic layer of the cerebellar cortex between 50 and 300 μm below the folial surface. Figure 47B is a photomicrograph of a parasaggital section through the vermis with the site of a P-cell response marked by a pontamine blue spot.

DISCUSSION

The latencies, magnitudes and temporal patterns of single Purkinje cell responses have been investigated with a view to establishing the routes and relative importance of mechanoreceptive projections from the maxillary sinus hair follicles. The organisation of these responses into functional groups within the cerebellar cortex was considered to be outside the scope of this investigation.

Eccles et al. (1968a) state that the size of the granular layer N_2-P_2 wave is an accurate measure of the size of the incoming mossy fibre barrage as the P-cell MF evoked response is subject to many influences. It was concluded in the results (p.3) that this wave corresponds exactly to the surface P-N wave illustrated in figures 39 and 40 at a latency of 10 - 14 ms. At the time of mossy fibre depolarisation of granule and Golgi cell dendrites the surface parallel fibres act as passive sources of current flowing to the active sink in the region of the granule layer glomeruli (Eccles, et al., 1967). The size of this wave was always small following both electrical and mechanical stimulation of the face and in figure 40 almost disappears in the averaging process.

This suggests that the MF response is small and of variable latency (causing the averaged response to blur). By contrast, the CF evoked wave was consistently large at a latency of 18 - 25 ms. and often prolonged over 10 - 15 ms. The relatively small MF response cannot be explained by inadequate stimulation of the primary afferent fibres which project to mossy fibre pathways as both MF and CF pathways receive low threshold group Ia and cutaneous input in the spinal tracts (Oscarsson, 1967a) and definitely receive low threshold cutaneous afferents in the trigemino-cerebellar tracts. The sequence of MF and CF responses to facial stimulation was as expected with the long synaptic delay at the inferior olive and slow conducting olivo-cerebellar fibres (5 - 20 M/sec.) causing the CF response to arrive at the cerebellum 6 - 10 ms. later than the response carried by the fast conducting (50 M/sec.) mossy fibres (Eccles et al., 1966a, 1968a). In absolute terms, however, the latencies of these responses were longer than expected, considering the short conduction distance from the face to the cerebellum (3 - 5 mm.). This was especially true of the mossy fibre evoked responses, many of which had a latency greater than 10 ms. to electrical stimulation and single-unit records often show latencies in the 14 - 20 ms. range (Fig.41). Electrically evoked MF responses from the forelimb have latencies as short as 4 ms. and these are attributable to inputs from the direct cuneocerebellar tract. No latencies shorter than 10 ms. were recorded in the present series of experiments. Eccles et al. (1972b) regard excitatory MF responses from forelimb nerves with

latencies of longer than 15 ms. as being transmitted via the lateral reticular nucleus and the reticulo-cerebellar pathway (Grant et al., 1966; Oscarsson & Rosen, 1966). The estimated time of conduction from the forelimb to LRN is 6 ms. and from the LRN to the cerebellum 1 ms. (Rosen & Scheid, 1973a), this means that the earliest forelimb-LRN response in the cerebellar cortex must arrive 3 ms. later than the cutaneous component of CCT. Rosen & Scheid state that given a response of latency 7 - 9 ms. from the forelimb it is not possible to decide whether or not the CCT or a reticulo-cerebellar tract is involved. As all of the cutaneous MF responses identified in this series of experiments were outside this range it must be assumed that a large proportion of these were transmitted via the reticulo-cerebellar tract. The relative weakness and variability of the MF response, especially during mechanical stimulation, may be due to a small volume of fibres ascending in this pathway or to an inhibition of the response at the level of LRN or the mossy fibre-granule cell relay. Inhibition at this relay, situated in the granular layer glomeruli, has been described in detail by Eccles et al. (1966d) and is due to the axo-dendritic inhibition of granule cells by Golgi cells directly activated by the ascending mossy fibres making contact with Golgi dendrites. Indirect inhibition at this site via the parallel fibre-Golgi cell route seems unlikely as the evoked potential records have shown the initial depolarisation of the granule cell dendrites to be small (and therefore few parallel fibres would be activated).

Eccles et al. (1967) regard Golgi cell inhibition as a method of sharpening the mossy fibre input which is diffused by the prolific branching of these fibres.

Unlike the spinocerebellar climbing fibre responses reported by Eccles et al. (1968a, 1971b, 1972c) the CF responses in a single cell or surface recording position were of relatively constant size and latency, especially the unit responses to mechanical stimulation of the vibrissae. Reasons put forward for the variability of the spinal CF response are the variable degrees of convergent excitation and inhibition at the inferior olive resulting in variable numbers of spikes in the bursting discharge of climbing fibres (Armstrong et al., 1968). There is also strong evidence of corticofugal control of this discharge at the level of the olive (Leicht et al., 1973b). The relative stability of the CF evoked responses observed in this series of experiments suggests that these conditions may not hold during excitation from the vibrissae.

Eccles et al. (1972b) report that for taps applied to the footpads the threshold for MF evoked P-cell responses is usually below 200 μm. of indentation. For CF evoked responses thresholds are often below 100 μm. (Eccles et al., 1972c; Leicht et al., 1973a). These stimuli were presumed to activate Pacini corpuscles preferentially. Tangential jets of air which activated only the rapidly adapting hair follicle receptors were also effective in evoking a CF evoked Purkinje cell response. The rapid onset (10 ms.), sustained movements

of the maxillary vibrissae employed in the present experiments were assumed to activate all types of mechano-receptor residing in the sinus hair follicle, as described in section II. No responses to vibratory stimuli were recorded when tuning fork stimulation was employed. Eccles et al. (1972c) who found this to be the case for tap evoked CF responses, put forward the inability of the inferior olive synapse to follow high frequencies of stimulation as the reason for this (Armstrong & Harvey, 1968) However, (they) describe a large projection of vibration sensitive Pacini corpuscles to the inferior olive from the limb nerves. It seems therefore that the capacity of la mediated receptors to follow high frequencies of mechanical stimulation is not functionally significant within the olivo-cerebellar pathway. The general conclusion to be drawn from investigations of both the spino and trigemino-cerebellar pathways is that there is a great convergence of input from all types of cutaneous and sinus body receptors in both MF and CF pathways onto the cerebellar Purkinje cell. The very narrow range from threshold to maximal excitation by different velocities of hair movement reported in this series of experiments suggests that a very large barrage of afferent activity from the sinus hair follicle arrives at the inferior olive over a short period of time and initiates a relatively constant and maximal CF burst which subsequently depolarises the target Purkinje cell.

The general sequence of Purkinje cell responses to electrical or mechanical stimulation was MF excitation,

CF excitation, a period of decreased activity and a return to normal spontaneous discharge after 250 - 300 ms. (Figs. 44, 45, 46). Eccles et al. (1972d) have suggested that MF responses are often expressed as a tonic inhibition of P-cell activity upon which phasic, excitatory CF responses are superimposed. They recognised, however, all degrees of tonic and phasic excitation and inhibition in both MF and CF responses, indicating that slowly adapting cutaneous receptors were involved in both afferent pathways to the cerebellar cortex. Purkinje cells activated by movement of the vibrissae showed no evidence of tonic activation or inhibition related to the duration of an applied hair deflection. The long period of inhibition often observed between 50 and 150 ms. following the stimulus onset is attributable to the direct inhibition of Purkinje cells by basket and stellate cells driven by adjacent bands of active parallel fibres (Eccles et al., 1967) by virtue of the long latency and duration of this effect. The complementary or antagonistic discharges recorded in figures 45 E,F and 45 G,H could be a rare example of 'cross-talk' between adjacent Purkinje cells mediated by the inhibitory recurrent collaterals of the P-cell axon.

The absence of a tonic effect related to the duration of sinus hair deflection was not expected and may be due to four factors:-

A. The absence of a direct cerebellar pathway which in the spinal cord has been shown to carry slowly adapting responses (Lundberg & Oscarsson, 1960; Mann, 1971) and which is suggested by the absence of short latency MF

responses to facial stimulation.

B. The activation of a phasic trigemino-reticulo-cerebellar pathway suggested by the long latency and variable MF responses observed in accordance with Eccles et al., (1968a, 1972b), Rosen & Scheid (1973a) and the results of section III.

C. The effects of anaesthetic on MF activity acting at the glomeruli, as described by Korlin & Larson (1970) and Gordon et al. (1972) or on the tonically driven activity of LRN cells as described by Rosen & Scheid (1973a). The former effect is relatively unimportant under the chloralose anaesthesia (Eccles et al., 1971a), used in most of the experiments described in this section.

D. The small number of stimulus presentations used in response averaging was not sufficient to reveal the low levels of inhibition which may obtain.

To summarise, the effects of sinus hair displacement on the discharge of Purkinje cells are mediated by both mossy and climbing fibre pathways. The mossy fibre evoked responses were weak and variable and of long latency, suggesting that the major route of MF responses is via the lateral reticular nucleus. Climbing fibre evoked responses were generally much more vigorous and, like the MF evoked responses, phasic in nature.

