AN INVESTIGATION OF NEUROENDOCRINE SYSTEMS BY METABOLIC MAPPING IN RODENT BRAIN

by

RUTH CAMPBELL SUTHERLAND

A Thesis submitted for the degree of Doctor of Philosophy.

University of Edinburgh

November 1983

To my parents

I declare that this thesis was composed by myself, and that all the experimental work described herewith was performed by myself, with the following exceptions:

1. Measurements of plasma osmolality and immunohistochemical staining of brain sections for neurophysin (Chaper III) were performed by Dr. John Morris, Department of Human Anatomy, University of Oxford;
2. Implantation of some electrodes in Chapter VI were performed by Dr. George Fink, MRC Brain Metabolism Unit, Department of Pharmacology, University of Edinburgh.

This thesis has not been, and will not be submitted in candidature for any other degree or professional qualification.

Ruth C. Sutherland 22.11.83

MRC Brain Metabolism Unit,
Department of Pharmacology,
1 George Square,
Edinburgh EH8 9JZ

Dr. George Fink 22/11/1983

MRC Brain Metabolism Unit,
Department of Pharmacology,
1 George Square,
Edinburgh EH8 9JZ

Published Results

Results obtained from this thesis have been published in the following form, and are included in Appendix VII:

1. Sutherland, R.C., Martin, M.J., McQueen, J.K. and Fink, G. (1983). Water deprivation results in increased 2-deoxyglucose uptake by paraventricular neurones as well as pars nervosa in Wistar and Brattleboro rats. Brain Res. 271, 101-108.
2. Sutherland, R.C., and Fink, G. (1983). The milk ejection pathway in brain studied with the 2-deoxyglucose method. Brain Res. 273, 291-296.

ACKNOWLEDGEMENTS

I would like to thank Dr. George Fink and Professor Bernard Ginsborg for providing me with the opportunity to work in the Medical Research Council; Brain Metabolism Unit at the Department of Pharmacology, University of Edinburgh. I would also like to thank Dr. Fink and Dr. Judith McQueen for their supervision of my work during the last three years, and their unfailing enthusiasm, support and advice was greatly appreciated. For their assistance with radioimmunoassay and glucose assay techniques I would like to thank Mrs. Graciela Sanchez-Watts, Ms. Elizabeth Nairn, Mr. John Bennie and Mr. John Sheward. My thanks also to Mrs. Jean Hunter and the animal house staff for their expert care of the animals. For their expertise and patience in the typing of this thesis I would like to thank Ms. Jo Donnelly, Mrs. Norma Brearley and Mrs. Celia Leitch.

My thanks to Dr. John Morris, Department of Human Anatomy, University of Oxford, for providing me with the mice and for his helpful advice, and also to Dr. Harry Charlton, Department of Human Anatomy, University of Oxford, for providing me with the voles. My thanks to Drs. G.D. Niswender and L.E. Reichert, National Institute of Arthritis, Diabetes, Digestive and Kidney Disease, Baltimore, U.S.A. for the materials used in the radioimmunoassays. I would also like to thank Dr. James McCulloch, Wellcome Surgical Institute, University of Glasgow, for allowing me the use of computer facilities.

Personal support provided by the Faculty of Medicine (Ethel and

Colin Gordon scholarship) is gratefully acknowledged. Finally, I would like to give my special thanks to my parents and sister, Honor, for their constant support and understanding throughout the course of my studies.

Glossary of Abbreviations for Neuroanatomical Structures used throughout this Thesis.

AAA	anterior amygdaloid area
ABL	basal amygdaloid nucleus
ACB	nucleus accumbens
ACE	central amygdaloid nucleus
AHA	anterior hypothalamic area
AL	lateral amygdaloid nucleus
AMYG	amygdala
AMYG CE+L	central and lateral amygdaloid nuclei
AMYG CO+M	cortical and medial amygdaloid nuclei
APVN	anterior region of the paraventricular hypothalamic nucleus
ARC	arcuate nucleus
AVT	anteroventral thalamic area
BST	bed nucleus of the stria terminalis
CA	anterior commissure
CC	corpus callosum
CG	midbrain central grey
CI	inferior colliculus
CP	caudate putamen
CS	superior colliculus
CX	visual cortex
DBB	diagonal band of Broca
DG	dentate gyrus
DHIPP	dorsal hippocampus
DHIPPG	dorsal hippocampus (granular layer)
DHIPPM	dorsal hippocampus (molecular layer)
DLF	dorsal longitudinal fasciculus
DMH	dorsomedial hypothalamic nucleus
DR	dorsal raphe nucleus
DVC	dorsal vagal complex
FI	fimbria
FRCX	frontal cortex
FX	fornix
HAB	habenular nucleus
HAB-l	lateral habenular nucleus
HAB-m	medial habenular nucleus
HIPP	hippocampus
HP	habenular-interpeduncular tract
IC	internal capsule
IP	interpeduncular nucleus
LC	locus coeruleus
LGEN	lateral geniculate body

LLM lateral lemniscus
LT lateral thalamic area

MBH medial basal hypothalamic area
MCHT medial corticohypothalamic tract
MDT dorsomedial thalamic area
ME median eminence
MFB medial forebrain bundle
MGEN medial geniculate body
MLM medial lemniscus
MM mamillary body

NR red nucleus
NTS nucleus of the solitary tract

OVLT organum vasculosum laminae terminalis

P pons
PD pars distalis
PH posterior hypothalamic area
PI pars intermedia
PIN pineal gland
PN pars nervosa
POA preoptic area
POA-l lateral preoptic area
POA-m medial preoptic area
PVH periventricular hypothalamic area
PVN paraventricular hypothalamic nucleus
PVT paraventricular thalamic area
PYRCX pyriform cortex

RA raphe nucleus
RE reuniens thalamic nucleus
RF midbrain reticular formation

S septum
SC spinal cord
SCN suprachiasmatic nucleus
SCX subicular cortex
SFO subfornical organ
S-l lateral septum
SM stria medullaris
S-m medial septum
SN substantia nigra
SON supraoptic hypothalamic nucleus
ST stria terminalis

TL lateral tegmentum
TM medial tegmentum
TSTH spinothalamic tract
TTS tectospinal tract
TV ventral tegmentum

V	ventricle
VHIPP	ventral hippocampus
VHIPPG	ventral hippocampus (granular layer)
VHIPPM	ventral hippocampus (molecular layer)
VLGEN	ventrolateral geniculate body
VmArc	ventromedial-arcuate hypothalamic area
VMH	ventromedial hypothalamic nucleus
VMT	ventromedial thalamic area
VT	ventral thalamic area
ZI	zona incerta

ABSTRACT

The aim of this thesis was to determine whether changes in the regional metabolism of the brain and pituitary gland accompanied several neuroendocrine events in the female rat. Regional brain metabolism was measured by quantitative autoradiography of [^{14}C] 2-deoxyglucose (2DG) content, and expressed relative to that of the corpus callosum ('relative metabolic activity'; rma).

The main findings of studies on the hypothalamo-neurohypophysial system were that water deprivation increased the rma of the pars nervosa (PN) and paraventricular nuclei (PVN) of normal rats, rats with hereditary diabetes insipidus (homozygous Brattleboro) and mice with hereditary nephrogenic diabetes insipidus. Measurements of plasma osmolality and the effects of desamino-D-arginine^{8}-vasopressin showed that the changes in rma of the PN and PVN, which were greatest in the water-deprived Brattleboro rats, were due mainly to changes in plasma osmolality. High resolution autoradiography with [^{3}H]2DG suggested that increased uptake of 2DG occurred around the edge and not in the perikarya of the PVN. Suckling in anaesthetised, but not conscious lactating rats increased significantly the rma of the PVN and SON, but not the PN. The rma of both nuclei and the PN was increased by electrical stimulation of the mammary nerve which also increased the rma of the spinothalamic tract in brainstem.

Studies on the effect of mating in the vole (*Microtus agrestis*), a reflex ovulator, showed that the rma of midbrain central grey (CG) and reticular formation (RF) in females that were allowed to come into contact with male voles was increased significantly compared with the rma of the CG and RF in females that were not allowed to come into contact with the males. The increase in the rma of the midbrain CG and RF occurred with lordosis whether or not intromission and/or LH release occurred.

A series of systematic studies were carried out on the effects of electrical stimulation of several hypothalamic nuclei, the hippocampus and amygdala with a view to determining the pathways activated by stimuli that have been used extensively to investigate neural control of the pituitary gland. The stimulation of each area had distinctive effects, and this study together with an investigation of the functional connections between the suprachiasmatic nuclei and the raphe nuclei and ventrolateral geniculate bodies confirmed and extended findings obtained by classical neuroanatomical, amino-acid autoradiographic and electrophysiological techniques.

Finally, a comparison was made between the rma of brain areas in the rat, vole and mouse.

Overall the studies showed that the 2DG technique is a powerful tool for determining functional discrete, but not diffuse, pathways in the brain that are concerned with the control of neuroendocrine and circadian activity.

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CHAPTER I

Introduction

1.1 Nature of this thesis

Studies of the central nervous system (CNS) have focussed on the localisation of function within, and the mapping of pathways related to specific functions. The approaches used have included histological degeneration and staining techniques, behavioural studies following ablation and stimulation, electrophysiological recording and histochemistry with fluorescent and immunocytochemical techniques and autoradiography of orthograde and retrograde axoplasmic flow. These techniques, while providing a vast amount of neuroanatomical and neurophysiological information, have the same disadvantages; usually only one pathway can be studied at any time, and demonstration of a pathway reveals only the potential for function or a function in an experimental situation, and not necessarily in the normal physiological state.

Functional activity within a tissue is intimately and directly related to energy consumption (Kennedy, Des Rosiers, Jehle, Reivich, Sharp and Sokoloff, 1975; Sokoloff, 1977). Thus, the 2-deoxyglucose technique (2DG), which measures energy metabolism within a tissue, can be used to determine functional activity within the components of the CNS in any given situation (1.3). The anatomical and electrophysiological properties of and neurotransmitter synthesis within the hypothalamo-hypophysial system have been studied extensively in relation to the control and mechanisms of hormone release (e.g. reviews by Cross, Dyball, Dyer, Jones, Lincoln, Morris and Pickering, 1975; Fink and Geffen, 1978; Brownstein, Russell and Gainer, 1980; Vale, Rivier and Brown, 1980; Swanson and Sawchenko, 1983). However, the metabolic activity associated with the

neuroendocrine control of the hypothalamo-hypophysial system has not been widely studied. The aim of this thesis was to investigate changes in metabolic activity associated with changes in functional activity, primarily in neuroendocrine systems but also throughout the rest of the brain, in situations known to cause or be associated with pituitary hormone release.

1.2 Function and neurohumoural control of the pituitary gland

1.2.1 The hypothalamo-neurohypophysial system

In the late 19th century the pituitary gland was commonly regarded as an organ without physiological function, probably the rudiments of an archaic sense organ (Macalister, 1889). However, the observation made by Oliver and Schäfer (1895) that extracts of whole pituitary glands, when injected intravenously into anaesthetised animals induced a rapid rise in blood pressure, instigated the start of neurohypophysial research. The action of pituitary extracts on blood pressure was confirmed by Von Cyon (1898), Livon (1898) and Howell (1898), who also showed that the pressor effect of pituitary extracts was located in the PN. Howell (1898) and Schäfer and Vincent (1899 a,b) also demonstrated that the pituitary extracts could have a depressor effect on blood pressure, an observation which was repeated in studies on the bird (Paton and Watson, 1912; Strahan and Waring, 1954; Woolley, 1959), and was used as an assay method for the standardisation of oxytocin (Coon, 1939).

For many years the PN extracts were thought only to have diuretic properties (Magnus and Schäfer, 1901; Schäfer and Herring, 1908). However, an antidiuretic action was demonstrated by Farini (1913) and Von den Velden (1913), who treated patients with diabetes

insipidus successfully with extracts of the PN. This result was confirmed by Romer, who also demonstrated the antidiuretic property of PN extracts in the anaesthetised rabbit (1914). The site of action of the antidiuretic effect was first shown by Starling and Verney (1924), who demonstrated the effect of PN extracts on the isolated kidney.

The effect of PN extracts on the uterus was first observed by Dale (1906) in the pregnant cat. He showed that PN extracts contracted the uterus in the cat, dog, guinea pig, rat and rabbit _in vitro_ and _in vivo_ (1909). These results were confirmed independently by Ott and Scott (1909) and by Blair Bell (1909), who introduced the use of pituitary extracts into obstetric practice.

Ott and Scott (1910) also demonstrated that PN extracts might be involved in the phenomena associated with milk let-down in the goat. Further studies in the cat and dog (Schäfer and Mackenzie, 1911) and in women (Heaney, 1913; Schäfer, 1913) suggested that neurohypophysial principles caused milk secretion, but Gaines (1915) was the first to differentiate clearly the effects of extracts of the PN on milk ejection and secretion.

Thus, the functions of PN extracts were determined in many animal species, but it was assumed that the pituitary gland was an independent endocrine organ.

The pituitary stalk and PN were known to contain nerve fibres from the brain (Cajal, 1894;1911), originating in the SON and PVN in mammals (Pines, 1925 a,b; Rasmussen, 1940; Romeis, 1940) and in the POA of lower vertebrates (Meyer, 1935). Although the influence of the CNS on the antidiuretic and oxytocin effects of the pituitary

gland had been demonstrated in several classical studies (Verney, 1926; Haterius and Ferguson, 1938; Haterius, 1940; Ferguson, 1941; Verney, 1947; Harris, 1947; Andersson, 1951; Cross and Harris, 1952), the concept of neurosecretion was suggested by Scharrer from his work on the midbrain of the fish (1928; 1930; 1932). Bargmann and Scharrer provided the basic evidence for neurosecretion in the mammal, and suggested that the synthesis of neurohypophysial hormones took place in the perikarya of the PVN and SON (Bargmann and Scharrer, 1951). The neurohypophysial hormones were then shown to be transported to the PN by axoplasmic flow, where they were stored and subsequently released into the blood (Bargmann and Scharrer, 1951; Ortmann, 1951; Schiebler, 1952). The PN was then recognised as a neuroendocrine organ which depended upon the CNS, particularly the hypothalamus, for its normal function. The hormones of the PN were characterised and synthesised by Du Vigneaud and his associates (Du Vigneaud, Gish and Katsoyannis, 1954; Du Vigneaud, Ressler, Swann, Roberts and Katsoyannis, 1954), and the first immunohistochemical studies, using anti-porcine neurophysin serum, were carried out by Livett, Uttenthal and Hope (1971). The latter pioneered the more recent sophisticated immunohistochemical analysis of the anatomy of the hypothalamo-neurohypophysial system (1.2.3).