A reticulo-cerebellar pathway receiving afferents from the descending tract of the spinal trigeminal nucleus was predicted on the basis of the results of section III, although the long latencies of activation of these MF evoked P-cell discharges suggest that the trigeminal cells identified by stimulation of the cerebellar cortex are not of the same pathway. Possible sensory projections from the main sensory(rostral) nucleus were not investigated.

The apparent absence of a direct, short-latency trigemino-cerebellar pathway was not expected. Such a pathway exists projecting from the mesencephalic nucleus of the trigeminal nerve (Pearson, 1949) which has connections with the spinal nucleus and is concerned with the proprioceptive control of the muscles of mastication (Sumino, 1971). This pathway joins the ventral spinocerebellar tract and continues ipsilaterally to the cerebellum, terminating bilaterally, probably in lobule IV, outside the recording area of these experiments. Vibrissae responses have also been reported in the paramedian lobules (Snider & Stowell, 1944) and may be substantially different from those of the main projection area in lobules V and VI of the vermis.

It appears from the results of this series of experiments that a powerful phasic input is provided by movement of the maxillary vibrissae, carried largely by climbing fibres. Comparing the efficacy of sinus hair stimulation with that of single cutaneous mechanoreceptors (Eccles et al., 1972 b-d; Leicht et al., 1973a) it seems that the sinus hair P-cell responses are evoked more reliably, although thresholds are similar. An explanation of this could be that the sinus hair follicle contains a large number of mechanoreceptors (>50) which, as discussed in section II, provide responses to a wide range of velocities and amplitudes of movement applied to the sinus hair. In a single movement of the hair a large proportion of these would be activated and afferent discharges would converge upon cells of the lateral reticular nucleus and, in particular, the inferior olive.

The fact that both MF and CF responses to single hair stimulation project onto the same Purkinje cell having travelled in spatially and temporally separated pathways is suggestive of an organisation of responses in terms of hair movement rather than individual sinus follicle receptor types.

The absence of large muscle groups involved in locomotion may account for the apparent lack of a fast, direct projection from the facial region to the vermis. In terms of cerebellar function it seems that the vibrissae act as 'amplifiers' by the transmission of small hair deflections to large numbers of receptors confined to a relatively small area with effective spatial summation of the responses. The fine grain of these projections and individual responses may reveal more subtle aspects of vibrissae function related to the integrated actions of the cerebellar Purkinje cell.

Fig. 39. The cerebellar surface potentials evoked by electrical stimulation of the face at the base of the vibrissae. The traces were photographed from the oscilloscope screen at 8 locations on lobules V and VI of the vermis, on both sides of the mid-line (broken vertical line).

p.v.v.= paravermal vein

f.p. = fissura prima.

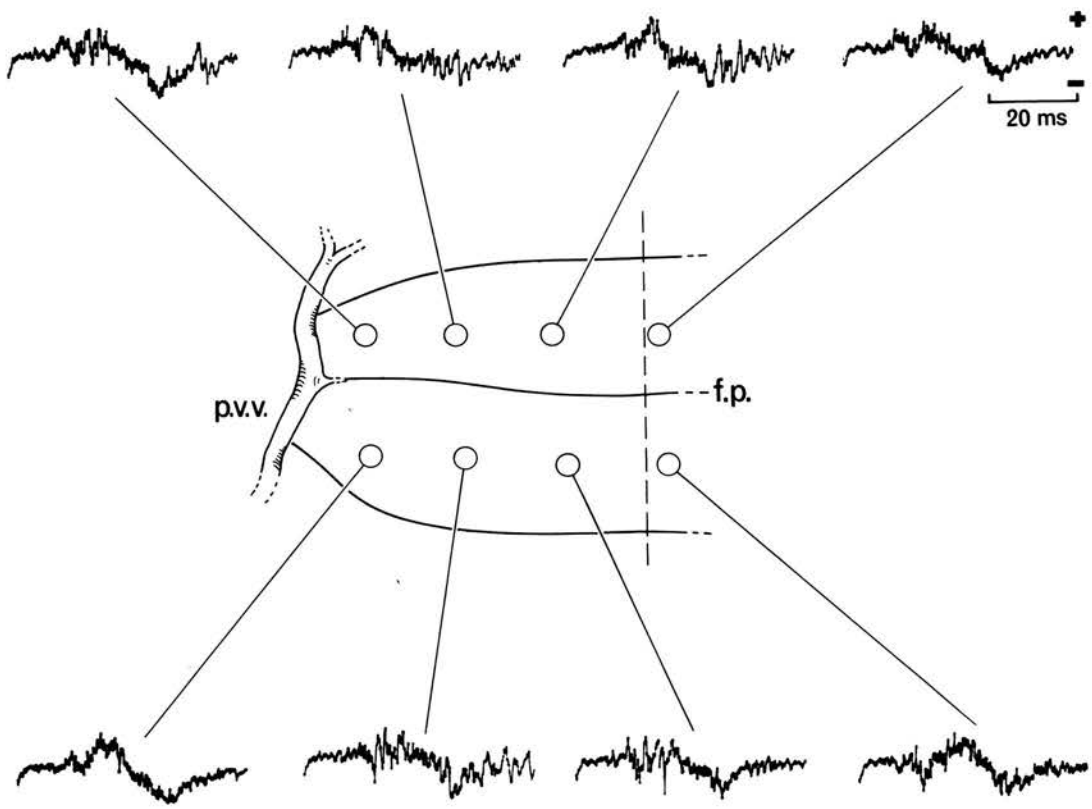

+
-
20 ms
p.v.v.
f.p.

Fig. 40. Cerebellar surface potentials evoked by stimulation of the face at the base of the vibrissae and averaged over 10 repetitions by the PDP 12 computer. At each stimulus presentation the recording channel was sampled 1000 times at 66 μs. intervals, the average of the sweeps was computed and stored on digital tape. These averaged potentials are plotted in the lower half of the figure, the recording locations are indicated in the top half of the figure. The ($\sim$) at locations 7 and 8 indicate that no response was visible at these points.

p.v.v. = paravermal vein

f.p. = fissura prima.

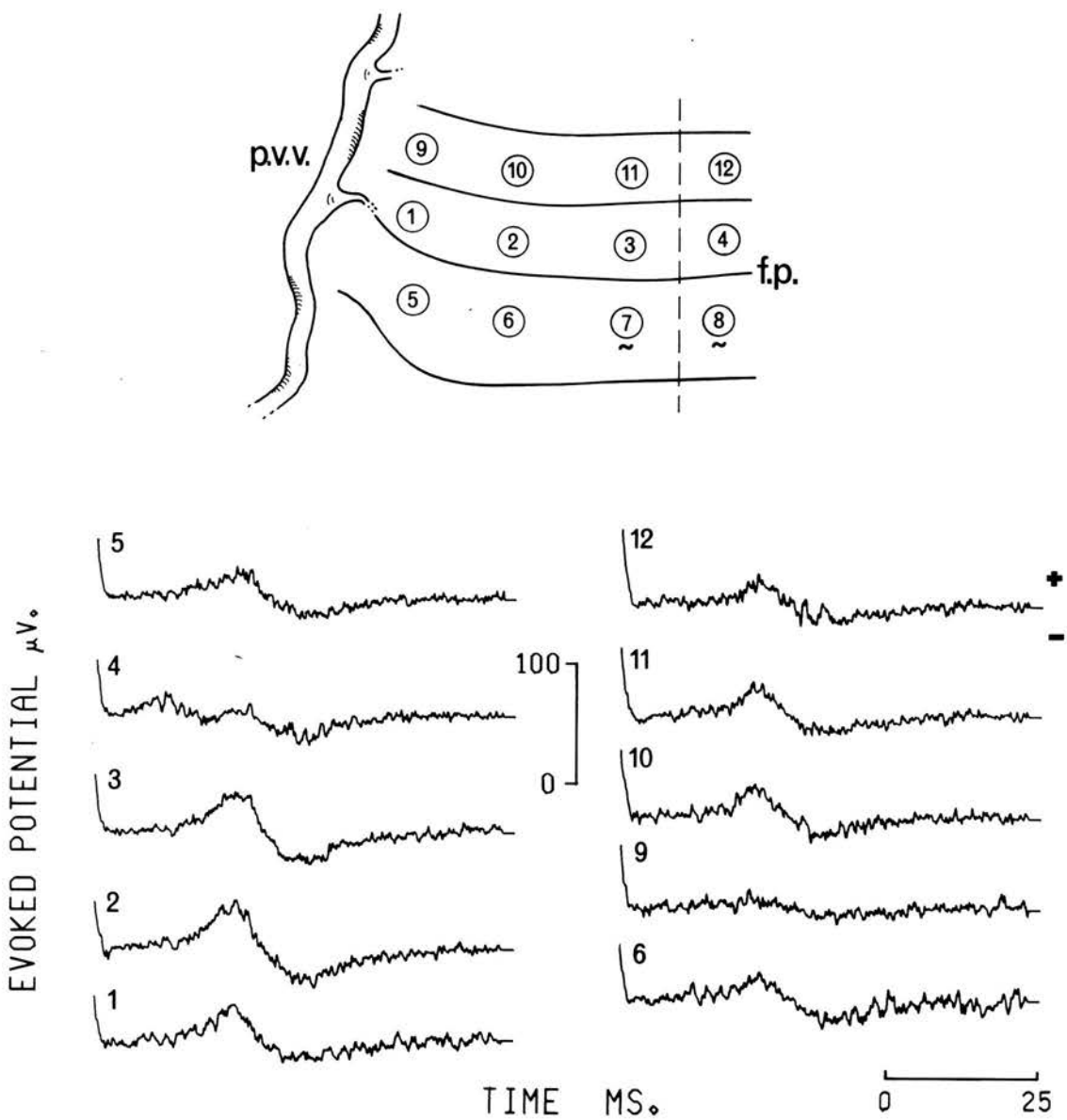

p.v.v.
f.p.
1
2
3
4
5
6
7
8
9
10
11
12
EVOKED POTENTIAL μV.
100
0
+
-
TIME MS.
0
25

Fig. 41. Spontaneously active and electrically evoked single-unit Purkinje cell discharge photographed from the oscilloscope screen.

A,B,C Spontaneous activity showing the simple and complex spike discharges.

D - I Electrically evoked activity.

For details of these responses see the text.

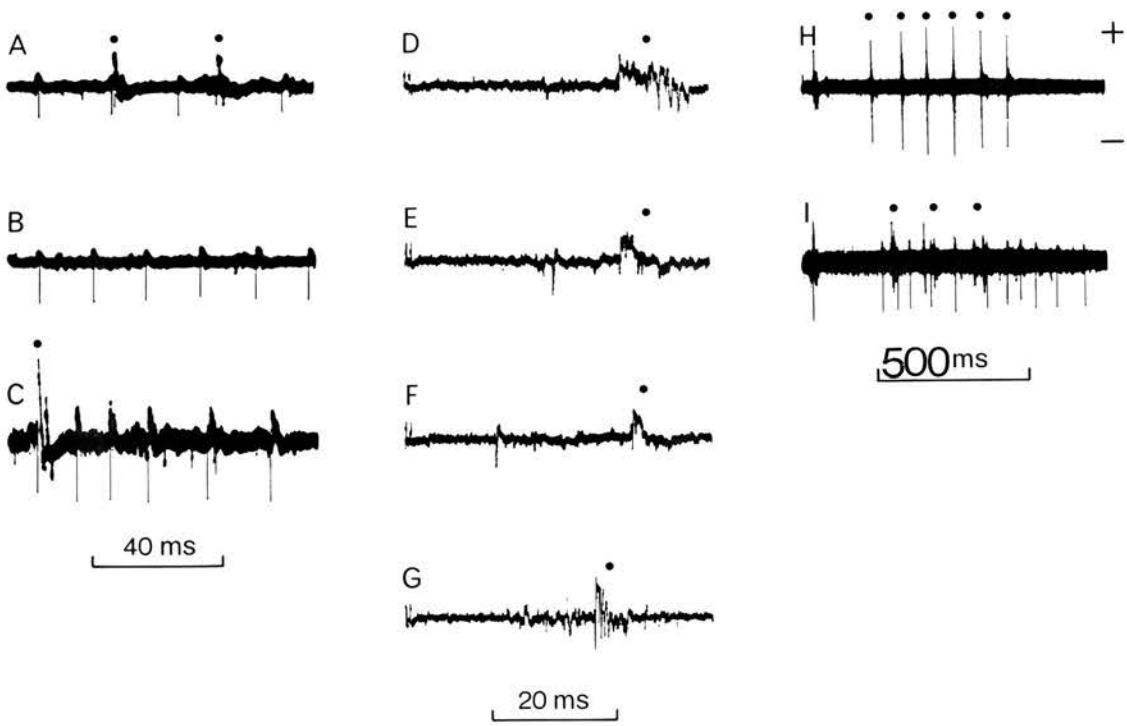

A
B
C
40 ms
D
E
F
G
20 ms
H
+
—
I
500ms

Fig. 42. The relationship between electrical activity recorded in the Purkinje cell layer and the distribution of spikes following a stimulus applied to the skin of the face at the base of the vibrissae.

A. The averaged field potential (10 sweeps) recorded in the region of an active Purkinje cell at a depth of 192 μm.

B. The post-stimulus time histogram of impulses evoked from the same Purkinje cell at a depth of 214 μm.

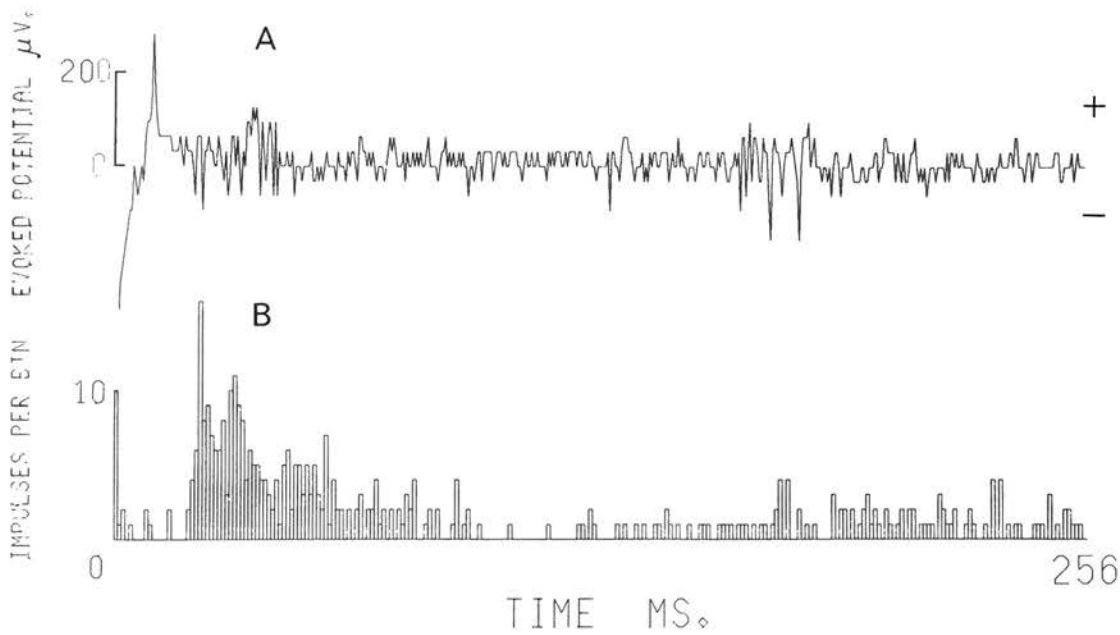

A
200
0
+
−
B
10
0
256
IMPULSES PER SIN
EVOKED POTENTIAL μV.
TIME MS.

Fig. 43. The responses of cerebellar Purkinje cells to mechanical stimulation of the maxillary vibrissae. Each movement shown was to an angle of 3^{o}. Climbing fibre responses are marked (●).