1.2.2 The hypothalamo-adenohypophysial system

The influence of exteroreceptive stimuli on reproductive function in animals has been known for many years. Light was recognised as being important for gonadal stimulation in birds (e.g. Rowan, 1926; 1930; Bissonette, 1933; Cole, 1933) and mammals

(e.g. Baker and Ranson, 1932; Bissonette, 1932; Marshall and Bowden, 1934; Browman, 1937; Hemmingsen and Krarup, 1937). On the basis of experiments involving hypophysectomy and the injection of extracts of the pituitary gland (Hill and Parkes, 1933; Marshall, 1936), the effects of light on gonadal function were thought to be mediated by the pituitary gland.

Sexual stimuli were also known to affect reproductive function, since coitus was shown to cause ovulation in the rabbit (Barry, 1839; Heape, 1905), ferret (Marshall, 1904) and cat (Courrier and Gros, 1933). Mechanical and copulatory stimulation of the uterine cervix in the rat led to a state of pseudopregnancy (Long and Evans, 1922; Shelesnyak, 1931), indicating that spontaneously ovulating species were also affected by sexual stimuli. Nutrition, temperature and sexual display were also shown to be important factors in the control of reproductive processes (Marshall, 1936; Harris, 1955).

The pituitary gland was known to be essential for reproductive function, since ovulation did not occur in hypophysectomised rabbits (Fee and Parkes, 1929). However, the fact that so many extero-receptive factors could affect reproductive processes led to the concept that the CNS could affect the pituitary gland through a direct anatomical connection. Vogt (1931) proposed that the sympathetic system was the most likely pathway, but studies involving sympathectomy and electrical stimulation of the sympathetic chain provided conflicting reports on the effects on pituitary gland function (Haterius, 1933a,b; Hinsey and Markee, 1933; Brooks, 1935; Friedgood and Pincus, 1935). Clinical studies

first implicated the hypothalamus in the control of reproductive and other functions controlled by the pituitary gland (e.g. Fröhlich, 1901; Cushing, 1932; Theobald, 1936), and experimental studies investigated this further. Electrical stimulation of the hypothalamus caused ovulation in the rabbit (Harris, 1937; Haterius and Derbyshire, 1937), and lesions of the anterior hypothalamus caused disturbances of reproductive processes in a variety of species (e.g. Fisher, Magoun and Ranson, 1938; Biggart and Alexander, 1939; Dey, Fisher, Berry and Ranson, 1940). Ovulation in the rabbit was shown only to occur following electrical stimulation if the stimulus was applied to the tuber cinereum, and not the PD (Markee, Sawyer and Hollinshead, 1946; Harris, 1948).

Although innervation of the pituitary stalk had been demonstrated (Cajal, 1894; Dandy, 1913; Chorobski and Penfield, 1932; Rasmussen, 1938), the control of PD secretion by these fibre systems seemed to be unlikely, since in some species the PD did not appear to be innervated (Rasmussen, 1938; Green, 1951). The concept that the pituitary gland was under neurohumoural control gained credence with the demonstration of a possible vascular link between the hypothalamus and the pituitary gland (Popa and Fielding, 1930; Wislocki and King, 1936). Observations on anaesthetised toads (Houssay, Biasotti and Sammartino, 1935), rats (Green and Harris, 1949) and mice (Worthington, 1955) showed that the direction of blood flow in these hypophysial portal vessels was from the hypothalamus to the pituitary gland. Experiments involving the sectioning of the pituitary stalk, however, caused disruption of reproductive function in some studies (e.g. Richter, 1933;

Harris, 1937; Brooks, 1938; Westman and Jacobsohn, 1938), but did not affect reproductive processes in others (e.g. Dempsey, 1939; Dempsey and Uotila, 1940). Harris (1950) showed that stalk sectioning alone was not sufficient to permanently disrupt reproductive function, since the portal vessels would regenerate and reform the vascular link between the hypothalamus and the pituitary gland. The regeneration of the vessels was confirmed by Harris and Jacobsohn (1952) and by Nikitovitch-Winer and Everett (1958; 1959), and could explain the varying effects of stalk sectioning on gonadal function previously obtained. However, the necrosis of the PD that occurs after cutting the pituitary stalk (e.g. Daniel and Pritchard, 1956; 1975) may contribute to the disruption of reproductive function and results should be interpreted accordingly with caution.

Transplantation of pituitary tissue also provided essential information for the neurohumoural hypothesis. When PD tissue was placed in the richly vascularised region of the anterior chamber of the eye in hypophysectomised animals, gonadal function did not return (Westman and Jacobsohn, 1940). Similarly, transplantations of pituitary tissue under the kidney capsule caused cessation of reproductive function (Nikitovitch-Winer and Everett, 1958). Only when the pituitary tissue was transplanted under the ME, and a vascular connection developed, did reproductive function return (Harris and Jacobsohn, 1952; Nikitovitch-Winer and Everett, 1958; 1959).

These different lines of investigation resulted in the hypothesis that neurones in the hypothalamus secreted substances which affected target cells in the PD by way of the hypophysial

portal vessels. The discovery that hypothalamic extracts could influence PD activity provided the crucial link for the central control of anterior pituitary function (e.g. Harris, 1972; Schally, Arimura and Kastin, 1973).

1.2.3 Anatomy of the hypothalamo-neurohypophysial system

Bargmann and Scharrer (1951) demonstrated that the PVN and SON were the hypothalamic nuclei in which the neurohypophysial hormones, vasopressin and oxytocin, were synthesised. The PVN and SON have since been studied in great detail using many different anatomical tracing and electrophysiological techniques, and appear to be important sites for the integration of information performed by the hypothalamus, as well as for synthesising, packaging and transporting the neurohypophysial hormones.

The SON is located adjacent to the lateral border of the optic chiasm. The cells are mainly large and bipolar, with few dendritic processes which are unbranching and confined to the SON (e.g. Lefranc, 1966; Leontovich, 1969; LuQui and Fox, 1976). The PVN appears to be composed of distinct magnocellular and parvocellular parts (e.g. Gurdjian, 1927; Krieg, 1932; Bodian, 1939). The PVN is currently thought to be composed of eight distinct cellular subdivisions, three of which are magnocellular. The magnocellular components form three dense clusters of magnocellular neurones, termed the anterior, medial and posterior divisions, which are embedded in a matrix of five parvocellular divisions, the periventricular, anterior, medial, dorsal and lateral parts (Fig. 1-A) (Swanson and Kuypers, 1980; Swanson and Sawchenko, 1983). As in the SON, the magnocellular neurones of the PVN are

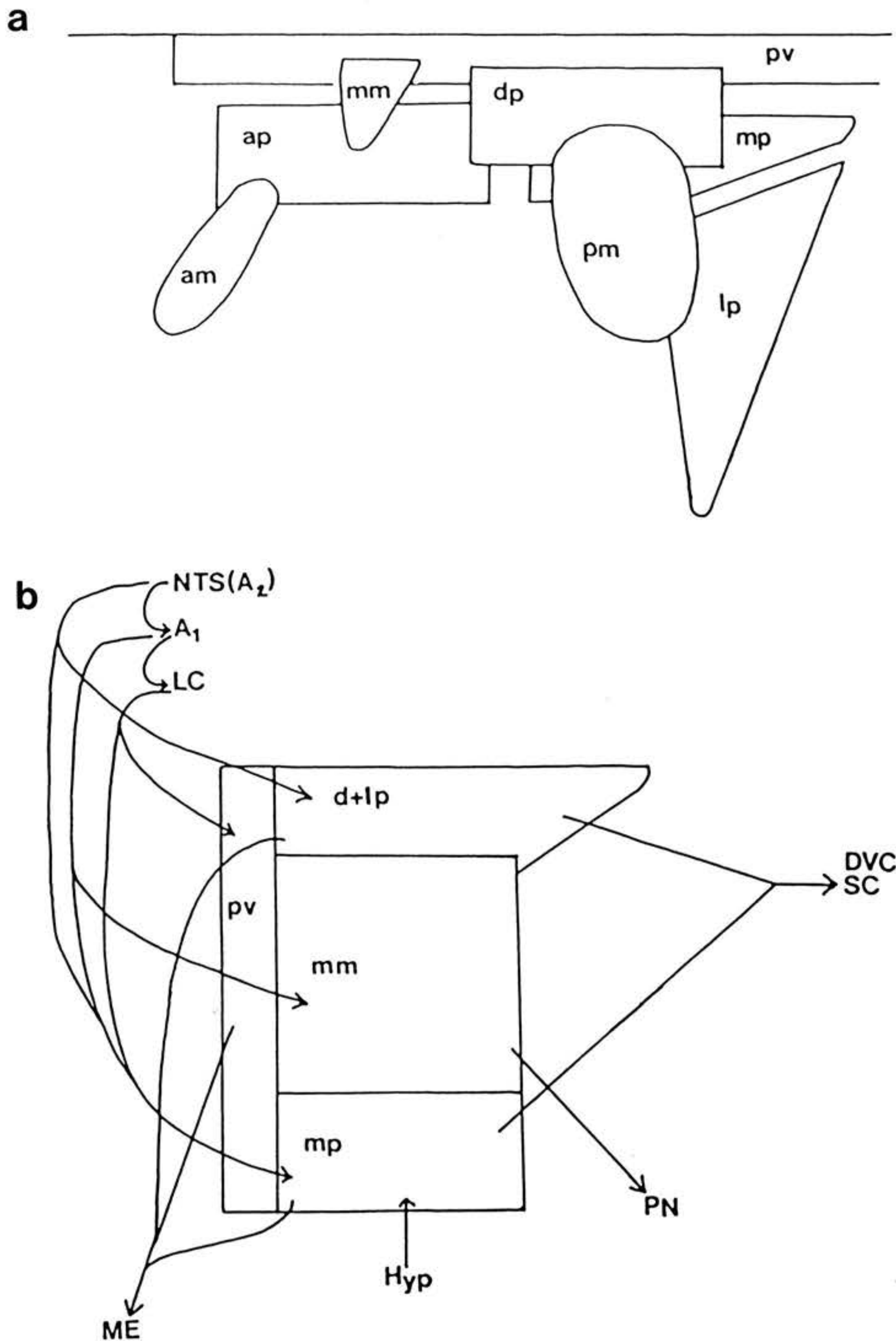


Fig. 1-A: schematic diagrams to show (a) the major cytoarchitectural subdivisions and (b) the major afferent and efferent projections of the paraventricular nucleus in the rat. Abbreviations: A_1, A_1 cell group of the ventral medulla; am, anterior magnocellular division; ap, anterior parvocellular division; dp, dorsal parvocellular division; DVC, dorsal vagal complex; Hyp, hypothalamus; LC, locus coeruleus; lp, lateral parvocellular division; ME, median eminence; mm, medial magnocellular division; mp, medial parvocellular division; NTS (A_2), A_2 cell group in the nucleus of the solitary tract; pm, posterior magnocellular division; PN, pars nervosa; pv, periventricular parvocellular division; SC, spinal cord.

Fig. 1-A (a) after Swanson and

Sawchenko (1983).

mainly bipolar with only a few dendritic branches (Armstrong, Warach, Hatton and McNeill, 1980; Van den Pol, 1982), and the dendrites tend to extend medially, suggesting that the dendritic mass of the neurones tends to remain within the same subdivision of the PVN (Armstrong et al., 1980; Van den Pol, 1982). However, collaterals from parvocellular neurones have been demonstrated to form contacts with dendrites from both parvocellular and magnocellular divisions (Van den Pol, 1982), providing the first clear evidence for an interaction between the cells of the major subdivisions of the PVN.

Before the availability of immunohistochemical techniques, it was not clear from conventional tracing techniques whether different neuronal populations within the PVN and SON synthesised the two neurohypophysial hormones. Oxytocin was thought to be synthesised primarily in the PVN, and vasopressin in the SON (Olivecrona, 1957; Lederis, 1961). However, the use of antisera against oxytocin and vasopressin demonstrated that the two nonapeptides are present in both hypothalamic nuclei, and occur in separate populations of neurones within the nuclei (Vandesande and Dierickx, 1975; Vandesande, Dierickx and De Mey, 1977; Dierickx, 1980). In the SON the topographical organisation of the oxytocin- and vasopressin-staining cells is quite distinct, with the oxytocin cells concentrated anterodorsally within the nucleus, and the vasopressin cells located posteroventrally (Swaab, Nijveldt and Pool, 1975; Swaab, Pool and Nijveldt, 1975; Sokol, Zimmerman, Sawyer and Robinson, 1976; Rhodes, Morrell and Pfaff, 1981; Swanson, Sawchenko, Bérod, Hartman, Helle and Vanorden, 1981). The organisation of the

oxytocin and vasopressin cells is equally as distinct in the PVN; the anterior and medial magnocellular divisions of the nucleus consist mainly of oxytocin cells (Rhodes et al., 1981; Sawchenko and Swanson, 1982a), and the vasopressin cells are found mainly in the dorsal and lateral parts of the posterior magnocellular division. There is general agreement that the posterior magnocellular division contains approximately equal numbers of oxytocin- and vasopressin-staining cells (Swaab et al., 1975a,b; Vandesande and Dierickx, 1975; Rhodes et al., 1981; Sawchenko and Swanson, 1982a). Perhaps surprisingly, a recent analysis of the distribution of oxytocin- and vasopressin-staining cells within the PVN demonstrated that of the total number of oxytocin- and vasopressin-containing cells, 31 and 21 , respectively, were located in parvocellular divisions of the nucleus (Sawchenko and Swanson, 1982a). This suggests that size and location are not sufficient criteria for distinguishing cells that contain the neurohypophysial hormones (Sawchenko and Swanson, 1982a).