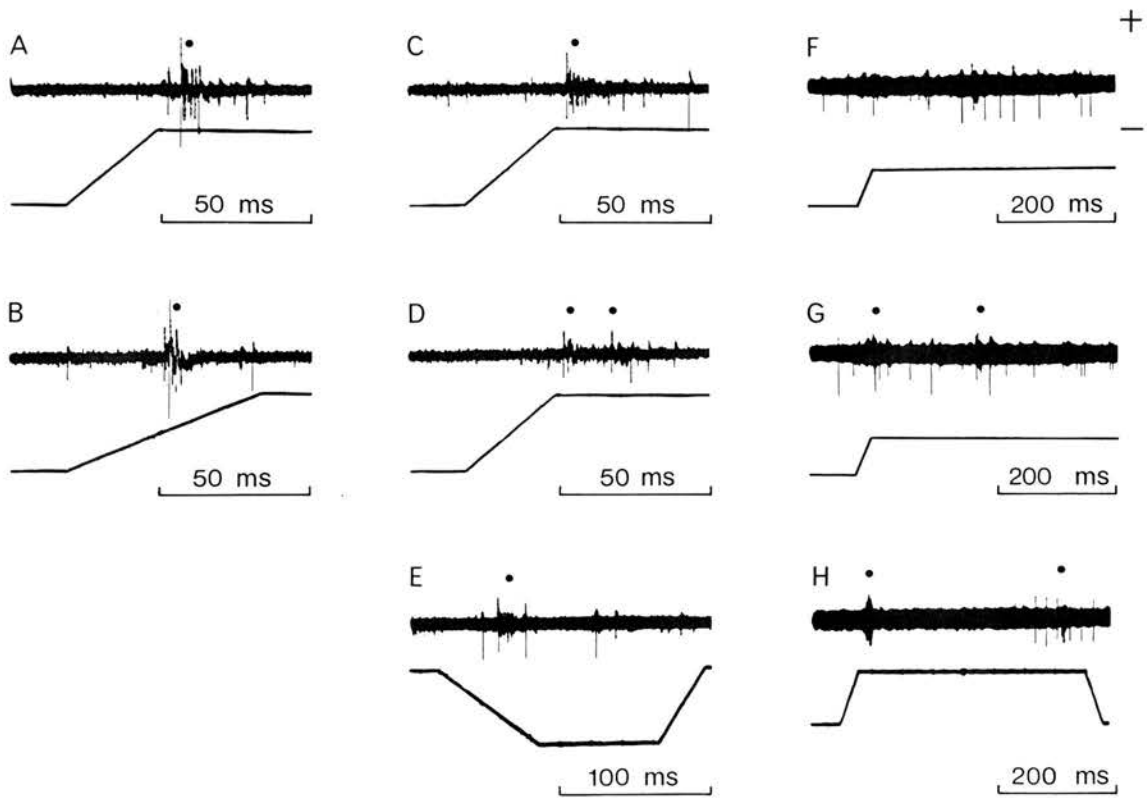

A
50 ms
C
50 ms
F
+
−
200 ms
B
50 ms
D
50 ms
G
200 ms
E
100 ms
H
200 ms

Fig. 44. The post-stimulus time responses of Purkinje cells to electrical stimulation of the face represented in two different ways. The left-hand column of graphs are post-stimulus time histograms (PSTH) of the impulse discharge collected for 500 ms. over 10 stimulus presentations. The integral of the PSTH, the cumulative frequency distribution (CFD) is presented in the right-hand column of graphs. In this distribution an increase in the rate of discharge is represented by an upward deflection of the curve, a decrease by a downward deflection and no activity by a flat curve. The slope of the curve at any instant represents the intensity of the response.

N.B. C,D include the stimulus artifact in bins 1 and 2 and G,H have a stimulus marker (↑) delayed by 20 ms.

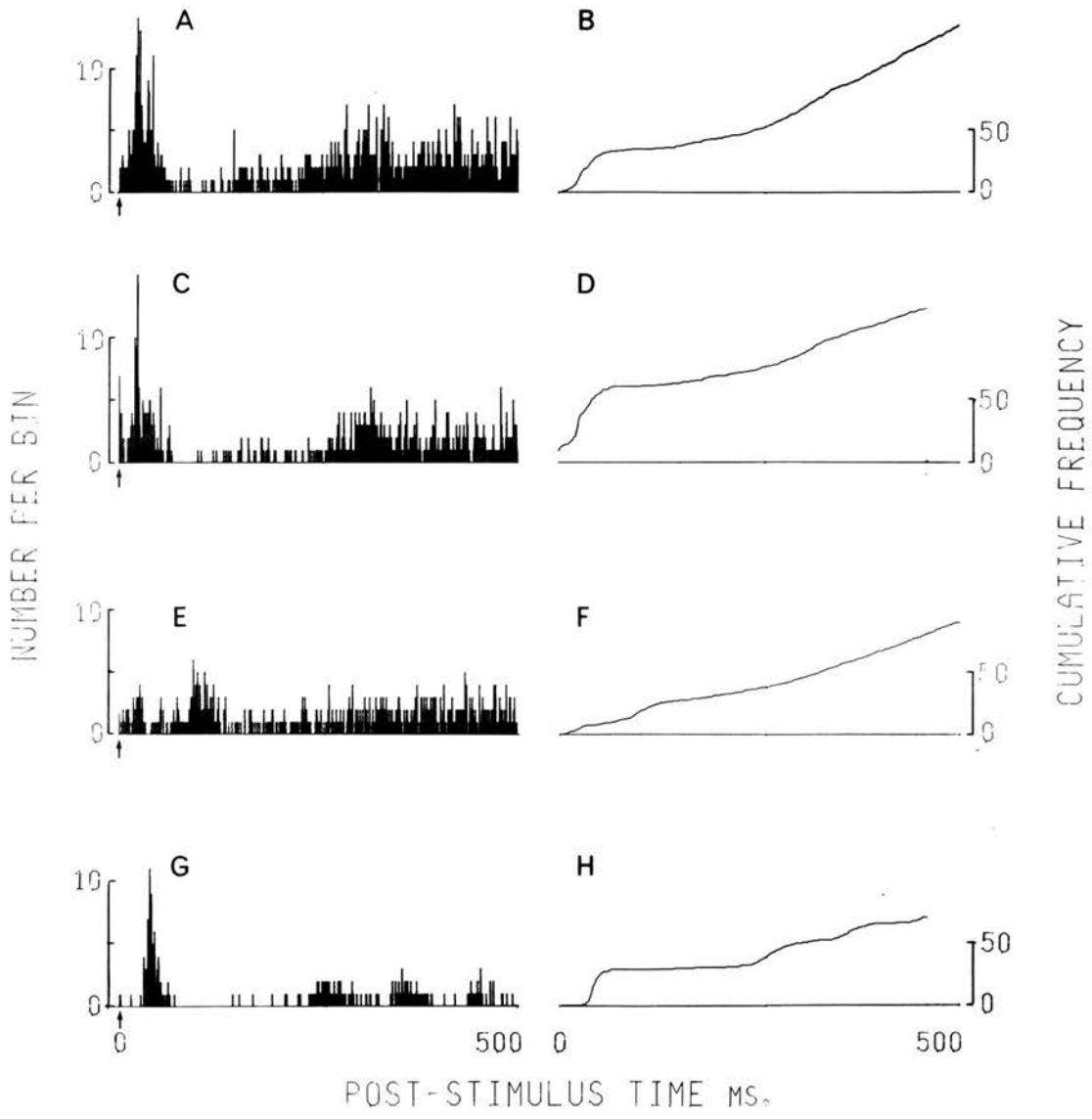

A
B
C
D
E
F
G
H
10
0
50
0
0
500
NUMBER PER BIN
CUMULATIVE FREQUENCY
POST-STIMULUS TIME MS.

Fig. 45. The PSTH and CFD of two Purkinje cells responding to movements of a maxillary vibrissa through an angle of 3^{o}, at a velocity of 300^{o}/sec., for the duration indicated by the solid line under the time axis. The slope of the broken line represents the rate of spontaneous activity. Details of these responses are given in the text.

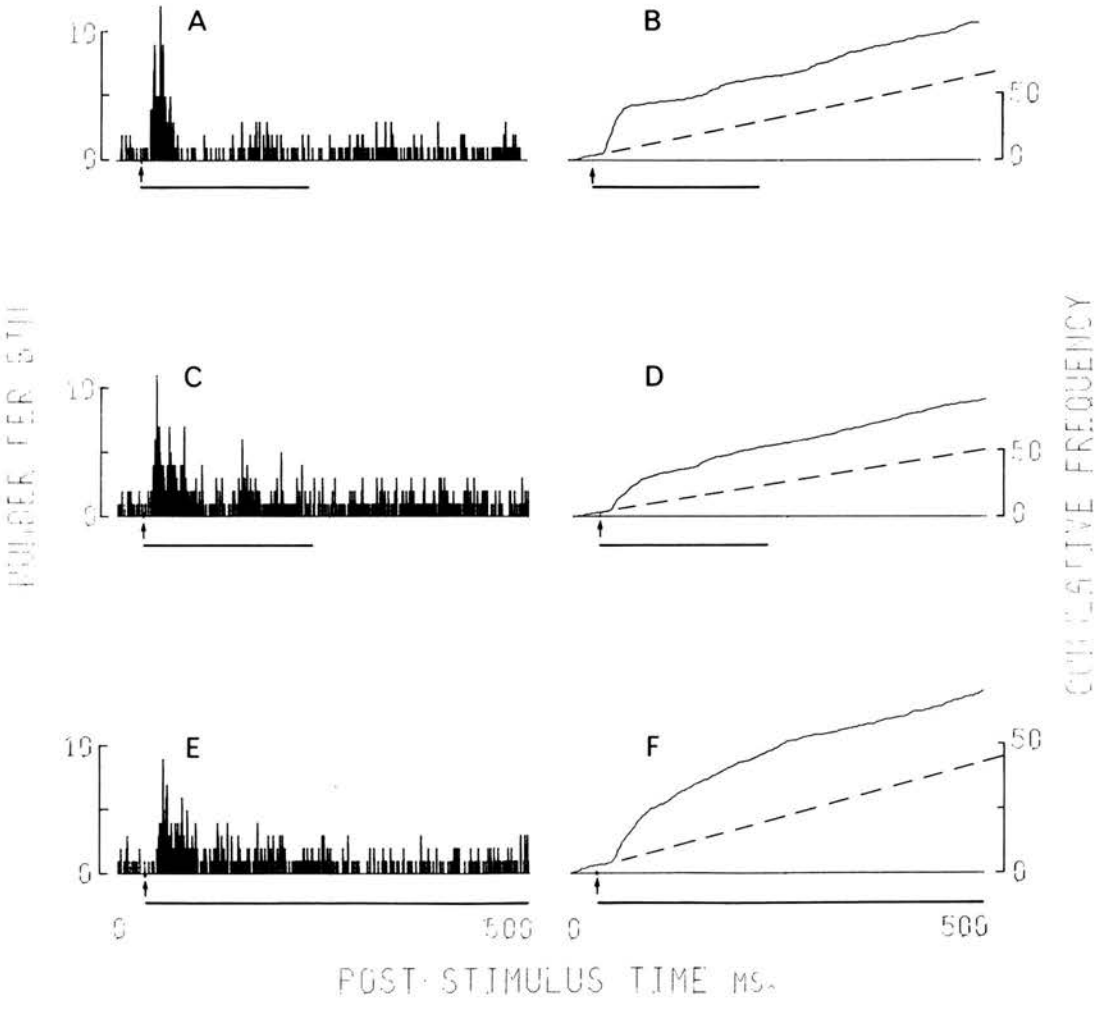

A
B
C
D
E
F
10
0
50
0
0
500
POST-STIMULUS TIME MS.