In addition to containing vasopressin and oxytocin, the cells and fibres of the PVN and SON have been shown to contain more than 30 putative transmitters and biologically active peptides, which have been summarised by Swanson and Sawchenko (1983). Although the locations of these substances within the PVN and SON have been studied in detail (e.g. Dierickx and Vandesande, 1979; Kahn, Abrams, Zimmerman, Carraway and Leeman, 1980; Kimura, McGeer, Peng and McGeer, 1981; Swanson et al., 1981a; Sawchenko and Swanson, 1982a), the function of these substances has yet to be determined.

Cajal (1894; 1911) first demonstrated convincingly that the SON projected to the PN. Since then, no additional pathways from the

SON have been firmly established. Using radiolabelled-amino acid tracing techniques it has been shown that fibres originating from the SON on one side of the brain course through the internal lamina of the ME on both sides of the midline, and, therefore, probably terminate in the PN on either side of the midline (Saper, Swanson and Cowan, 1978). There have also been reports of Gomori-stained fibres passing between the PN and the PI (Christ, 1966) but these fibres have not been studied with autoradiographic and immunohistochemical techniques.

Despite the obvious morphological similarities between cells of the SON and PVN, a paraventriculo -hypophysial pathway was not demonstrated for some years after the description of a supraoptico-hypophysial pathway (Cajal, 1894; 1911; Ingram, Fisher and Ranson, 1936). The existence of this pathway has since been confirmed using retrograde tracers such as horseradish peroxidase (HRP) and true blue (Sherlock, Field and Raisman, 1975; Kelly and Swanson, 1980; Swanson and Kuypers, 1980), and it has also been demonstrated that some parvocellular neurones project to the PN (Swanson and Kuypers, 1980). The fibres of the paraventricular-hypophysial tract leave the PVN laterally or ventrally, course above or below the FX and arch posteroventrally to the ME (Krieg, 1932; Lefranc, 1966; Vandesande and Dierickx, 1975). It is not yet known whether there is any segregation of oxytocin-containing and vasopressin-containing neurones in the tract. In addition to oxytocin and vasopressin and their associated neurophysins, this tract probably also contains enkephalins, since enkephalin-immunoreactivity has been demonstrated in the PVN (Rossier, Battenberg, Pittman, Bayon, Koda, Miller,

Guillemin and Bloom, 1979) and co-existing with vasopressin and oxytocin in individual terminals in the PN (Martin and Voigt, 1981).

Electrophysiological recordings of the neurosecretory cells demonstrated that some cells projecting to the PN fired with characteristic intermittent bursts of activity (phasic discharge) (Wakerley and Lincoln, 1971; Dreifuss and Kelly, 1972). The phasic discharges were subsequently shown to occur almost exclusively in vasopressin-containing neurones, with oxytocin-containing neurones rarely firing in a phasic pattern (Lincoln and Wakerley, 1974; Dreifuss, Harris and Tribollet, 1976; Poulain, Wakerley and Dyball, 1977).

A direct neuronal input from the PVN to the external lamina of the ME has been demonstrated (Rinne, 1960) and has since been shown to originate mainly in the periventricular and anterior parvocellular divisions of the PVN, although some cells in the magnocellular areas of the PVN have been lightly labelled (Lechan, Nestler, Jacobson and Reichlin, 1980; Weigand and Price, 1980). The Gomori-stained (Wittkowski and Bock, 1972) and neurophysin-stained (Silverman and Zimmerman, 1975) secretory granules in the ME are smaller than those in the PN, which suggests that the external lamina of the ME may be innervated by a separate group of parvocellular neurones. The fibres projecting to the ME are thought to be vasopressinergic (Dierickx, Vandesande and De Mey, 1976), and probably mediate the effects of vasopressin on the release of adrenocorticotrophic hormone (ACTH) (Gillies and Lowry, 1982).

The PVN is also known to project to extrahypothalamic areas of the brain, which is probably important for the integration of

visceral information. Long fibres from mainly the parvocellular divisions of the PVN have been demonstrated to project to the DVC and SC (Hancock, 1976; Saper, Loewy, Swanson and Cowan, 1976; Swanson and Kuypers, 1980). These efferents are thought to be mainly oxytocinergic (Nilaver, Zimmerman, Wilkins, Michaels, Hoffman and Silverman, 1980; Sawchenko and Swanson, 1980; 1982a). However, the oxytocinergic and vasopressinergic neurones only account for about 20% of the cells that project from the PVN to the autonomic centres in the medulla and SC (Sawchenko and Swanson, 1980, 1982a), which suggests that additional cell types may contribute to these projections. Immunohistochemical studies have shown that these additional cell types include cells that contain tyrosine hydroxylase and not dopamine-β-hydroxylase or phenyl-ethanolamine-N-methyltransferase, therefore presumably dopaminergic (DA) (Swanson et al., 1981a), somatostatin and metenkephalin (Sawchenko and Swanson, 1980, 1982a).

The PVN and SON receive afferent inputs from a variety of brain areas, including the hypothalamus, limbic system and the brain stem. Probably the best documented neural input to the PVN and SON is by noradrenergic (NA) neurones, which have been shown by a variety of neuroanatomical methods to originate in the A_1 and A_2 cell groups in the medulla and in the LC (Carlsson, Falck and Hillarp, 1962; Ungerstedt, 1971; Sakumoto, Tohyama, Satoh, Kimoto, Kinugasa, Tanizawa, Kurachi and Shimizu, 1978; Swanson and Hartman, 1980; Berk and Finkelstein, 1981a; Tribollet and Dreifuss, 1981). These three NA cell groups project primarily to the parvocellular divisions of the PVN and SON (Jones and Moore, 1977; Koh and

Ricardo, 1979; Sawchenko and Swanson, 1981a; 1982b), although the A_1 region of the medulla does project to the magnocellular divisions of the PVN and SON, areas in which the vasopressin-staining neurones are located (Sawchenko and Swanson, 1981a; 1982b). The PVN and SON receive projections from most hypothalamic nuclei, but only the fibres orginating in the DMH and POA-m terminate in the magnocellular divisions of the PVN and SON (Sawchenko and Swanson, 1981b). The SFO and the area around the anteroventral third ventricle are now recognised as important structures in the control of water balance (e.g. Fitzsimons, 1972; Andersson, 1978; Johnson and Buggy, 1978; Johnson, Hoffman and Buggy, 1978; Fitzsimons, 1979; Brody and Johnson, 1980; Johnson, Bealer, McNeil, Schoun and Möhring, 1980; Simpson, 1981; Negro-Vilar and Samson, 1982; Bealer, Crofton and Share, 1983; Mangiapane, Thrasher, Keil, Simpson and Ganong, 1983). A variety of techniques including HRP (Silverman, Hoffman and Zimmerman, 1981; Tribollet and Dreifuss, 1981) and autoradiography (Miselis, Shapiro and Hand, 1979; Miselis, 1981; Sawchenko and Swanson, 1981b) have shown that the SFO and the surrounding area project to the PVN and SON, and electrophysiological studies have demonstrated that stimulation of the SFO primarily excites phasically discharging (putative vasopressingergic) cells in the SON (Renaud, Arnauld, Cirino, Layton, Sgro and Siatitsas, 1981). The hypothalamus and associated structures therefore project to the areas of the PVN and SON whose principal outputs are directed towards the ME and autonomic centres in the brain stem. The limbic system, primarily the S, AMYG and VHIPP have been shown to project to the PVN and SON by retrograde

tracing techniques (Berk and Finkelstein, 1981a; Silverman et al., 1981; Tribollet and Dreifuss, 1981). However, the radiolabelled-amino acid tracing method has failed to demonstrate these connections (Krettek and Price, 1978; Swanson and Cowan, 1977; 1979), and the discrepancies are probably due to problems in the interpretation of results obtained by the HRP method.

The main afferent and efferent projections of the PVN, based on the review by Swanson and Sawchenko (1983) are shown in Fig. 1-A, which demonstrates the potential of the PVN for integrating information from many hypothalamic and autonomic areas of the brain.

1.2.4 Physiology of the hypothalamo-neurohypophysial system

1.2.4.1 Control of water balance

The main physiological stimuli for the release of vasopressin are a reduction or redistribution of blood volume and an increase in plasma osmolality. Thus, the release of vasopressin is regulated by both volume and osmotic control systems (reviews by Bisset and Jones, 1975; Bisset, 1976).

1.2.4.1.1 Volume control

Haemorrhage, carotid occlusion, hypotension and exercise are effective stimuli for the release of vasopressin (e.g. Rydin and Verney, 1938; Weinstein, Berne and Sachs, 1960; Beleslin, Bisset, Haldar and Polak, 1967; Clark and Roche e Silva, 1967; Roche e Silva and Rosenberg, 1969). Alterations in blood volume must therefore be detected by 'volume receptors', which then influence magnocellular activity, to cause the release of vasopressin. Stimulation of the left atrial stretch receptors by inflation of a balloon in the left atrium in anaesthetised dogs was followed by diuresis (Henry, Gauer

and Reeves, 1956), which suggested that the atrial receptors might function as volume receptors. Studies involving cold blockade of electrical activity in the vagus nerve (Henry and Pearce, 1956) demonstrated that the afferent fibres from these stretch receptors were located in the vagus; activity in the vagus increased when the atrium was stretched and was accompanied by diuresis, while haemorrhage reduced activity in the vagus and was accompanied by antidiuresis. Distension of the left atrium has been demonstrated to cause a rapid decrease in plasma vasopressin concentration (Share, 1965; Ledsome, Ngsee and Wilson, 1983). Electro-physiological recordings from the atrial stretch receptors themselves showed that the mean firing rate of the receptors increased when the blood volume increased, and decreased when the blood volume decreased (Gupta, Henry, Sinclair and Von Baumgarten, 1966). Since vagal activity causes diuresis, it seems likely that the vagus exerts a tonic inhibitory control over vasopressin. A similar inhibitory control is exerted through baroreceptors in the carotid sinus and aortic arch, mediated through afferents in the carotid sinus nerves and the vagi (Share and Levy, 1962). Excitation of chemoreceptors by anoxia or reduced blood flow through the carotid and aortic bodies also stimulates the release of vasopressin (Share and Levy, 1966). Therefore, the release of vasopressin caused by haemorrhage, carotid occlusion and hypotension is probably due to inhibition of the atrial stretch receptors and baroreceptors, and an increase in the activity of the chemoreceptors. The afferent vagal and carotid sinus nerves terminate in the NTS in the brain stem, where a pathway (possibly

cholinergic) (Bisset, Feldberg, Guertzenstein and Rocha e Silva, 1975) is thought to relay the stimulus to the A_1 cell group in the medulla. The A_1 cell group projects directly to the PVN and SON through a NA pathway (Sawchenko and Swanson, 1982b), the function of which is probably to inhibit the release of vasopressin (Blessing and Reis, 1982; Blessing, Sved and Reis, 1982). Noradrenaline, acetylcholine (Ach) and DA have been shown to stimulate vasopressin release in vivo (Olsson, 1970; Kühn, 1974; Bridges, Hillhouse and Jones, 1976; Moos and Richard, 1982), and a dual system for the control of phasic discharges and vasopressin release by NA has been suggested, with a facilitatory α-receptor mechanism and an inhibitory β-receptor mechanism (Bhargava, Kulshrestha and Srivastava, 1972; Wakerley, Noble and Clarke, 1983).

1.2.4.1.2 Osmotic control

The importance of an osmotic stimulus for the release of vasopressin has been known for many years (Chambers, Melville, Hare and Hare, 1945; Verney, 1946; 1947). However, the site of the osmoreceptors has yet to be firmly established. Injections of hypertonic saline into the carotid artery increased activity in the region of the vasopressin secreting neurones (Von Euler, 1953; Cross and Green, 1959), and was subsequently shown to cause the release of vasopressin and to excite neurones in the SON and PVN (Dyball, 1971). Since cells in the SON respond to iontophoretic application of hypertonic saline (Leng, 1980) it has been suggested that cells in the SON are osmo- or sodium receptors (Andersson, 1977; Mason, 1980; Abe and Ogata, 1982; Noble and Wakerley, 1982), as originally suggested by Jewell and Verney (1957). However, other workers

believe that the receptors are located in areas near, and not within the SON (Hayward, 1977; Schrier, Berl and Anderson, 1979; Bie, 1980), possibly in areas such as the circumventricular organs or OVLT, which are outside the blood-brain barrier (Dellman and Simpson, 1979). The possibility exists that the cells of the SON are depolarised by osmotic stimuli, but need synaptic input from other neurones for the generation of action potentials (Leng, Mason and Dyer, 1982).

1.2.4.2 Oxytocin release and milk ejection

In several species the milk ejection reflex is the only mechanism by which substantial quantities of milk can be released from the mammary gland (Cross and Harris, 1952; Cross, 1955a,b; Benson and Cowie, 1956; Grosvenor and Turner, 1957; Pickford, 1960). Afferent impulses ascend from the nipple to the hypothalamus, where transient increased activity in the oxytocin-containing cells of the PVN and SON (Wakerley and Lincoln, 1973) causes the release of oxytocin from the PN, and subsequent milk ejection. Although the nature of the afferent path of the milk ejection reflex is still disputed, the hypothalamic, neurohypophysial and mammary gland components of the reflex arc have been well documented (e.g. reviews by Benson and Fitzpatrick, 1966; Cross, 1966; Cowie and Tindal, 1971; Cross et al., 1975). There are several classical papers which laid the basis for our understanding of the reflex. These were that extracts of neurohypophysial tissue contained milk producing activity (Ott and Scott, 1910) which acted on mammary tissue (Gaines, 1915), in particular on the contractile myoepithelial cells that surround the acini in the gland

(Richardson, 1949). Oxytocin was first characterised and synthesised by Du Vigneaud et al. (1954b). The size of the suckling litter was found to affect the pattern of milk ejection in the rat (Lincoln, Hill and Wakerley, 1973) and rabbit (Fuchs and Wagner, 1963).