Fig. 46. The PSTH and CFD graphs of two adjacent Purkinje cells responding to a 3^{o} movement of a maxillary vibrissa applied at a velocity of 300^{o}/sec. and sustained for the duration of the solid line drawn under the time axis. Details of these responses are given in the text.

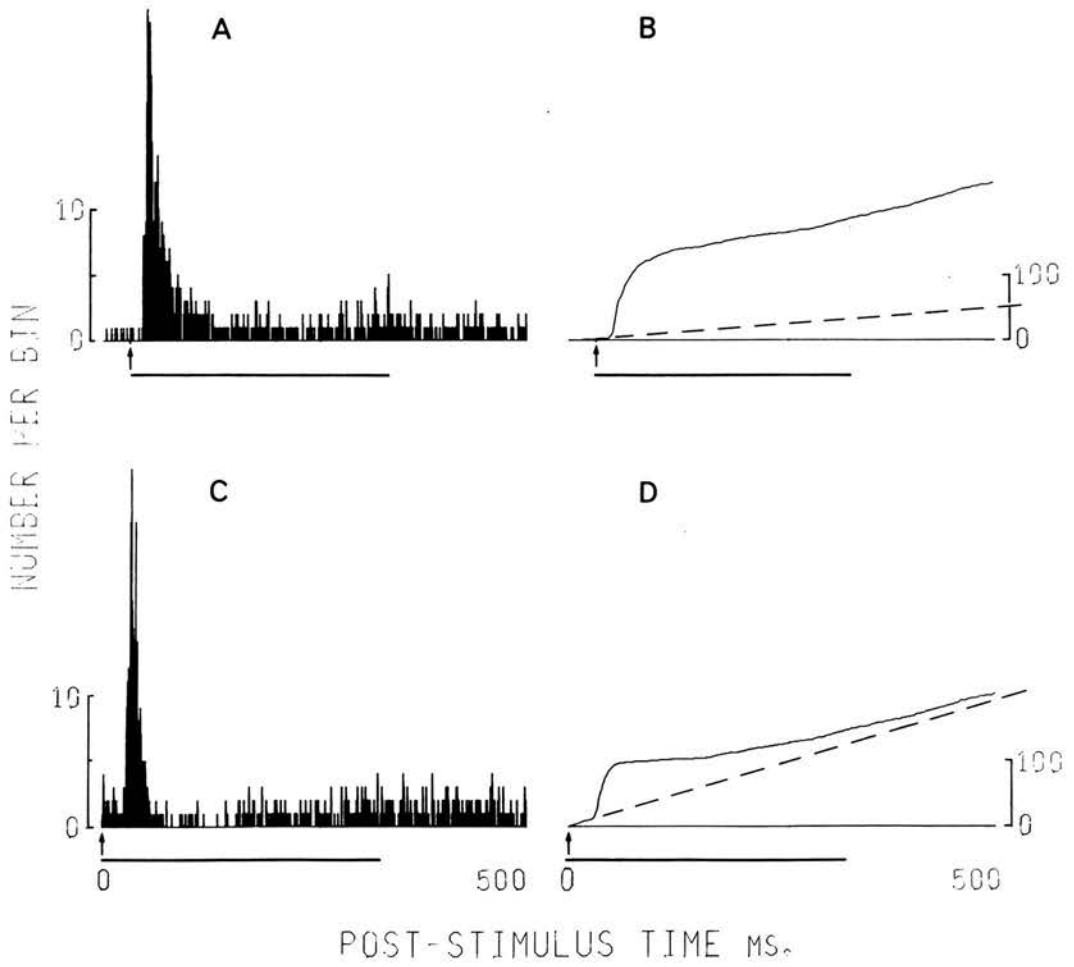
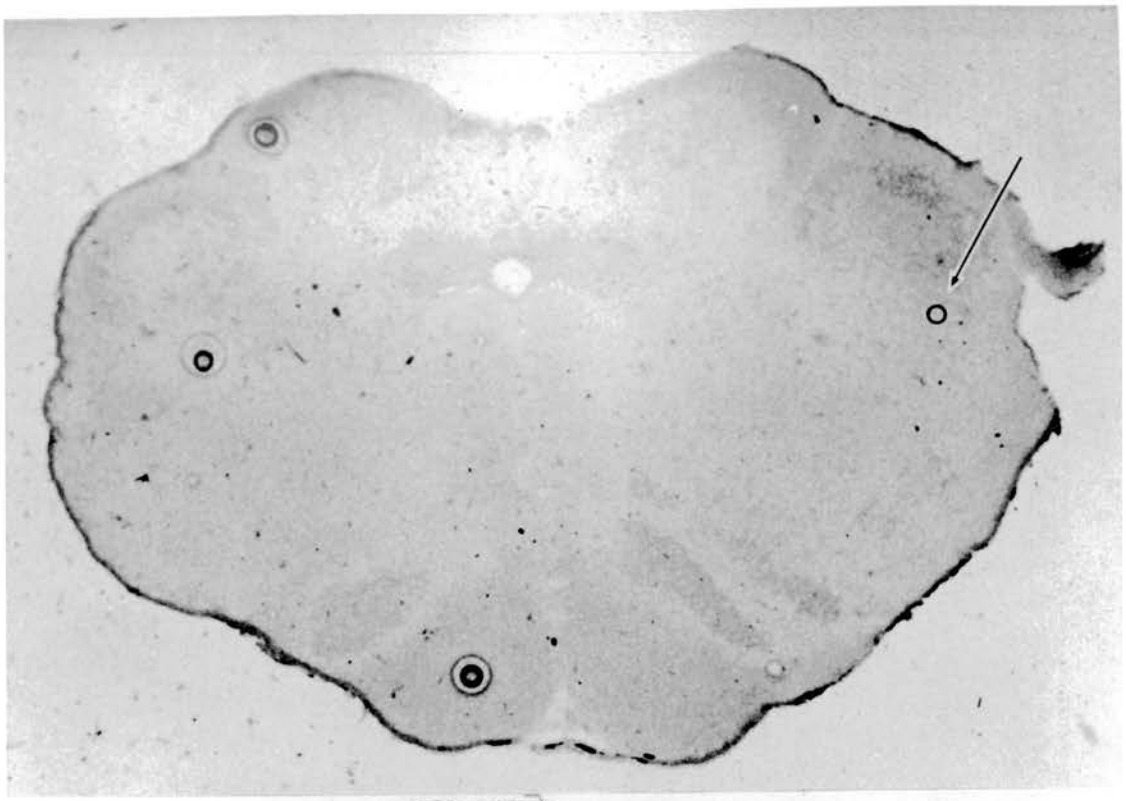
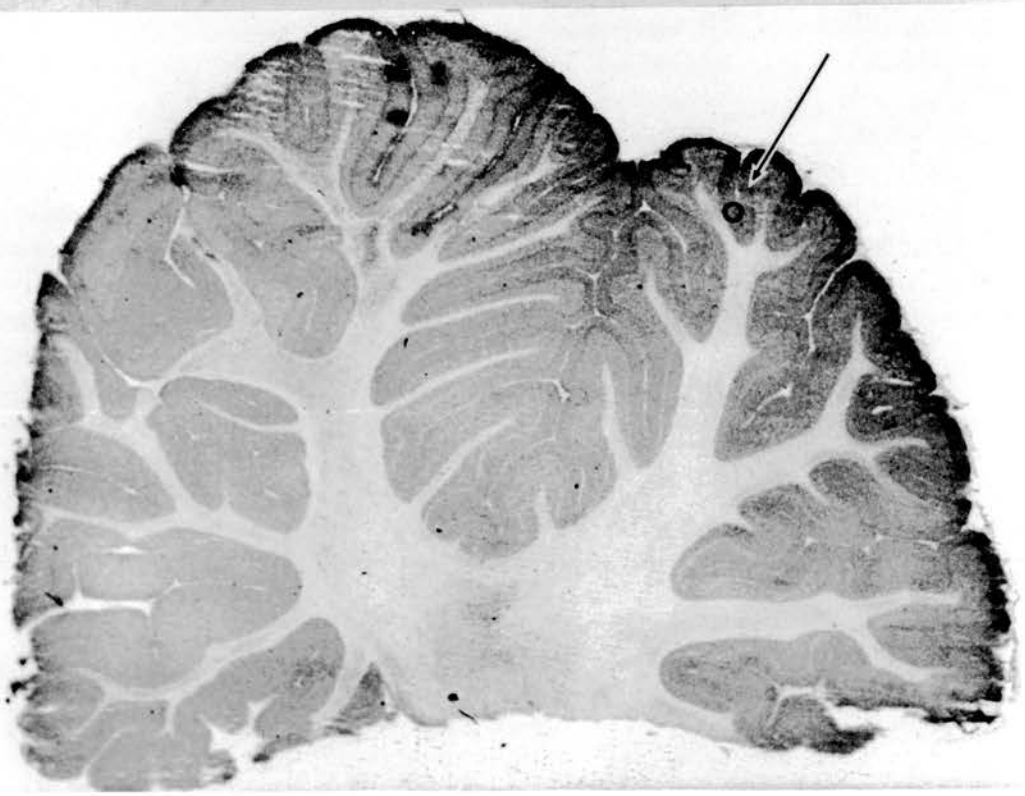