The anatomy of the afferent pathway that conveys the suckling stimulus to the hypothalamus has been disputed. Studies on the guinea pig, rabbit and goat suggested that the path through the midbrain was discrete and coincided with the TSTH (Tindal, Knaggs and Turvey, 1967; 1969; Knaggs, McNeilly and Tindal, 1972), while other studies in the rat and rabbit suggested that the pathway was diffuse and scattered across the TM and TL (Urban, Moss and Cross, 1971; Voloschin and Dottaviano, 1976; Juss and Wakerley, 1981). The course of the pathway and the 'gating' mechanism which must operate to convert the constant suckling stimulus to transient magnocellular activity (Wakerley and Lincoln, 1973) remain to be investigated more fully. Stimulation of the anterolateral pathways of the SC always caused milk ejection (Dyer and Poulain, 1982), which suggested that some 'gating' probably occurs at the level of the SC.

Oxytocin release associated with milk ejection can also be initiated by stimuli other than suckling. Thus, auditory stimuli from other nursing rats can cause oxytocin release in rats (Deis, 1968), as can the approach of the milker in cows (Cleverley and Folley, 1970) and the sound of the baby crying in nursing women (Newton, 1961). Stress can inhibit the milk ejection reflex in some species (Cross, 1953; 1955a,b), probably mediated by sympathetic adrenergic pathways, since adrenaline (Adr) inhibits the milk

ejection reflex (e.g. Chan, 1965; Bisset, Clark and Lewis, 1967). The role of DA in the control of the milk ejection reflex appears to be different depending on the preparation used. Studies involving electrical stimulation of the PN *in vitro* showed that DA inhibited oxytocin release (Barnes and Dyball, 1982), while studies in which extracellular recordings were made from hypothalamic slices showed that DA increased the firing rate of putative oxytocinergic cells (Mason, 1983). Dopamine has also been shown to facilitate milk ejection *in vivo* (Clarke, Lincoln and Merrick, 1979; Moos and Richard, 1982), and systemically administered bromocriptine and α-ergocriptine did not inhibit the milk ejection reflex (Russell, Harrison and MacNeilly, 1981). Whatever the transmitters involved, the fact that oxytocin release can be conditioned by various stimuli and prevented by stress demonstrates that although the ejection of milk occurs as a reflex, other brain areas can influence the release of oxytocin.

Oxytocin can also be released in response to experimental manipulation of the genital tract (Haterius and Ferguson, 1938; Ferguson, 1941; Debackere, Peeters and Tuyttens, 1961; Fitzpatrick, 1966a,b) and coitus (Harris and Pickles, 1953; Hays and Van Demark, 1953), although the precise nature of the stimulus causing oxytocin release in mating is difficult to determine (Harris, 1955). The physiological function of oxytocin released by mating is not clear, but by stimulating uterine contractions may assist in the transport of sperm along the female genital tract (Fitzpatrick, 1966a).

Oxytocin release is also associated with parturition, and milk ejection has been shown to coincide with uterine contractions in

women (Gunther, 1948) and with delivery of the young in rabbits (Cross, 1958; Fuchs, 1964). The use of assay techniques have demonstrated the association of oxytocin release with parturition in several species of domestic animal (Fitzpatrick and Walmsley, 1962; Knaggs, 1963; Fitzpatrick and Walmsley, 1965). Oxytocin undoubtedly has a role in the expulsion of the foetus, but it now seems likely that oxytocin does not initiate parturition (e.g. Liggins, 1979).

1.2.5 Anatomy and physiology of the hypothalamo-adenohypophysial system

The neuroendocrine control of adenohypophysial hormones has been the subject of several recent reviews (e.g. Fink and Geffen, 1978; Vale et al., 1981). Hence, attention will be focussed on the control of gonadotrophin release and prolactin release, since the control systems for these two hormones are especially relevant to the metabolic studies reported in this thesis.

1.2.5.1 Neuroendocrine control of gonadotrophin release

The development of the concept of neurohumoural control of adenohypophysial function (Harris, 1955; 1972), and the subsequent isolation and characterisation of luteinising hormone releasing hormone (LHRH) (Matsuo, Baba, Nair, Arimura and Schally, 1971) provided the basis for the elucidation of neuroendocrine control of gonadotrophin release. In spontaneously ovulating mammals such as the rat, ovulation is triggered by a surge of luteinising hormone (LH) from the PD, which is preceded by an increase in plasma oestradiol-17β concentration (Knobil, 1974; Legan and Karsch, 1975) and is accompanied by an increased secretion of ovarian progesterone (Feder, Brown-Grant and Corker, 1971; Aiyer,

Fink and Greig, 1974). Oestradiol-17β increases the responsiveness of the pituitary gland to LHRH (Aiyer and Fink, 1974; Aiyer et al., 1974b) and initiates a surge of LHRH into hypophysial portal plasma (Sarkar, Chiappa, Fink and Sherwood, 1976; Sarkar and Fink, 1979; Sherwood, Chiappa, Sarkar and Fink, 1980; Ching, 1982). The responsiveness of the pituitary gland to LHRH is increased further by the priming effect of LHRH (Aiyer, Chiappa and Fink, 1974; Wang, Lasley, Lein and Yen, 1976; Fink and Pickering, 1980), and the increased secretion of ovarian progesterone (Aiyer and Fink, 1974; Fink and Henderson, 1977a; Turgeon and Waring, 1981). Although oestradiol and progesterone can facilitate pituitary responsiveness by a direct action on the gonadotrophs (Drouin, Lagacé and Labrie, 1976; Fink and Henderson, 1977b), the action of oestradiol at the level of the hypothalamus is probably also important in increasing pituitary responsiveness by way of the priming effect of LHRH (Speight, Popkin, Watts and Fink, 1981). The priming effect of LHRH probably serves to co-ordinate the surge of LHRH with the increase in pituitary responsiveness, to ensure a massive ovulatory surge of LH (Fink, 1979; Rusak and Zucker, 1979).

The neural signal responsible for the release of LHRH is thought to originate in an area of the rostral diencephalon which includes the POA and SCN (e.g. Taleisnik and McCann, 1961; Halász and Pupp, 1965; Brown-Grant and Raisman, 1972; Van Rees, 1972; Brown-Grant and Raisman, 1977; Wiegand, Terasawa, Bridson and Goy, 1980) and in which the majority of the LHRH-containing perikarya are located (Barry, Dubois and Poulain, 1973; Barry, Dubois, Poulain and Leonardelli, 1973; Sétáló, Vigh, Schally, Arimura and Flerkó, 1976;

Kawano and Daikoku, 1981). The relative importance of the SCN compared with the POA in the generation of the neural signal is still disputed (Rusak and Zucker, 1979). Although the SCN does send LHRH-containing fibres to the ME (Réthelyi and Halász, 1970; Swanson and Cowan, 1975), the variability of the effects of lesions of the SCN and of electrical stimulation of the SCN on gonadotrophin release may, in fact, be due to the close proximity of the SCN to the retrochiasmatic area in which LHRH-containing pathways converge (Réthelyi, Vigh, Sétáló, Merchenthaler, Flerkó and Petrusz, 1981).

The most prominent neural projections to the hypothalamus come from the limbic system (Nauta, 1963; De Groot, 1966; Raisman, 1966). It is not surprising, therefore, that brain areas such as the AMYG and VHIPP have been implicated in the control of gonadotrophin release (e.g. Bunn and Everett, 1957; Velasco and Taleisnik, 1969a,b; Lawton and Sawyer, 1970; Gallo, Johnson, Goldman, Whitmoyer and Sawyer, 1971; Brown-Grant and Raisman, 1972; Kawakami, Kimura and Wakabayashi, 1972; Chiappa, Fink and Sherwood, 1977). Similarly, other neural pathways such as the retino-hypothalamic projection (Moore, Karapas and Lenn, 1971) influence gonadotrophin release by mediating the effect of light on reproductive processes (for references see section 1.2.2). The precise nature of the interactions between the LHRH neuronal system and other intrinsic and extrinsic neurones of the hypothalamus is not clear. The hypothalamus is densely innervated by NA fibres (Fuxe and Hökfelt, 1969; Jacobowitz and Palkovits, 1974), which originate in the LC and TL (Dahlström and Fuxe, 1964; Ungerstedt, 1971; Swanson and Hartman, 1980). In general, NA neurones are

thought to facilitate gonadotrophin release in a variety of animal models (e.g. Kalra and McCann, 1973; Krieg and Sawyer, 1976; Martinovic and McCann, 1977; Sarkar and Fink, 1981), although manipulations of this system did not affect reflex ovulation in the rabbit (Fink, Smith, McMaster, Osborne and Chiappa, 1975), and lesions of the main NA projections to the hypothalamus have only a transient inhibitory effect on LH release and ovulation (Clifton and Sawyer, 1979; 1980).

The anatomy of DA neuronal systems in the brain has been the subject of many reviews (e.g. Moore and Bloom, 1978; Palkovits, 1981). Three DA pathways are of importance for neuroendocrine studies; 1) the mesocortical system, with cell bodies located in the TV and SN which project to the AMYG (Fallon and Moore, 1976a,b) and possibly to the VMH, SCN and ME (Kizer, Palkovits and Brownstein, 1976); 2) the incerto-hypothalamic system, with cell bodies in the PH, ZI and PVH (Lindvall, Björklund, Moore and Stenevi, 1974; Björklund, Lindvall and Nobin, 1975), and 3) the tuberoinfundibular system, with cell bodies in the ARC and PVH (Dahlström and Fuxe, 1964; Hökfelt and Fuxe, 1972), which project to the PN (Smith and Fink, 1972), the PI (Smith and Fink, 1972) and the ME (Björklund, Falck, Hromek, Owman and West, 1970; Smith and Fink, 1972; Björklund, Falck, Nobin and Stenevi, 1973; Björklund, Moore, Nobin and Stenevi, 1973). The precise role of DA neurones in the control of gonadotrophin secretion has not been established. It would appear that depending on the experimental preparation and the method used, DA can be shown to either facilitate (Schneider and McCann, 1970) or inhibit (Fuxe and Hökfelt, 1970; Löfström, 1977; Fink and

Geffen, 1978; Sarkar and Fink, 1981) gonadotrophin release. This may reflect the different types of DA receptors in the CNS (Kebabian and Calne, 1979).

Studies using a variety of techniques have shown that most of the serotonergic (5-HT) innervation of the brain originates in the RA (Dahlström and Fuxe, 1964; Ungerstedt, 1971; Kuhar, Aghajanian and Roth, 1972; Parent, Descarries and Beaudet, 1981; Consolazione and Cuello, 1982). Within the hypothalamus, 5-HT innervation is most dense in the SCN and ARC (Saavedra, Palkovits, Brownstein and Axelrod, 1974), although most hypothalamic nuclei have some detectable 5-HT. Studies involving pharmacological manipulation of 5-HT levels or administration of 5-hydroxytryptophan have suggested that 5-HT has an inhibitory role in the control of gonadotrophin release (Wilson and McDonald, 1974; Wilson, Horth, McNeilly and McDonald, 1975; Coen and Mackinnon, 1976; Héry, Laplante and Kordon, 1976). Whether 5-HT plays a functional role in the control of the normal oestrous cycle is still unknown (e.g. Schneider and McCann, 1970).

1.2.5.2 Neuroendocrine control of prolactin release

The secretion of prolactin (PRL) is thought to be regulated primarily by an inhibitory mechanism, an idea which was first suggested by the presence of apparently functional lactotrophs in pituitary tissue grafted under the kidney capsule, and thereby removed from hypothalamic influence (Nikitovitch-Winer and Everett, 1958; 1959). This was supported by the finding that plasma concentrations of PRL were increased after deafferentation of the MBH (Donoso, Bishop, Krulich, Fawcett and McCann, 1971; Blake,

Scaramuzzi, Norman, Hilliard and Sawyer, 1973) or pituitary stalk section (Diefenbach, Carmel, Frantz and Ferin, 1976; Kanematsu and Sawyer, 1973) and that hypothalamic extracts contained a prolactin inhibiting factor (PIF) (Amenomorri and Meites, 1970; Meites, Lu, Wuttke, Welsch, Nagasawa and Quadri, 1972). The release of PRL can be stimulated by thyrotrophin releasing hormone (TRH) and another hypothalamic factor (PRF), presumed to be a peptide (Reichlin, Saperstein, Jackson, Boyd and Patel, 1976; Vale et al., 1980), and partially inhibitied by somatostatin (Mueller, Chen and Meites, 1973; Rivier and Vale, 1974). However, the physiological role, if any, of TRH, PRF and somatostatin in the control of PRL secretion is not known. Numerous studies have implicated monoamines in the control of PRL release. In general, catecholamines are thought to inhibit, and indoleamines stimulate PRL release (Meites et al., 1972; Lawson and Gala, 1974; Clemens, Sawyer and Cerimele, 1977; Weiner and Ganong, 1978; Clemens and Shaar, 1980). Dopamine has long been thought to be the main PIF (e.g. Macleod, 1976; Neill, 1980). Suckling, which causes the release of PRL (e.g. Amenomori, Chen and Meites, 1970; Burnet and Wakerley, 1976) has been shown to decrease the DA content of the hypothalamus (Mena, Enjalbert, Carbonnel, Priam and Kordon, 1976; Chiocchio, Cannata, Cordero Funes and Tramezzani, 1979) and to inhibit activity in monoaminergic neurones (Neill, 1974; Tindal, 1974), while removing the pups from the doe reduced the plasma concentration of PRL and increased the concentration of DA in hypophysial portal plasma (Ben-Jonathan, Neill, Arbogast, Peters and Hoefer, 1980). Recent data, however, have raised the question as to whether DA is the <u>only</u> PIF, and

whether indeed it acts as a PIF at all concentrations and under all conditions (e.g. Denef, Manet and Dewals, 1980; Leong, Frawley and Neill, 1983).

Purification of hypothalami for PIF activity resulted in the finding that the greatest PIF activity was located in fractions containing catecholamines (Schally, Redding, Arimura, Dupont and Linthicum, 1977). Fractions devoid of catecholamines but with PIF activity contained γ-amino butyric acid (GABA), which, therefore, may supplement the inhibitory action of catecholamines on PRL release (Schally et al., 1977; Dow, Fink, Grieve and Mitchell, 1982). In addition to TRH, oestrogen, neurotensin and vasoactive intestinal polypeptide (VIP) may act as PRF (Chen and Meites, 1970; Judd, Rigg and Yen, 1979; Enjalbert, Arancibia, Priam, Bluet-Pajot and Kordon, 1982; Enjalbert, Arancibia, Ruberg, Priam, Bluet-Pajot, Rotsztejn and Kordon, 1980). However, the control of PRL release appears to vary between animal species, since a PRF appears to be dominant in birds (Meites et al., 1972).

In sum it would appear that the neural control of PRL release might be mediated by both inhibiting (e.g. DA, GABA and somatostatin) and releasing factors (e.g. TRH, PRF, VIP, neurotensin and oestrogen) which may interact in a complex manner at the level of the pituitary lactotroph. This point is illustrated, for example, by the complexity of the interactions between inhibiting and releasing factors which are thought to be involved in the PRL surge induced by suckling (e.g. Burnet and Wakerley, 1976; Grosvenor and Mena, 1980; Grosvenor, Mena and Whitworth, 1980; Leong et al., 1983).

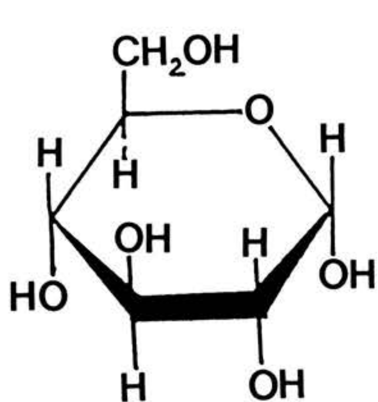
1.3 2-Deoxyglucose technique for measuring cerebral metabolic activity

1.3.1 Development of the technique

Early studies in which cerebral energy metabolism was investigated obtained measurements of the average rate of energy metabolism in the brain as a whole. The methods used were the nitrous oxide technique (Kety and Schmidt, 1948) and its modifications (Scheinberg and Stead, 1949; Lassen and Munck, 1955; Eklöf, Lassen, Nilsson, Norberg and Siesjö, 1973; Gjedde, Caronna, Hindfelt and Plum, 1975), which demonstrated changes in cerebral metabolic rate related to gross or diffuse alterations in cerebral function and/or structure, such as those occurring in post natal development, ageing, senility, anaesthesia and convulsive states (Kety, 1950, 1957; Lassen, 1959; Sokoloff, 1960). Changes in metabolism presumed to be associated with perhaps more subtle alterations of cerebral activity, such as deep slow-wave sleep, performing mental arithmetic, sedation, schizophrenia and LSD-induced psychosis, however, were not detected using these techniques (Kety, 1950; Lassen, 1959; Sokoloff, 1969), possibly due to the redistribution of regional activity within the brain, rather than a change in metabolism of the brain as a whole. Thus, in order to study cerebral metabolism (and therefore functional activity) in detail, a method was needed for the measurement of local changes in metabolic activity.

A quantitative autoradiographic technique was developed to measure the local tissue concentrations of diffusible radioactive tracers which were not metabolised, such as ^{131}I-labelled

trifluoroiodomethane. This technique was used to determine the rates of blood flow in all the structural components seen in autoradiographs of sections of the brain (Landau, Freygang, Rowland, Sokoloff and Kety, 1955; Freygang and Sokoloff, 1958; Kety, 1960; Reivich, Jehle, Sokoloff and Kety, 1969). Oxygen consumption is the most direct measure of energy metabolism; however, the fact that O_2 is highly volatile and that its radioactive isotopes have a short half life, make it difficult to use O_2 as a tracer in autoradiographic measurements of brain metabolism. Based on the premise that CNS tissue is dependent on the aerobic catabolism of glucose to meet its energy requirements (Sokoloff, 1977; Siesjö, 1978) and the fact that glucose utilization is stoichiometrically related to O_2 consumption (Kety, 1957; Sokoloff, 1976), a modification of the technique used to measure blood flow was developed using 2DG to determine local cerebral metabolic rate (Sokoloff, Reivich, Kennedy, Des Rosiers, Patlak, Pettigrew, Sakurada and Shinohara, 1977). Glucose itself could not be used as it is converted to carbon dioxide and water which are cleared rapidly from the CNS. However, 2DG, an analogue of glucose (Fig 1-B) has biochemical properties which make it extremely suitable for use in measuring cerebral metabolism. The entry of 2DG into the CNS has been shown to be mediated by the same carrier mechanism as for glucose (Bachelard, 1971; Olendorf, 1971), and, once in cerebral tissue, 2DG acts as a substrate for hexokinase, which converts 2DG into 2-deoxyglucose-6-phosphate (2DG-6-P) (Sols and Crane, 1954). 2-Deoxyglucose-6-phosphate is not a substrate for glucose phosphate isomerase, or glucose-6-phosphate dehydrogenase (Wick, Drury, Nakada



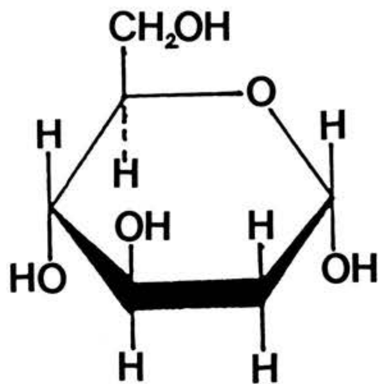


Fig. 1-B: diagram to show the structure of D-glucose and 2-deoxy-D-glucose.

and Wolfe, 1957; Bachelard, 1971) and is therefore not metabolised by way of the glycolytic and tricarboxylic acid pathways or the pentose phosphate shunt. 2-Deoxyglucose-6-phosphate is a substrate for glucose-6-phosphatase, but studies *in vitro* have shown that the activity of this enzyme is low in mammalian brain, and appears to be associated with cerebral capillaries rather than with cerebral tissue itself (Hers, 1957; Raggi, Kronfeld and Kleiber, 1960; Goldstein, Wolinsky, Csejtey and Diamond, 1975). Thus 2DG-6-P becomes trapped within the tissue, and, if radioactively labelled, can be measured, either by quantitative densitometry of autoradiographs prepared from serial sections throughout the brain, or by liquid scintillation counting of areas of tissues prepared by gross dissection or the punch microdissection technique of Palkovits (1973).

1.3.2 Measurement of rates of local cerebral glucose utilisation

1.3.2.1 Theory

Following the introduction of $[^{14}C]$2DG into the circulation, the quantity of $[^{14}C]$2DG-6-P accumulated in an area of brain at any given time is equal to the integral of the rate of $[^{14}C]$2DG phosphorylated by hexokinase in that area during that interval of time (Sokoloff, 1981). Since glucose and 2DG compete for hexokinase, this integral is related to the amount of glucose that has been phosphorylated over the same time interval, depending on the time courses of the relative concentrations of $[^{14}C]$2DG and glucose in the precursor pools, and the Michaelis-Menten kinetic constants for hexokinase with respect to $[^{14}C]$2DG and glucose. The amount of glucose phosphorylated during the interval of time

equals the steady-state flux of glucose through the phosphorylation step multiplied by the duration of the interval, and the net rate of flux of glucose through this step equals the rate of glucose utilisation (Sokoloff, 1981).

The rates of local glucose utilisation (LCGU) can, therefore, be described by a single mathematical operational equation, (Fig. 1-C), provided the following assumptions and/or conditions are met: 1) the tissue compartment is homogeneous with respect to blood flow, concentrations of glucose, $[^{14}C]$2DG, glucose-6-phosphate, $[^{14}C]$2DG-6-P and rates of transport of glucose and $[^{14}C]$2DG between plasma and tissue; 2) carbohydrate metabolism in the brain is in a steady state throughout the experimental time period, in that plasma glucose concentration and rate of cerebral glucose consumption are constant; 3) $[^{14}C]$2DG and $[^{14}C]$2DG-6-P are present only in tracer amounts; 4) free $[^{14}C]$2DG and glucose are present in a single compartment which serves as the precursor pool for the hexokinase reaction and the carrier-mediated transport between plasma and tissue; 5) the capillary plasma concentrations of $[^{14}C]$2DG are approximately equal to, or bear a constant relationship with the arterial plasma concentrations, and 6) there is negligable loss of $[^{14}C]$2DG-6-P from the tissue within the experimental time period (Sokoloff et al., 1977).

The rate constants for $[^{14}C]$2DG transport and phosphorylation have, as yet, only been determined in conscious albino rats (Sokoloff et al., 1977) and in conscious monkeys (Kennedy, Sakurada, Shinohara, Jehle and Sokoloff, 1978). The 'isotope-effect' correction factor (lumped constant) is a constant of proportionality

Fig. 1-C. The operational equation for the 2-deoxyglucose method for measuring rates of local cerebral glucose utilisation.

After Sokoloff et al. (1977).

$$R_i = \frac{\overbrace{\underbrace{C_i^*(T)}_{\text{Total } ^{14}\text{C in Tissue at Time, T}} - \underbrace{k_1^* e^{-(k_2^*+k_3^*)T} \int_0^T C_p^* e^{(k_2^*+k_3^*)t}\, dt}_{^{14}\text{C in Precursor Remaining in Tissue at Time, T}}}^{\text{Labeled Product Formed in Interval of Time, 0 to T}}}{\underbrace{\left[\frac{\lambda \cdot V_m^* \cdot K_m}{\Phi \cdot V_m \cdot K_m^*}\right]}_{\text{"Isotope Effect" Correction Factor}} \underbrace{\left[\underbrace{\int_0^T \left(\frac{C_p^*}{C_p}\right) dt}_{\text{Integrated Plasma Specific Activity}} - \underbrace{e^{-(k_2^*+k_3^*)T} \int_0^T \left(\frac{C_p^*}{C_p}\right) e^{(k_2^*+k_3^*)t}\, dt}_{\text{Correction for Lag in Tissue Equilibration with Plasma}}\right]}_{\text{Integrated Precursor Specific Activity in Tissue}}}$$

C_i^*, total [^{14}C] concentration in a single homogenous tissue of the brain;

C_p^*, concentration of [^{14}C]deoxyglucose ([^{14}C]2DG) in arterial plasma;

C_p, concentration of glucose in arterial plasma;

K_1^*, rate constant for the carrier-mediated transport of [^{14}C]2DG from plasma to tissue;

K_2^*, rate constant for the carrier-mediated transport of [^{14}C]2DG back from tissue to plasma;

K_3^*, rate constant for phosphorylation of [^{14}C]2DG by hexokinase;

$K_{1,2,3}$, equivalent rate constants for glucose;

T, time at the termination of the experiment;

λ, ratio of the distribution space of [^{14}C]2DG in the tissue to that of glucose;

Φ, fraction of glucose once phosphorylated that continues down the glycolytic pathway;

K_m^*, V_m^*, K_m, V_m, Michaelis-Menten kinetic constants of hexokinase for [^{14}C]2DG and glucose respectively;

t, individual time interval.

between the steady state rates of [^{14}C]2DG and glucose phosphorylation by the brain when it is exposed to equal concentrations of both in arterial plasma. The lumped constant has been determined for the albino rat, monkey, cat and dog, and appears to be species dependent and is not significantly altered by anaesthesia (Sokoloff, 1979).

1.3.2.2 Methodology

The methodology has been published in detail by Sokoloff et al. (1977). The animals are prepared surgically for the experiment under halothane anaesthesia. Polythene catheters are inserted into the femoral artery and vein, and the animal is immobilised in a plaster cast. The animal is then allowed to recover for at least 2 h. The 2DG is injected into the vein over 30 sec, while arterial blood samples are collected at frequent, timed intervals. The concentration of glucose and [^{14}C]2DG in each plasma sample is then determined by enzymatic assay and liquid scintillation counting, respectively. Approximately 45 min after the injection of 2DG, the animal is killed and the brain removed and frozen in cooled organic solvent. Serial sections 20 μm thick are then cut from the brain, and exposed to X-ray film for 5-14 days in light-tight X-ray cassettes. A series of [^{14}C]methyl methacrylate standards of known [^{14}C] concentration, precalibrated for 20 μm tissue sections, are exposed in each X-ray cassette. Local tissue concentrations of [^{14}C] are then determined by quantitative densitometry, using the standards to construct a standard curve.

For the calculation of LCGU, the plasma concentrations of glucose and [^{14}C] and the rate constants for the transport and

phosphorylation of 2DG are used in the operational equation to determine the final concentration of $[^{14}C]$2DG remaining in the tissue at the end of the experimental time period, and the final integrated precursor-specific activity in the tissue. These two variables, the $[^{14}C]$ concentration of each brain area and the lumped constant are then used in the operational equation to calculate the LCGU of each brain area.

1.3.2.3 Limitations and possible constraints of the technique for measuring absolute rates of local cerebral glucose utilisation

In the CNS a small proportion of the glucose available for metabolism is used for the synthesis of proteins and lipids. Since the entry of glucose into these pathways occurs after the phosphorylation step, the LCGU within a brain area could be affected by each of these pathways. Under normal conditions however, only 2% and 0.3% of the total cerebral glucose flux is directed towards lipid and protein synthesis, respectively, (Maker, Clarke and Lajthe, 1972), and, therefore, the redirection of glucose into these pathways is not a major constraint on the validity of the 2DG technique.

Glucose is normally the primary energy source for the brain. However, in conditions of high glucose utilisation or low glucose availability, such as might occur in animals undergoing seizures or starvation, alterations in the normal catabolism of glucose can occur. Thus glycogen and ketone bodies can be mobilised as alternative substrates (Rogers and Hutchins, 1972; Ruderman, Ross, Berger and Goodman, 1974; Gjedde and Crone, 1975), and lactate can

be formed. Since elevated rates of phosphorylation will be needed to maintain a constant level of energy production if lactate is formed, the measurements of glucose phosphorylation will no longer bear the same relationship with energy production (Siesjo, 1978). These considerations however, are not normally encountered in most experimental situations. Errors in the measurement of LCGU are more likely to occur from incomplete phosphorylation of [^{14}C]2DG, such as might occur if the experimental time period is reduced significantly from 45 min.