A
B
C
D
10
0
100
0
NUMBER PER BIN
CUMULATIVE FREQUENCY
0
500
POST-STIMULUS TIME MS.

Fig. 47. A. Transverse section through the medulla at the level of the obex showing the position at which a trigemino-cerebellar unit was recorded.

B. Parasaggital section through the vermis showing the position of recording in the Purkinje cell layer.

The recording sites were marked with a Pontamine sky-blue spot and sections stained with luxol blue as described in appendix II.





SECTION V

General Discussion

Throughout this study the maxillary vibrissae have been regarded as tactile organs in the sense that they convey detailed information relating to the contact or imminent contact between external objects and the face of the cat. The possible use of these hairs as a composite organ in orientation and searching behaviour, especially in the dark, is stressed.

The innervation of the sinus hair follicles was shown, in section II, to consist of mainly fast myelinated axons which may be divided into four main groups according to the properties of the responses evoked by movement of the sinus hair. These responses were taken to originate in the morphologically distinct receptor terminals described by Andres (1966), and shown to vary according to the amplitude, velocity and direction of hair movement. The range of stimuli to which sinus hair follicle responses may be obtained is very wide. For example, the high velocity rapidly adapting (HVRA) discharge attributed to the lamellated Golgi-Mazzoni corpuscles in the sinus hair follicle may respond faithfully to vibratory stimuli of 1000 Hz or more whereas the slowly adapting St II(A) response may persist unchanged over the duration of a hair deflection maintained for several hours. Between these extremes there is a range of responses to different amplitudes and velocities of movement in the dynamic and static discharges of the slowly adapting responses, the low velocity threshold rapidly adapting responses and less sensitive HVRA responses. By using finely controlled mechanical stimuli as described by Brown & Iggo (1967) it is possible

to define and differentiate between the four main types of sinus hair follicle responses on a quantitative basis.

The sinus hair follicle may thus be regarded as a detector of mechanical disturbances, with individual components (receptors) 'tuned' to a restricted range of frequencies of movement, able to code both amplitude and velocity by the frequency of afferent discharge and the types of active receptors. The range of stimulus frequencies which may be detected extends from greater than 1000 Hz to constant or 'DC' deflection of the sinus hairs. The various mechanical components of a stimulus which, in nature, may affect several hairs, are 'filtered out' by the appropriate receptor structures according to their mechanical and membrane properties. The coded discharges of each of these is then transmitted to the central nervous system via the trigeminal sensory complex.

The suggestion was made in section I that the dissociation of the various components of the primary afferent response may be related to their functional role and that functional specificity might be revealed by the distribution and onward projections of these responses at the level of the trigeminal sensory complex. The slowly adapting discharges were proposed as candidates for a particular role in cerebellar function, since the information relating to degree of sinus hair deflection over a long period of time may be of use in orientation and searching behaviour and would therefore be expected to project to the cerebellum. The cutaneous SA I mechanoreceptor has been shown to project to the anterior lobe of the cerebellum

via the dorsal spino-cerebellar tract (Mann, 1971) and moreover, the involvement of this receptor in a strictly 'sensory' function has been challenged (Harrington & Merzenich, 1970).

The transmission of the slowly adapting discharge to the spinal trigeminal nucleus has been shown to take place, but with no apparent organisation beyond that imposed by a rough somatotopic localisation within all transverse planes of the rostro-caudal axis as originally described by Kruger & Michel (1962). No systemic rostro-caudal variation of receptive field size was encountered within the spinal trigeminal sub-nuclei as has been suggested by Wall & Taub (1962) and Darian-Smith et al. (1963).

Analysis of the statistical properties of the slowly adapting discharges have shown that various aspects of the impinging stimuli are transduced by the receptor and transmitted as discriminable levels of neural activity. The consistent relationship between rate of discharge and the intensity (amplitude/velocity) of an applied stimulus is described most parsimoniously by a power function for both the first and second-order discharges. The variability and serial order statistical measures of the two main slowly adapting discharge types imply considerable differences in the generator processes involved, differences which may be related to morphological features of the terminal structures.

The proportions of slowly to rapidly adapting responses falls from approximately 66:33 in the primary afferent nerve

to 21:79 in that part of the trigeminal sensory complex sampled. Those second-order cells which receive input from the sinus hair follicle slowly adapting receptors, as described in section III, showed an increase in variability measured in both the distribution and serial-order statistics of the discharge. This increased variability, coupled with an ostensibly unchanged relationship between stimulus and response in the first and second order discharges, indicates a loss of stimulus information in the order of 20%. The distinctive features of the two main types of discharge; variability, adaptive properties and directional sensitivity, are, however, preserved in the slowly adapting responses of cells in the trigeminal nucleus. The ability of the experimenter to recognise the essential features of the primary afferent input to these cells depends largely on the statistical methodology employed providing the necessary quantitative criteria.

The cells with slowly adapting responses to movements of the sinus hairs did not have a direct projection to the anterior lobe of the cerebellum and therefore the proposal made in section I that there exists a trigemino-cerebellar pathway, relaying in the spinal nucleus and homologous with DSCT, must now be rejected.

Neurones which respond phasically to light brushing of the facial hairs, including the vibrissae, form the great majority of cells within the trigeminal sensory complex, almost half of these responding to high frequency vibratory stimuli and therefore receiving input from the sinus hair follicle HVRA receptor - probably the

Golgi-Mazzoni corpuscle. The rapidly adapting responses were also distributed evenly over the rostro-caudal axis of the spinal nucleus. All morphologically distinct sub-nuclei (Olszewski, 1950; Eisenman et al., 1963) have the potential, therefore, of participating in the function of fine touch discrimination. Mosso & Kruger (1973) found cells of the pericornual rim of the caudal nucleus which respond specifically to noxious and thermal stimuli applied to the facial skin. The presence or absence of these cells in the rostral sub-nuclei has not been investigated in the present work. Their absence from these regions might resolve the long-standing controversy over the segregation of 'medial lemniscal' and 'anterolateral' function within the spinal trigeminal nucleus which may be traced to the neurosurgical investigations of Sjöqvist (1938). Such cells appear to share the properties of those reported in the dorsal horn of spinal segments by Christensen & Perl (1970). If found only in the caudal trigeminal nucleus these cells would be decoupled from their afferent input by trigeminal tractotomy at the level of the obex. Previous investigations of the trigeminal nucleus (Kruger et al., 1961; Wall & Taub, 1962; Eisenman et al., 1963) have failed to report these specifically nociceptive and thermoreceptive cells because of inadequate stimulation and sampling techniques.

The original proposition that the vibrissae were involved in the operations of the cerebellum is upheld by the finding that in the ventral zone of the trigeminal sensory complex there are cells which possess a direct projection to the anterior lobe of the cerebellum

(latency $<$2ms.). These cells respond phasically to movement of the maxillary vibrissae with extensive receptive fields often including one or more limb. The highly convergent input to these cells with both inhibitory and excitatory receptive fields and their location ventral to the spinal trigeminal nucleus indicates that these were cells of the lateral reticular nucleus forming part of a trigemino-reticulo-cerebellar pathway.

It is possible to utilise the unique properties of the cerebellar Purkinje cell discharge to establish the routes of afferent excitation involved in trigemino-cerebellar function. A consideration of the latencies, receptive fields and discharge patterns of evoked Purkinje cell responses reveals that two main afferent pathways are involved. The long latency of the mossy fibre responses and their extensive receptive fields to cutaneous stimulation is consistent with those of a reticulo-cerebellar pathway (Grant et al., 1966; Oscarsson, 1967a; Rosen & Scheid, 1973b).