The lumped constant is a major determinant of the amount of [^{14}C]2DG left in the brain at the end of the experimental time period. Any alteration in the value of the lumped constant will be reflected in the [^{14}C] concentration in the brain. The lumped constant, however, depends on the relative distribution of glucose and [^{14}C]2DG, and their relative kinetic constants for hexokinase, so the relationship of [^{14}C]2DG to glucose is likely to remain fairly constant even in situations where the distribution and metabolism of either is altered (McCulloch, 1982). The value of the lumped constant has been shown to remain constant even under conditions of marked changes in glucose utilisation, such as those induced by barbiturates (Sokoloff et al., 1977) and in conditions of altered cerebral blood flow (Sokoloff, 1979). Severe hyper- and hypoglycaemia can alter the value of the lumped constant (Schuier, Orzi, Suda, Kennedy and Sokoloff, 1981; Suda, Shinohara, Miyaoka, Kennedy and Sokoloff, 1981), and the value appears to vary between animal species (Sokoloff et al., 1977; Kennedy et al., 1978; Sokoloff, 1979).

The fact that the kinetic constants for the transport and phosphorylation of [^{14}C]2DG are not known precisely for each animal impose restrictions on the experimental design employed. At least 45 min must be allowed to elapse between the time the [^{14}C]2DG is administered and the time at which the animal is killed, to minimise the contribution these constants make to the operational equation (Fig. 1-C). If the animal is killed 45 min after administration of the [^{14}C]2DG, considerable variation in the constants can be tolerated by the equation (Sokoloff, 1979). The long time interval necessitated by the technique places a limitation on the types of experiments in which the 2DG method can be used.

The concentration of plasma glucose and the rate of glucose utilisation must remain constant within the experimental time period, which can impose further limitations on the technique. A modified operational equation, in which moderate changes in plasma glucose concentrations can be accommodated has been derived (Savaki, Davidsen, Smith and Sokoloff, 1980) to circumvent the problem of mild fluctuations in plasma glucose concentration. However, large fluctuations in plasma glucose concentrations cannot be accommodated even by the modified equation. To ensure that the rate of glucose utilisation remains constant throughout the time period is more difficult in some experimental situations (e.g. seizures). However, the method is biased towards the glucose utilisation that occurs in the first 15 min after the injection of [^{14}C]2DG (McCulloch, 1982) which allows some flexibility especially in the later stages of the experimental time period.

Especially relevant to this thesis is the impracticality of the 2DG technique for measuring LCGU in many neuroendocrine studies. The stress involved in surgery immediately before the experiment, and the immobilisation of the animal in a plaster cast for the duration of the experiment, are likely to severely disrupt the normal hormonal state of the animal (e.g. Neill, 1970; Brown and Martin, 1974; Lawson and Gala, 1974).

In addition, the physical presence of the plaster cast would not enable the investigation of some neuroendocrine events, such as the suckling-induced milk ejection reflex and the reflex release of gonadotrophins.

1.3.3 Measurement of relative metabolic activity

In view of the fact that the Sokoloff method for the measurement of LCGU has several limitations for its application to certain physiological, and especially neuroendocrine studies (1.3.2.3), modifications of the technique are now widely employed. These modified methods have been applied with success to a wide variety of experimental situations (e.g. Sharp, Kauer and Shepherd, 1975; Collins, 1978; Schwartz, Smith, Davidsen, Savaki, Sokoloff, Mata, Fink and Gainer, 1979; Greer, Stewart, Kauer and Shepherd, 1981; Kilduff, Sharp and Heller, 1982). The content of $[^{14}C]$ 2DG in a given brain area is estimated by measurement of the optical density (OD) of the area relative to that of standards of known $[^{14}C]$ content. Different manipulations of the data have been used to determine changes in the 2DG uptake by a brain area; for example, 2DG uptake by a brain area has been expressed qualitatively from the OD (e.g. Mikoshiba, Kohsaka, Takamatsu and Tsukada, 1981), expressed

as an OD relative to the other side of brain (e.g. Sharp et al., 1975) and expressed as a percentage change in the OD ratio of a brain area to the OD of a white matter area in experimental compared with control animals (e.g. Kilduff et al., 1982). The method used in this thesis was to compare the [^{14}C] concentration of a brain area with that of an area of white matter in which the [^{14}C] concentration is unlikely to change significantly. The ratio between the two [^{14}C] concentrations is termed the relative metabolic activity (rma) of that area. Since relative, and not absolute rates of LCGU are measured by the modified technique, no assumptions are made regarding the stability of plasma glucose concentrations or rate of glucose utilisation, and, therefore, a possible source of error is avoided. The assumption that the LCGU and rma of white matter areas does not change is a limitation of the modified technique. The LCGU of the IC has been shown to be increased in conscious animals following electrical stimulation of the cortex (Sharp, Bzorgchami and Kilduff, 1983). However, if the ratio of relative OD or [^{14}C] concentrations of two topographically distinct white matter areas remains constant, that is, changed by the same amount, the use of a white matter area as an internal standard is unlikely to yield misleading information, and the advantages of using a white matter area as an internal standard outweigh the disadvantages. By using an internal standard, differences in the amount of 2DG administered, section thickness and film development are minimised, which makes comparisons of rma between animals more meaningful.

1.3.4 High resolution 2-deoxyglucose techniques

The resolution of the [^{14}C]2DG technique has been estimated as being no greater than 100 μm (Sokoloff, 1981) and, therefore, the precise site of 2DG uptake (e.g. in perikarya, dendrites, glia, axons or terminals) cannot be determined. The resolution has been improved slightly by the use of [^{3}H]2DG (Sharp, 1976; Alexander Schwartzman, Bell, Yu and Renthal, 1981), since the beta particles emitted by [^{3}H]2DG are of a lower energy and, therefore, travel for a much shorter distance than those emitted by [^{14}C]2DG. However, the main limitations of the resolution of the 2DG technique are due to the grain size of the X-ray film, and the diffusion of water-soluble 2DG-6-P in the brain during the freezing and cutting procedures. There have been many methods developed to extend the resolution of the [^{3}H]2DG technique to light and electron microscopic levels, using dipping and strip emulsion autoradiography rather than using X-ray film. These methods have included the apposition of frozen tissue sections with frozen emulsion (Sharp, 1976), thaw-mounting sections (Pilgrim and Wagner, 1981; Toop, Burke, Dum, O'Donovan and Smith, 1982) and perfusions or incubations of the tissue with osmium-aldehyde or aldehyde (Des Rosiers and Descarries, 1978; Basinger, Gordon and Lam, 1979; Pujol, Sans and Calas, 1981), or fixation of the tissue with a fixative for carbohydrate (Durham, Woolsey and Kruger, 1981) before embedding the tissue in resin or paraplast for sectioning. These methods have improved the resolution of the 2DG technique to a level at which it can be used at a cellular level. Since all of the methods could still result in some movement of 2DG-6-P, either by transient

thawing of the tissue, or in the dipping procedures, a method was developed (Ryan and Sharp, 1982) using sections of vapour-fixed tissue, to which photographic emulsion was applied by the dried-loop technique of Caro and Van Tubergen (1962) and Caro (1969). Using this method the 2DG was localised in neuronal perikarya, dendrites, fibres and terminals, and in glial cells. However, it should be recognised that different cellular components may be labelled in different functional systems.

1.4 Aims of This Thesis

The 2DG technique has been used with great success to map the functional organisation of parts of the visual, olfactory and somatosensory systems, and to characterise the metabolic effects of manipulations of the central neurotransmitter systems (reviews by Hand, 1981; Sokoloff, 1981; McCulloch, 1982). However, at the time at which the work for this thesis began, the application of the 2DG technique to investigations of neuroendocrine systems was limited (e.g. Schwartz and Gainer, 1977; Schwartz et al., 1979; Schwartz, Davidsen and Smith, 1980).

The aims of this thesis were to use the 2DG method to investigate changes in metabolic activity throughout the brain associated with changes in neuroendocrine functional activity. Specifically, 1) the effects of water deprivation and the administration of synthetic vasopressin on the metabolic activity of primarily the hypothalamo-neurohypophysial system in Wistar and Brattleboro rats, and in a strain of mouse with nephrogenic diabetes insipidus; 2) the changes in metabolic activity throughout the brain associated with the milk ejection reflex and the release of PRL in

the rat; 3) the effects on metabolic activity in the brain of electrical stimulation of areas of the 'neuroendocrine brain', and 4) the pattern of cerebral metabolic activity associated with mating and reflex ovulation in the vole *Microtus agrestis*.

CHAPTER II

Materials and Methods

MATERIALS AND METHODS

2.1 Animals

(a) Rats

The animals used were adult female Wistar Cobs (Caesarian barrier sustained) rats purchased from Charles River U.K. Ltd. (Margate, Kent), adult female homozygous Brattleboro and adult female Piebald Virol Glaxo (PVG) rats bred in the department. The animals were maintained under conditions of controlled lighting (14 h light, 10 h dark) and temperature (22°C) and were allowed free access to food (Diet 41B Oxoid, Basingstoke, Hampshire) and tap water unless otherwise specified. Wistar Cob rats were kept in the animal house after transportation for a minimum of 24h before experimentation.

The stage of the oestrous cycle was determined on the day of experimentation, or for a longer period beforehand (Chapter VI) by inspection of vaginal smears. The days of the four-day cycle were termed metoestrus, dioestrus, pro-oestrus and oestrus, and were distinguished by different cytological characteristics; metoestrus, a mixture of cornified cells and nucleated epithelial cells and leucocytes; dioestrus, mainly leucocytes; pro-oestrus, mainly nucleated epithelial cells with a few leucocytes and cornified cells; oestrus, cornified cells.

(b) Mice

Adult female and male Severe DI +/+ and Non-severe DI +/+ diabetes insipidus mice were obtained from a breeding colony at the Department of Human Anatomy, University of Oxford. Adult, female normal house mice were obtained from the departmental breeding

colony. Mice were maintained under conditions of controlled lighting (14 h light, 10 h dark) and temperature (22°C) and were allowed free access to food (Oxoid Breeding Diet, Basingstoke, Hampshire) and tap water unless otherwise specified. Mice transported from Oxford were kept in the animal house for a minimum of 24h before experimentation.

(c) Voles (*Microtus agrestis*)

Adult female and male voles were obtained from a breeding colony at the Department of Human Anatomy, University of Oxford. The voles were allowed free access to food (guinea pig diet, S.D.S. Ltd., Witham, Essex, supplemented with carrots) and tap water, and were kept in the animal house for a minimum of 5 days before experimentation.

2.2 Experimental and surgical techniques

2.2.1 Autoradiography with 1-[^{14}C] 2-deoxyglucose

Autoradiographs were prepared according to the method of Sokoloff et al. (1977). 1-[^{14}C] 2-deoxyglucose (Amersham International; specific activity 60 mCi/mmol in 3% ethanol) was injected intravenously (i.v.) (see sections 2.2.4 and 2.2.5) in experiments using rats(150-250μCi/kg body weight), and intraperitoneally (i.p.) in experiments using mice and voles(400-500μCi/kg body weight). The 2DG was diluted with 0.9% saline solution and injected as a bolus with a final volume of 0.5 ml i.v. and 0.15 ml i.p. After 45 min the animals were killed by decapitation and the brain and pituitary removed *en bloc*; the lower jaw was detatched and the basioccipital bone removed. The basisphenoid and overlying soft palate were carefully removed with

rongeurs to expose the pituitary gland and the ventral surface of the brain. The dura mater was then cut rostrally along both trigeminal nerves and medially across the optic nerves. The optic and trigeminal nerves were severed and the brain and attached pituitary gland removed from the skull. The tissue was then frozen in 2-methylbutane cooled to -45 - -60°C in dry ice for 5 min before mounting in Tissue Tek II (Miles, Illinois) on a cork disc (22 mm diameter) frozen onto a cryostat chuck. The tissue was mounted in the orientation described by de Groot (1959), and allowed to equilibrate for 30-60 min in a cryostat (Brights) at -22°C. Serial 20 µm coronal sections were then cut and mounted on coverslips (22 x 40 mm, No. 1). In all brains every section was taken from the hypothalamus, the region between the rostral edge of the optic chiasm and immediately rostral to the mamillary body. In addition, in experiments involving brain stimulation by electrodes and brain lesions, all sections were collected throughout the area of electrode and lesion placement for autoradiography or staining. Fewer sections were taken from the remainder of the brain. In the case of rat brain, 2 consecutive 20 µm sections in every 200 µm throughout the brain were collected. Sections from mice and vole brains were collected at smaller intervals; 2 consecutive 20 µm sections in every 100 µm throughout the brain. The sections were dried rapidly for a minimum of 5min on an hot plate at 60°C, glued sequentially on card and exposed to SB-5 X-ray film (Kodak, Rochester, N.Y.) for 7 days at -15°C. A series of [^{14}C] methyl methacrylate standards precalibrated for 20 µm tissue sections (section 2.3.1) (concentration range 51-1057 nCi/g, Radiochemical

Centre, Amersham) were inserted in a well cut in the card to be processed with each autoradiograph. The autoradiographs were developed in complete darkness using Kodak DX80 developer at 1:5 dilution for 4 min at 20°C , placed in a stop bath containing 2% glacial acetic acid and fixed in a solution containing Kodak Rapid Fixer A 375 ml and Kodak Rapid Fixer B 42 ml in 1500 ml tap water for a minimum of 3 min. The autoradiographs were then washed thoroughly in tap water and dried.

2.2.2 Autoradiography with 2-[1,2-^{3}H] 2-deoxyglucose

2-[1,2-^{3}H] 2-deoxyglucose (New England Nuclear; specific activity 37 Ci/mmol) was injected i.v. (see section 2.2.4) at a dosage of 800 μCi/kg body weight (0.5 ml final volume with sterile 0.9% saline solution). After 45 min the animals were anaesthetised with sodium pentobarbitone (see section 2.2.3) and perfused through the heart with saline for 1 min and with the fixative described by MacClean and Nakane (1974) for 4 min. The fixative contained 0.01 M sodium periodate, 0.075 M lysine and 2% paraformaldehyde in 0.037 M phosphate buffer (Appendix II). The brain was removed and a block of tissue containing the hypothalamus, as described in Section 2.2.1, was cut from the brain. The hypothalamus was left in 50% ethanol overnight, and then transferred to 70% ethanol for 4 h, 95% ethanol for 4 h, butanol I for 1 h and butanol II overnight. The tissue block was embedded in paraplast and 15 μm sections were cut on a microtome (Leitz). The sections were float mounted onto chrome-gelatin coated slides (Appendix I) and dried overnight. Paraplast was removed by xylene and the sections were then transferred through a series of graded alcohols to distilled water

and dried overnight at 37°C.

L4 Nuclear research emulsion (Ilford Ltd., Basildon, Essex) was applied to the slides in complete darkness, using the dryloop technique of Caro and Van Tubergen (1962) and Caro (1969). The slides were dried and stored in light-tight boxes at -40°C for 14-18 weeks. The slides were then developed in D19 (Kodak) for 5 min at 20°C and fixed in Unifix solution B (Kodak) for 5 min, washed and dried. Sections were then stained using celestine blue and Meyers haematoxylin stains (Appendix III).

2.2.3 Anaesthetics

The anaesthetics used for surgical and experimental procedures were as follows:

a) Althesin (Glaxo, Greenford, Middlesex)-9 mg alphaxalone (3α-hydroxy-5α-pregnane-11,20-dione) and 3 mg alphadalone acetate (21-acetoxy-3α-hydroxy-5α-pregnane-11,20-dione) per ml of isotonic aqueous vehicle was administered i.p. at a dosage of 0.4-0.6 ml/100 g body weight.

b) Avertin - tribromoethanol in amylene hydrate 1 g/ml (Aldrich Chemical Company Inc., Milwaukee, U.S.A.)diluted in absolute alcohol (2:8 ml) and diluted further in 90 ml of 0.9% saline solution to give a final concentration of 10mg/ml, was injected i.p. at a dosage of 0.25 g/kg body weight.

c) Ether-diethylether (May and Baker Ltd., Dagenham, Essex). Anaesthesia was induced by placing the animals into a sealed plastic box lined with a pad of cotton wool saturated with ether. Anaesthesia was maintained by administering ether by a drop bottle onto a piece of thin cotton wool which served as a face mask.

d) Halothane (Fluothane, ICI Pharmaceuticals Ltd., Macclesfield, Cheshire). Anaesthesia was induced by placing the animals into a plastic box with a 5% Halothane/oxygen mixture circulating through using a 'vapor-Halothane' vapouriser. Anaesthesia was maintained using a 1.5% Halothane/oxygen mixture administered through a face mask.

e) Sodium pentobarbitone (Sagatal, May and Baker Ltd., Dagenham, Essex) 60 mg/ml in alcohol:propylene glycol vehicle (10:20 v/v) was injected i.p. at a dosage of 40 mg/kg body weight.

f) Urethane-ethyl carbamate (B.D.H.) dissolved in 0.9% saline solution (10 g/100 ml) was injected i.p. at a dosage of 1 g/kg body weight.

2.2.4 Intravenous injection of 2-deoxyglucose into, and collection of venous blood samples from conscious animals

2-Deoxyglucose was injected into, and blood samples collected from conscious animals by way of an intra-atrial cannula that had been inserted according to the method of Greig (1971) 12-24 hr previously under halothane anaesthesia (section 2.2.3). The cannula consisted of an intravenous portion of silastic tubing 0.5 mm I.D., 0.93 mm O.D. (Cat. No. 602-135 Dow Corning Corporation, Michigan, U.S.A.) 32-35 mm in length, depending on the weight of the animal, connected to an extravenous portion of Tygon-tubing 0.25 mm I.D. 2.25 mm O.D. (Technicon Instrument Corporation, Michigan, U.S.A.) by an 8 mm stainless steel pin made from a 23 gauge hypodermic needle. The cannula was soaked overnight in chlorohexidine (Hibitane, ICI Pharmaceuticals Ltd., Macclesfield, Cheshire) 1:250 dilution, washed through and filled with sterile 0.9% saline solution containing

heparin 50 IU/ml from a 1 ml syringe which was attached to the Tygon tubing by a 23 gauge hypodermic needle. A 30mm incision was made approximately 10 mm anterior to the right clavicle and the tissue retracted to expose the external jugular vein. Four silk sutures (3/0) were placed under the vein and the most rostral suture tied and placed under gentle traction. A small cut was made in the vein 4-5 mm rostral to the pectoralis major using iridectomy scissors, and the Silastic portion of the cannula introduced into the vein and fed into the atrium. The patency of the cannula was checked by drawing a small amount of blood into the syringe and injecting an equivalent volume of the heparinised saline solution back into the vein. The two caudal sutures were tied around the pin and Silastic tubing, and the two rostral sutures were tied around the pin and Tygon tubing, holding the cannula securely in place. The syringe was then removed from the cannula and the Tygon portion plugged with a stainless steel plug (8 mm x 0.5 mm). The Tygon tubing was brought around to the back of the neck under the skin and externalised through a small incision. After rechecking the patency, the cannula was trimmed to a 20-30 mm length and plugged, and the skin incisions were sutured. To enable sampling and injecting of 2DG with minimal disturbance to the animal, an extension to the cannula was made. The extension consisted of a length of Tygon tubing with an 8 mm stainless steel pin inserted at one end, filled with heparinised saline solution from a 1 ml syringe connected by a 23 gauge hypodermic needle. The plug was removed from the cannula and the extension attached using the pin. The extension could then be brought out of the top of the cage.

For the injection of 2DG, blood was drawn up into the syringe, the cannula was clamped between thumb and forefinger and the syringe removed and replaced with the syringe containing the 2DG. The 2DG was then injected into the cannula. Blood samples were withdrawn similarly, except that the syringe filled with heparinised saline was replaced with an heparinised sampling syringe. After sampling, the syringe filled with heparinised saline was re-attached to the Tygon tubing and a volume of saline injected equivalent to the volume of blood removed (usually 0.4ml).

2.2.5 Intravenous injection of 2DG into, and collection of venous blood samples from anaesthetised animals

The right external jugular vein and left femoral vein were used to inject 2DG into, and collect blood samples from anaesthetised animals. The external jugular vein was exposed and a 25 gauge hypodermic needle attached to an heparinised 1 ml syringe introduced into the vein through the pectoralis major muscle. This minimised trauma to the vein and so allowed repeated sampling. Blood samples were replaced with an equivalent volume of 0.9% saline solution. 2-Deoxyglucose was injected using the same approach, and care was taken to draw blood into the syringe before injecting the 2DG to prevent injection of any air. The left femoral vein was used in one study (Chapter V) for the collection of venous blood samples and for the injection of 2DG. The vein was cannulated under the anaesthetic used in the experiment, urethane (2.2.3). The cannula consisted of a 200 mm length of polythene tubing, O.D. 0.75 mm (Portex, Hythe, Kent) connected to an 1 ml syringe filled with heparinised saline 50 IU/ml. An incision was made in the skin of the rear left leg,

extending into the skin of the abdomen. The underlying fascia was removed and the lower abdominal wall was retracted to expose the femoral vessels and nerve, and the inguinal ligament. The femoral nerve was carefully dissected away from the vessels, and the artery and vein were separated using fine forceps. Three silk sutures were placed under the vein, and the most caudal suture was tied and placed under gentle traction. A small cut was made in the vein through which the cannula was introduced into the vein. The sutures were tied around the cannula, and the patency of the cannula was checked by drawing a small volume of blood into the syringe and injecting an equivalent volume of heparinised saline back into the vein. The skin incision was then sutured around the cannula.

For the injection of 2DG into and the collection of blood samples from the vein, the same procedure was followed as was used in conscious animals into which an intra-atrial cannula had been inserted (2.2.4).

2.2.6 Collection of arterial blood samples from anaesthetised animals

The femoral artery was exposed and separated from the vein and nerve as described in section 2.2.5. Three silk sutures (4/0) were placed under the artery and the most caudal suture was tied and placed under gentle traction. A microvascular clamp was placed on the artery rostral to the sutures, and a small cut was made in the artery just rostral to the tied suture. The cannula, consisting of a length of polythene tubing 200 mm long, O.D. 0.75 mm (Portex, Hythe, Kent) connected to an 1 ml syringe filled with heparinised saline 100 IU/ml, was introduced into the artery. When the tip of

the cannula reached the microvascular clamp the sutures were tied around the cannula, and the clamp was released. The caudal suture was then tied round the cannula to hold it securely in place. The patency of the cannula was checked by drawing a small volume of blood into the syringe and injecting an equivalent volume of heparinised saline back into the artery. The cannula was then cut to a convenient length for sampling, and the free end was clamped with artery forceps.

To collect arterial blood samples the forceps were removed from the cannula and 4-6 drops of blood were allowed to escape onto a piece of gauze. The free end of the cannula was then placed into an LP2 tube (Luckhams Ltd., Burgess Hill, Sussex) and 50-100 µl of blood was collected. An 1 ml syringe filled with heparinised saline was then attached to the cannula by a 26 gauge hypodermic needle, and 100 µl of heparinised saline was injected back into the artery.

2.2.7 Separation of blood samples

Blood samples collected in heparinised syringes (see sections 2.2.4, 2.2.5) were transferred to ice-cooled LP2 tubes (Luckhams Ltd., Burgess Hill, Sussex) and centrifuged at 4°C for 10 min. The plasma was pipetted into PT0944 tubes (Luckhams Ltd., Burgess Hill, Sussex) and stored at -20 - -40°C.

2.2.8 Electrode manufacture

Platinum unipolar and twin unipolar electrodes were used in the experiments. Electrodes were made from 11-13 mm and 6-8 mm lengths of wire (0.25 mm diameter) straightened and tightly encased in capillary glass tubing. The glass was fixed in place using nail varnish, leaving 0.3 mm of wire exposed at one end. To the other

end of the wire 50 mm of multistranded insulated copper wire was soldered. The whole assembly was mounted in a teflon jig and fixed in place with acrylic cement (Howmedica, London). The electrodes were either 11 mm or 6 mm in length, and twin unipolar electrodes were parallel and separated by either 1 mm or 2 mm.

2.2.9 Electrode implantation in brain and stimulation parameters

2.2.9.1 Areas of brain excluding the median eminence

Animals were anaesthetised with Avertin supplemented with ether (section 2.2.3), and placed in a stereotaxic apparatus with the incisor bar 5 mm above the inter-aural line (De Groot, 1959). A midline skin incision exposed the frontal and parietal bones which were then cleaned of periosteum. A hole approximately 4 x 4 mm was drilled in the skull above the appropriate brain area using a dental drill. Stainless steel screws were inserted into the skull near the edges of the hole, and the dura mater was cut to facilitate electrode entry. The electrode was carefully lowered into the appropriate area using a triplanar micromanipulator (for various co-ordinates and type of electrode see section 6.2). The electrode assembly was fixed in place using dental cement (Simplex Rapid, Dental Fillings Ltd., London N16) applied around the screws and the hub of the electrode. When the cement had hardened the animal was removed from the stereotaxic apparatus, and the skin was sutured over the electrode assembly. The insulated copper wire(s) was externalised through a small incision in the skin at the back of the neck, and the wire(s) coiled and bound with masking tape. Animals were allowed to recover for a minimum of 5 days before experimentation, and were given chlorotetracycline hydrochloride

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(Aureomycin, Cynamid G.B. Ltd., Gosport) 50mg/l in the drinking water for 3-5 days following surgery.

The parameters of stimulation were based on the parameters used by Fink and Aiyer (1974) and Jamieson and Fink (1976), and consisted of trains (30 sec on and 30 sec off) of accurately balanced biphasic square wave pulses with a frequency of 50 Hz, pulse duration of 1 msec and pulse amplitude of 500 μA (1 mA peak to peak, see section 6.2). The stimulus was produced by a constant current stimulator (Digitimer Neurolog, Welwyn Garden City) and was monitored on a calibrated oscilloscope (Scopex 14D-10). To determine the exact placement of the electrode tip(s), 1 20 μm cryostat tissue section in every 5 collected throughout the region containing the electrode track(s) was used for staining. The sections were stained with luxol fast blue and cresyl violet stains (Appendix IV) and the location of the electrode tip(s) was identified using a microscope. Damage to the tissue caused by the electrode track(s) was also identified.

2.2.9.2. Median eminence

The procedure for the implantation of electrodes in the ME was based on the transpharyngeal approach for the exposure of hypophysial portal vessels described by Green and Harris (1949) and modified by Worthington (1966). The animal was anaesthetised with Althesin (section 2.2.3) and immobilised in the supine position on a board. A midline incision was made in the skin of the lower jaw, and the skin was retracted to expose the pretracheal muscles. These muscles were divided with a midline incision and a silk thread was passed under the exposed trachea. A polythene tube (15 mm long) was

inserted into the trachea through a small transverse incision, and tied in place with the thread. Silk guy sutures were placed through the tip of the tongue and through each of the flaps of the divided lower lip. The lower jaw was split with a midline incision and the cut extended through the muscles of the floor of the mouth on either side of the tongue in order to mobilise the tongue. A ligature was placed around the base of the tongue and the tongue was withdrawn and secured in place over the chest of the animal. The remaining procedure was carried out using a binocular microscope (Zeiss).

The exposed soft palate was incised with an iris cautery along the midline, longitudinally from the caudal edge of the hard palate to the epiglottis, and laterally to the pterygoid processes of the sphenoid. The mucosa overlying the basisphenoid was detached using a pledget of cotton wool. For exposure of the ME a hole was drilled in the outer table of the basisphenoid bone approximately 1 mm caudal to the transverse venous sinus. The hole was carefully extended rostrally to the basisphenoid-presphenoid suture. The inner table was then 'polished' using a fine drill head, until thin enough to be removed using watchmaker forceps. A V-shaped incision was then made in the dura mater with a piece of razorblade held in a chuck. The cuts were started over the pituitary gland and extended rostrally so that the apex of the cut was rostral to the ME. The resulting flap of dura mater and arachnoid mater was retracted caudally to expose the ME, portal vessels and PD.

Unipolar electrodes (11 mm) were used in these experiments, and were inserted into the ME with the aid of a micromanipulator. The parameters of stimulation were identical to the parameters of

stimulation used in the previous section (2.2.9.1).