The absence of a direct trigemino-cerebellar pathway homologous with DSCT or VSCT, projecting as mossy fibres with a short latency of action upon the cells of the cerebellar cortex, was not expected. Such a pathway may have relayed in the main sensory nucleus which was not investigated. A direct pathway to another area of the cerebellum outside lobules V and VI of the vermis is also possible as Snider & Stowell (1944) reported a small area of projection from the vibrissae to the paramedian lobules.

The weak and diffuse nature of the mossy fibre projection to lobules V and VI is complemented by a powerful climbing fibre input, as measured by the large and consistent complex discharge. This discharge is only evoked by climbing fibres which are axons of cells of the inferior olive (Eccles et al., 1966a). This pathway is probably relayed in the contralateral inferior olive if it represents a facial equivalent of the contralateral ventral flexor reflex tract (Oscarsson, 1967a,b; 1973). A relatively simple test of this would be to make a mid-line section and so interrupt any tract passing (probably via the medial lemniscus) to the contralateral inferior olive.

The responses of this pathway were phasic and excitatory which is largely consistent with the observations of Eccles et al. (1972c) and Leicht et al. (1973a) who suggest that there is a large projection of the responses of Pacini corpuscles via this pathway. Eccles et al. (1972d) suggest that the climbing fibre input may serve as a phasic control imposed on the tonically active mossy fibre evoked discharges. It is probable therefore that the Golgi-Mazzoni corpuscle of the sinus hair follicle is involved in the trigemino-olivo-cerebellar pathway although the dynamic responses of the slowly adapting receptors might also take part in the excitation of inferior olivary neurones (Armstrong et al., 1968; Crill, 1970). This would provide a reliable response to a wide range of stimulus velocities.

Consistent with the absence of a pathway homologous

with DSCT is the absence of tonic excitatory or inhibitory actions by mossy fibre input on the Purkinje cell discharge as described by Eccles et al. (1972 b,d). The reticulo-cerebellar pathway is regarded by Oscarsson (1967a) and Rosen & Scheid (1973c) as conveying information relating to the 'transmittability' of segmental interneurones or the states of excitation and inhibition which obtain at spinal levels. Climbing fibre projections from the spinal cord and probably the facial region, seem to have a more specific effect on Purkinje cell activity and the organisation of cutaneous climbing fibre evoked responses into functionally rather than purely topographically related groupings has been reported (Eccles et al., 1972c; Leicht et al., 1973a). However, in these papers as in the present results a convergence of both mossy and climbing fibre input was commonly observed.

The powerful climbing fibre projection from the vibrissae may reflect their role as a high gain signalling device relating the position of external objects relative to the head and utilised in conjunction with kinesthetic, proprioceptive and exteroceptive information from the spino-cerebellar pathways. The absence of fast conducting pathways from the facial region might then be explained by the absence of large muscle groups involved in locomotion within the facial segments. The condensation of a large number of receptors of different responses properties within the sinus hair follicles ensures a maximal response to a wide range of mechanical stimuli.

In terms of cerebellar function the abrupt, high

frequency climbing fibre discharge may act as an instant 'read out' of the integrated activity of Purkinje cells relayed to motor nuclei and the cerebral cortex. This facility may provide the ability to correct ongoing motor activity in the light of new information received from the periphery and the evolution of new motor patterns (Eccles *et al*, 1967; Llinas, 1971).

In this interpretation of the results of this study an attempt has been made to view the function of mechano-receptive afferents in as wide a sense as possible, recognising the possibility of parallel processing of tactile information in separate functional pathways. The experimental work has been directed towards establishing the role of tactile information in the synthesis and control of motor activity by its influence on cells of the cerebellar cortex. This in itself would seem to pre-define the function of such an influence by the presumption of a purely motor operation of the cerebellum. However, in terms of the <u>perception</u> of the spatial and temporal relationships between the body and external objects the cerebellum may not act simply as a high order motor ganglion but as an integral part of a more broadly defined somatosensory system.

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APPENDIX I

NEURAL DATA MONITOR

DESCRIPTION:

The tape provided contains 8 programmes which have proved useful in the analysis of simple neural activity using the PDP-12 computer. Each programme is loaded by a monitor system which, in turn, can be called from any of the programmes. The object being to cut the amount of time spent in finding the appropriate programmes during an experiment.

START-UP procedure:

The monitor may be called from the DIAL system by loading the programme STARTUP , which is stored on the system tape, independent of the MONITOR system. More usefully, the MONITOR may be BOOTSTRAPPED by the following sequence of steps.

1. Mount the MONITOR SYSTEM on tape unit 8. Select REMOTE and WRITE LOCK.
2. Mount the DATA TAPE on tape unit 1. Select REMOTE and WRITE ENABLE.
3. Set the CONSOLE SWITCHES to:-

   Ø 7 Ø Ø (LSW) Ø Ø Ø Ø (RSW)

4. Set the MODE switch to LINC
5. Press I/O preset
6. Press DO (Wait for tape)
7. Press START 20

The screen will display an INDEX of the programmes available, numbered 1 to 8.

To select the required programmes type its number, followed by linefeed.

The programmes available are:-

1. COLLECT 72:

   A programme to collect inter-spike interval data in real-time and store on linc tape. (page 4)

2. DATA 72:

   A programme to analyse sequential histograms and post-stimulus-time rasters. (page 7)

3. MAGSPY:

   A modified version of the basic DEC programme to view collected data on linctape. (page 9)

4. NEWTAPE:

   A utility programme which will clear all 1600 blocks of a linctape to zero. (page 9)

5. TAPEDUMP:

   A programme to type out data stored on the linctape octal or decimal format. (page 9)

6. SIGAVGI:

   The DEC programme initialised to one channel of input for collecting evoked potentials and storing them on linctape. (page 10)

7. SINPRE:

   A programme used in conjection with SIGAVGI which converts the double-precision file stored by SIGAVGI into a single-precision file which is stored on specified tape blocks. (page 15)

8. DIAL:

   The LAP 6 - DIAL system under which other stored programmes are accessible. (page 15)

THE MONITOR SYSTEM OCCUPIES TAPE BLOCKS Ø - 160, DO NOT OVERWRITE THIS AREA.

In core, the MONITOR occupies memory quarter 7 starting at absolute location 14Ø20 in LINC mode.

PROGRAMME DESCRIPTIONS

1. COLLECT 72:

This programme measures the time between events via the real-time clock and stores these times sequentially and as a histogram. The time-intervals may be measured between action potentials if they are collected on clock channel 3, or between a trigger pulse on channel 3 and the action potentials on channel 2. Clock channel 1 may be used to input an external time base

THE INPUTS:

A. For the collection of successive inter-spike intervals and the construction of an interval histogram.

- ACTION POTENTIALS TO CLOCK CHANNEL 3 INPUT.
  (OPTIONAL: CLOCK OUTPUT 3 TO ANALOG CHANNEL 10)
  (OPTIONAL: If selected, External time base to CLOCK CHANNEL 1)

B. For the collection of post-stimulus-time rasters and the construction of a post-stimulus-time histogram.

- ACTION POTENTIALS TO CLOCK CHANNEL 2
- TRIGGER PULSE TO CLOCK CHANNEL 3
  (OPTIONAL: If selected, External time base to CLOCK CHANNEL 1)
  (OPTIONAL: CLOCK OUTPUT 2 or 3 to ANALOG CHANNEL 10)

When called, the programme will type out

"CHECK KW12 TRIGGER, SET BIN WIDTH, THEN HIT L/F:"

At this point the programme will stay in a loop waiting for an input event on ANY of the clock channels. (test this by switching to LINE on any clock channel)

Step I: On receiving a supra-threshold event the screen will display the input to ANALOG CHANNEL 10 so enabling the threshold level and the polarity of any CLOCK INPUT to be checked, as long as the appropriate CLOCK OUTPUT is fed to ANALOG CHANNEL 10.

Step 2: Having satisfied the triggering requirements of each channel the BIN WIDTH or SAMPLING RATE of the real-time clock must be determined by the settings of the first 3 bits of the right switches (Bits 0, 1, 2)

BIT Ø	DOWN	=	0. 1 MSEC BINWIDTH
BIT 1	DOWN	=	1 MSEC BINWIDTH
BIT 2	DOWN	=	10 MSEC BINWIDTH
ALL BITS	UP	=	BINWIDTH DETERMINED BY FREQUENCY OF PULSES ON CLOCK CHANNEL 1 FROM AN EXTERNAL SOURCE.

Each time data collection begins these 3 bits will be interrogated. The binwidth may therefore be left unchanged throughout several data collection runs, or changed according to the frequency of the nerve activity. The programme is written such that inter-spike intervals or post-stimulus-times longer than 500 x BINWIDTH are counted but not stored. (The overflow count is stored in location 511 of the HISTOGRAM). This limitation is imposed by the C.R.T. display.

Step 3: Having set the binwidth, type LINEFEED

The computer will respond with:-

"FROM BLOCK":

Step 4: Now type in a 4-digit block number from Ø - 156Ø with leading zeros in place (e.g. Ø123), and type RETURN.

Numbers less than 2Ø will be set to 2Ø so preserving blocks Ø - 2Ø of the tape. Numbers greater than 156Ø or containing illegal characters (for example: 'O' as in FOOL). Will cause a "?" to be typed and the words "FROM BLOCK:" to be re-typed.

On receiving the RETURN, the computer will type:-

"IDENTIFY:"

Step 5: Type in the unit identification (up to 35 characters) ending with RETURN.

The programme will now store each 256 successive intervals on the data tape (unit 1) whilst continually displaying the successive intervals or the distribution histogram according to the sense switch settings explained below:-

Sense switch Ø DOWN - freeze data collection.

Sense switch 1 DOWN - display successive intervals

" " 1 UP - display a histogram

Sense switch 2 DOWN - display the input to analyse channels 10 and 14 as a free run oscilloscope.
(Data collection frozen)

In the successive interval mode (SNS 1 DOWN) the ORDINATE is Ø - 512 x BINWIDTH MSEC., the ABSCISSA is Ø - 512 EVENTS.

In the interval histogram mode (SNS 1 U P) the ORDINATE is Ø - 512 EVENTS per BIN, the ABSCISSA is Ø - 512 x BINWIDTH MSEC.

Step 6: When the required number of events have been collected HIT ANY KEY ON THE TELETYPE. THE NEXT EVENT will cause the display to disappear for a few seconds whilst the data is permanently stored on linctape. If nerve discharge has stopped switch the source knob of clock channels 2 or 3 to line frequency to provide the trigger event. (Don't forget to switch it back!)

The computer will then display the data collected (according to SNS 1 as well as the unit identification and the BLOCK NUMBER of the stored INTERVAL HISTOGRAM.

labelled BN = - - - - - on the screen.
(During data collection BN = the starting block on tape)
During this display cycle (after collection) the computer will respond to teletype input. (Unless frozen by SNS Ø, or SNS 2). The instructions which may be issued are:-

CNTRL/C ; continue data collection on successive tape blocks. (2 BLANK blocks are left between each collection)
CNTRL/R : To overwrite the data you have just collected if it was unsatisfactory.
CNTRL/B : To change the block number completely for the next data collection.
CNTRL/T : To re-set the KW12 trigger level (back to step 1)
CNTRL/M : Return to the MONITOR
CNTRL/D : Return to DIAL

The BIN WIDTH may be changed at this point by altering RSW 0, 1, 2 as in STEP 2.
Illegal input to the teletype will cause a "?" to be typed.
This programme occupies most of the lower 4K of memory and starts at Ø4Ø2Ø in 8 mode.
The minimum interval between events is about 500 microseconds at which point spikes may be missed when data is transferred to the tape every 256 events.

THE DATA STORAGE

Each 256 successive events are copied from alterating halves of the display area (SNS 1 DOWN) when a key is struck the collection stops, the remaining portion of the current display half is cleared of old data and stored. The interval histograms occupies the next two tape blocks and the identification and starting block (BN = - - - - -) information is stored on the next block (1 ASC II character per word). Two blank blocks will be left before the next data collection where other relevant data may be stored off-line.
The successive interval data will never exceed 1ØØØ OCTAL, all data is stored in 12-BIT binary format.

2. DATA 72

When called, this programme will display the contents of the two display buffers which probably contain rubbish. All data must be loaded from linctape (UNIT 1) and will be dumped into absolute core locations 1ØØØ - 1777 (viewed with SNS I UP = THE DATA AREA.) All calculations are performed on data moved to absolute core locations 2ØØØ - 2777 (viewed with SNS I DOWN = THE CALCULATION AREA.

PRINCIPLES of OPERATION

The programme is designed to calculate simple statistics of post stimulus time rasters and successive interval data. The data in the DATA AREA may be segregated by the use of horizontal and vertical cursors, and operated upon by a number of commands:-

R: Read 2 blocks of data from block - - - - A 4 - digit octal block number between Ø and 1574 must be typed in, leading zeroes included. There is no error detection for this routin

P: Move all data from the DATA AREA to the CALCULATION AREA.

J: Move all data between the vertical censors to the CALCULATION AREA.

I: Transform all post-stimulus time raster data to successive intervals (DIFFERENTIATE).

M: Transform all post-stimulus time raster data between the horizontal and vertical censors to the CALCULATION AREA.

X: Scale all data up by 2. (data will be set to zero when 512 exceeded).

Z: Scale all data down by 2.

T: Display data on double tune-base (cursors only operate on normal-time data).

N: Display data on normal-time base.

C: Clear the data in the DATA AREA to zero, between the vertical censors. This does not transfer data to the CALCULATION AREA.

L: View the stored LABELLING of the Data Set after reading the appropriate tape block into the DATA AREA. (The last block of each collection run). Return to the normal display by hitting ANY KEY.

D: Return to the DIAL EDITOR on tape unit Ø.

#: Calculate the number of stimulus presentations in the selected raster sample in the CALCULATION AREA (Selected with M or I) and the mean number of SPIKES/RESPONSE (Only accurate when there are single points on the baseline of the CALCULATION AREA after typing M or I, representing the start of each stimulus presentation.

$ Calculate the NUMBER OF SPIKES, MEAN, STANDARD DEVIATION and COEFFICIENT OF VARIATION of successive interval or TRANSFORMED post-stimulus-time data. That is, after typing P, J, I & M.

CNTRL/R Return to the MONITOR.

THE SENSE SWITCHES:

SNS Ø DOWN = view cursors only

SNS 1 DOWN = view the CALCULATION (AREA (2000-2777))

SNS 1 UP = view the DATA AREA (1000 - 1777)

SNS 2 DOWN = remove the cursors from the display.

THE CURSORS:

Analog channels Ø and 4 move the horizontal cursors, channel 7 positions both horizontal cursors.

Analog channels 1 and 5 move the vertical cursors, channel 3 position both vertical cursors.

The 4-digit octal numbers displayed in the upper left-hand corner of the screen are the DECIMAL values of the distances between the two vertical (upper) and two horizontal (lower) cursors.

For all options which use the cursors these numbers must lie between Ø and 512. If the cursors are positioned the wrong way around a negative number will result which will cause the computer to return to the display without executing the option selected. (- 1 = 4095). Re-set the cursors to values between Ø and 512 and repeat. (Remember zero lies in the middle of the screen in the vertical direction for the horizontal cursors).

Direct readings of latencies and interspike intervals may be measured by the cursors without using the calculation routines.

The calculation routines do not analyse distribution or post-stimulus time HISTOGRAMS,

The programme occupies most of the lower 4K of memory and starts at absolute location 2ØØ in 8 mode.

3. MAGSPY

This programme is a modified version of DEC-12-USZA which accepts 16ØØ word data tapes when loaded the block number and unit number will be asked for on the display leading zeroes need not be typed in. RUBOUT erases single characters, ALT/MODE erases all characters. CNTRL/D will return control to the MONITOR. Terminate input with LINEFEED and the tape data will be displayed.

Analog Knob Ø controls the speed and direction of tape motion.

Knob 4	scales data DOWN
ALT/W	displays binary words
ALT/A	displays ASCII characters
ALT/O	displays octal values
ALT/F	displays full-size characters
ALT/H	displays half-size characters
CNTRL/D	returns control to the MONITOR.

4. NEWTAPE

When loaded NEWTAPE displays:-

"MAGZERO
puts zero on tape I
press LF to continue"

The programme will clear a tape of old data, programmes or MARK 12 checknumbers-make sure you clear the correct tape.
The programme automatically returns control to the MONITOR.

5. TAPEDUMP

The programme will type out any data stored on the linctape and is self-explanatory. The format for data may be OCTAL or DECIMAL always select MEMORY QUARTER Ø.
After dumping the data the screen will display
the question: "PRINT MORE"
The response Y will re-start tapedump
The response N will re-load DIAL
It is not possible to return directly to the MONITOR from this programme.
This programme accepts only 777 blocks of tape.

APPENDIX II.

The preparation of material for histology.

1 - 40μ sections cut using the cryostat microtome at -20°C.

2 - 70% alcohol for 1 - 2 minutes.

3 - Luxol blue; 1% in 70% alcohol for 2 - 3 hours.

4 - 70% alcohol dip.

5 - 30% alcohol for 1 minute.

6 - Cresyl violet for 10 minutes.

7 - Tap water dip.

8 - Distilled water 1 - 2 minutes.

9 - 50% alcohol for long enough to wash out excess cresyl violet.

10 - 70% alcohol dip.

11 - 95% alcohol for 1 - 2 minutes or longer to decolourise.

12 - Absolute alcohol 1 for 1 - 2 minutes.

13 - Absolute alcohol 2 for 1 - 2 minutes.

14 - Xylene for 5 minutes.

15 - Mount in DePex.

If the cresyl violet counterstain is not required steps 5 - 10 inclusively may be ommitted.