2.2.10 Brain lesioning

2.2.10.1 Electrothermal lesioning

Animals were anaesthetised with Avertin supplemented with ether (section 2.2.3) and the cranium and dura mater over the appropriate brain area exposed. An unipolar glass-insulated platinum electrode was introduced into the brain as described in Section 2.2.9.1(see section 6.2 for co-ordinates). The electrode was connected to a Grass LM4 lesion maker and an indifferent electrode placed in the rectum. For the lesion, a 3-5 mA current was passed with a frequency of 100 KHz for 20 sec. The skin incision was sutured and the animal was allowed to recover for a minimum of 3 days and a maximum of 7 days before experimentation. Animals were given Aureomycin in the drinking water as described in section 2.2.9.1.

To determine the exact site of the lesion, tissue sections were collected and stained as described in section 2.2.9.1, and the lesion site was located with the aid of a microscope.

2.2.10.2 Knife cut lesioning

Animals were anaesthetised with Avertin supplemented with ether (section 2.2.3) and the cranium and dura mater over the appropriate brain area exposed. A knife made from a piece of razor blade 12 mm in length, 0.6 mm in width, was introduced into the brain just rostral to the RA (for co-ordinates see section 6.2), using a similar procedure to the method described in section 2.2.9.1. Bleeding was controlled by applying pressure to the cranium with cotton wool, and the skin incision was sutured. The animal was allowed to recover for a minimum of 3 days and a maximum of 7 days

before experimentation, and was given Aureomycin in the drinking water as described in section 2.2.9.1. The lesion site was located using stained tissue sections that were collected and stained as described in section 2.2.9.1.

2.2.11 Mammary nerve exposure and stimulation

The mammary nerve was exposed according to the method of Mena, Pacheco, Aguayo, Clapp and Grosvenor (1978). Animals were anaesthetised with urethane (section 2.2.3) and the abdominal skin cut from the outer edge of the paravertebral muscles to the nipple of an abdominal mammary gland, to expose the segmental nerve. The deeper branches of the nerve were cut near the limits of the mammary parenchyma and dissected free to the point where the nerve penetrated the lumbar muscles. The nerve was threaded into a perspex chamber between 2 ring electrodes (approximately 2 mm diameter, 2 mm separation) made from fine silver wire. The animals were allowed to rest for 45-60 min after dissection. The mammary nerve was stimulated using a constant current stimulator (Digitimer Neurolog, Welwyn Garden City). The parameters of stimulation used were based on the parameters used by Mena et al. (1978) and were 1 msec pulses at 20 Hz and 10 V for 10 s followed by 10 s of rest.

2.2.12 Ovariectomy

Animals were anaesthetised with ether and bilateral ovariectomy was carried out through bilateral flank incisions.

2.2.13 Steroid injection

Oestradiol benzoate (OB) (Paines and Byrne Ltd., Greenford, Middlesex) in ethyl oleate was diluted with arachis oil (Hopkins and Williams, Romford, Essex) and administered subcutaneously at a dose of 10 μg (0.2 ml).

2.3 Analytical techniques

2.3.1 Quantitative autoradiography

2.3.1.1 Measurement of relative metabolic activity

The quantitative autoradiographic technique described by Reivich et al. (1969) and modified by Sokoloff et al. (1977) was used to determine the relative [^{14}C] concentrations in localised regions of the brain.

Optical density (OD) is a measure of the absorption of light, which, obeying Lambert-Beer's Law is a function of the intensity of incident light and the concentration of absorbent material in the section.

$$I = I_o e^{\alpha ct}$$

where

I = measured light

I_o = incident light

α = negative constant

c = concentration of absorbent material

t = thickness of section

$$\log \frac{I}{Io} = \alpha ct$$

and the ratio $\frac{I}{Io}$ = Transmission (T)

Optical density = $\log \frac{1}{T} = \log \left(\frac{Io}{I}\right)$

The measurement of OD can therefore be related to the concentration of absorbent material within a section of constant thickness.

The transmission of light through the portion of autoradiograph representing each standard was measured using a Quantimet 800

(Cambridge Instruments). The measured transmission, expressed as an unit of brightness on a scale of 0 to 64 (where 0 = zero transmission and 64 = total transmission), was converted to an OD using the equation described above;

$$\text{Optical density} = \log \left(\frac{Io}{I}\right)$$ where Io = brightness value measured from the background film.

The [^{14}C] concentrations of the standards, matched with standards calibrated and tissue matched by Dr. Louis Sokoloff (National Institute of Mental Health, Bethesda, Maryland) according to the method of Reivich et al. (1969), could then be used to construct a standard curve for OD and [^{14}C] concentration for each autoradiograph. Each curve was constructed using a 3rd order linear regression, using the method of least squares. A representative standard curve is shown in Fig. 2-A. Selected brain structures were identified on the autoradiograph using a rat atlas (Pellegrino, Pellegrino and Cushman, 1967), and a mean OD obtained for each area using a minimum of 10 determinations over 5 sections. In some experiments areas in the 'right' and 'left' sides of the brain were measured separately, and in other experiments the mean OD of an area was calculated from combined right and left sides. The [^{14}C] concentration was then computed for each area using the standard curve.

The [^{14}C] concentration for each area was expressed as a ratio to the [^{14}C] concentration of the CC, and termed the rma. The CC was used as an internal standard for each brain to minimise differences in film development and 2DG uptake by the brain.

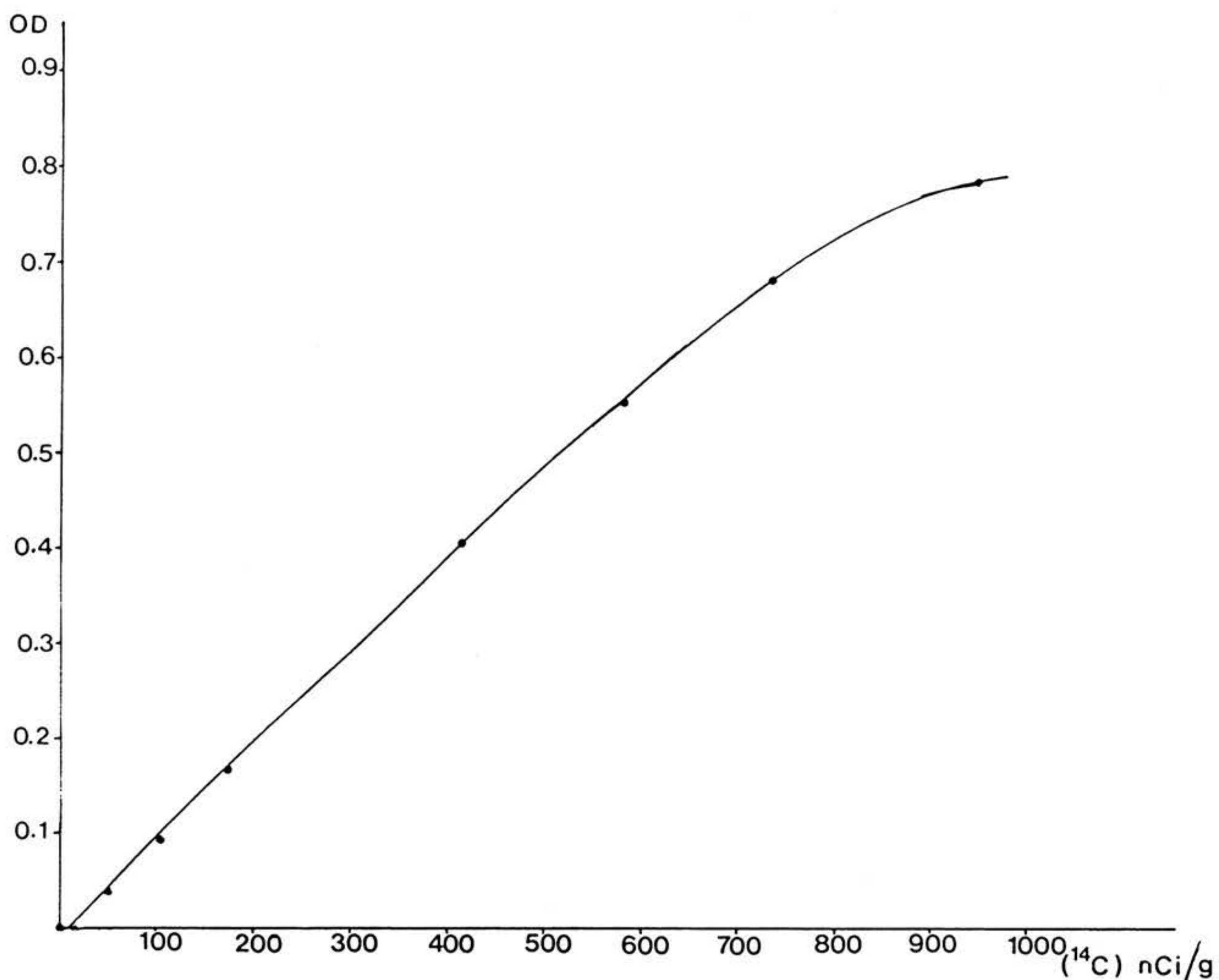


Fig. 2-A: standard curve for optical density (OD) and [^{14}C] concentration determined from standards calibrated for 20 μm tissue sections.

2.3.1.2 Measurement of local rates of glucose utilisation

The arterial plasma concentrations of glucose and [^{14}C] 2DG (2.3.3 and 2.3.4) and the rate constants for [^{14}C] 2DG transport and phosphorylation (Sokoloff et al., 1977) were used in the formula described by Sokoloff et al. (1977) to determine the final concentration of [^{14}C] 2DG remaining in the tissue, and the final integrated precursor specific activity in the tissue. These two variables, the [^{14}C] concentration of each brain area (2.3.1.1) and the lumped constant (Sokoloff et al., 1977) were then used in the operation equation described by Sokoloff et al. (1977) to calculate the LCGU of each area (see Introduction for details). A Cromenco System Three computer was used for the calculations, using a programme devised by Dr. James McCulloch (Wellcome Surgical Institute, University of Glasgow).

Significance of differences in rma and LCGU was assessed using the unpaired Students t-test, and analysis of variance and Duncan's multiple range test (Harter, 1960).

2.3.2 Radioimmunoassay

2.3.2.1 Introduction

Radioimmunoassay (RIA) techniques were used to measure the concentration of PRL and LH in rat plasma. Several reviews have described the basic principles, theory and techniques of RIA of peptide hormones (Midgley, Niswender and Rebar, 1969; Kirkham and Hunter, 1971; Rodbard, 1971; Ekins, 1974; Yalow, 1980) and therefore only the relevant methodology is described here.

Rat PRL and ovine LH were labelled with I^{125} (NaI^{125}, Amersham International, Bucks.) using modifications of the

chloramine-T method of Greenwood, Hunter and Glover (1963) (Appendices V (b) and VI (b)). The double antibody technique of Utiger, Parker and Daughaday (1962) was used to separate 'free' and 'bound' hormone. Details of the preparation of stock solutions, methods for radioisotope labelling and assay protocols are given in Appendices V and VI.

The 'bound' hormone was counted using an automatic Gamma Counter (Berthold Mag 310, Scotlab, Lanarkshire) and the concentration of hormone was determined by linear regression from standard curves, logit B/Bo on the ordinate, log concentrations on the abscissa (B/Bo = counts per minute (cpm) of standard or sample minus background/cpm of total bound minus background, logit B/Bo

$= \text{Ln}\left(\frac{B/Bo}{1-B/Bo}\right)$).

The lower limit of assay sensitivity was determined by calculating mean cpm minus 2 times standard deviation of the total bound tubes (Rodbard, 1971). Quality control for PRL and LH RIA was determined by monitoring variation of the total counts (labelled hormone), blanks (zero antiserum added), total bound (zero unlabelled hormone) as a percentage of total counts (% bound) and the equivalent concentrations of the 20%, 50% and 80% B/Bo.

Samples for all RIA's were aliquoted into LP3 assay tubes (Luckhams Ltd., Burgess Hill, Sussex) using Gilson Pippetman (Scotlab Instrument Sales Ltd., Lanarkshire). Reagents were aliquoted using a Dispensette (Brand) or 'Micromedic' automatic pipetting systems.

2.3.2.2 Radioimmunoassay of Prolactin

Double antibody RIA kits supplied by the National Institute of

Arthritis, Diabetes, Digestive and Kidney Disease (NIADDK), Baltimore, U.S.A., were used to measure the concentration of PRL in rat plasma samples. The hormone used for iodination was rat-PRL-1-5 (NIADDK), and to prepare reference standards was rat-PRL-RP-1 (NIADDK) (range 0.5-64 ng/ml). The hormone specific antibody was anti-rat-PRL-S8 (NIADDK) used at a working dilution of 1:5000. The second antibody was anti-rabbit gamma globulin (ARGG) supplied by the Scottish Antibody Production Unit, Law Hospital, Lanarkshire, used at a working dilution of 1:20. The assay protocol has been described by Pickering and Fink (1979) and is outlined in Appendix V. A representative standard curve and quality control data for the assay are shown in Fig. 2-B and Table 2-1 respectively. The lower limits of sensitivity of the assays for 20 μl samples ranged from 12-20 ng/ml.

2.3.2.3 Radioimmunoassay of luteinising hormone

Luteinising hormone was measured in rat plasma samples using the ovine-ovine RIA method developed by Niswender, Midgley, Monroe and Reichert (1968). Ovine pituitary LH (LER-10565-C2) used for iodination was provided by Dr. L.E. Reichert Jr. (NIADDK), and ovine LH (NIH-LH-S18) used to prepare the standards (range 0.25-16 ng/ml) was also provided by the NIADDK. The ovine LH specific antibody (GDN-15) was raised in rabbits and was provided by Dr. G.D. Niswender (NIADDK). The antibody was used at a working dilution of 1:60000. The second antibody was ARGG (Scottish Antibody Production Unit, Law Hospital, Lanarkshire) and was used at a working dilution of 1:20. The LH RIA protocol used in this thesis has been described by Aiyer et al. (1974b) and Chiappa and Fink (1977), and is outlined

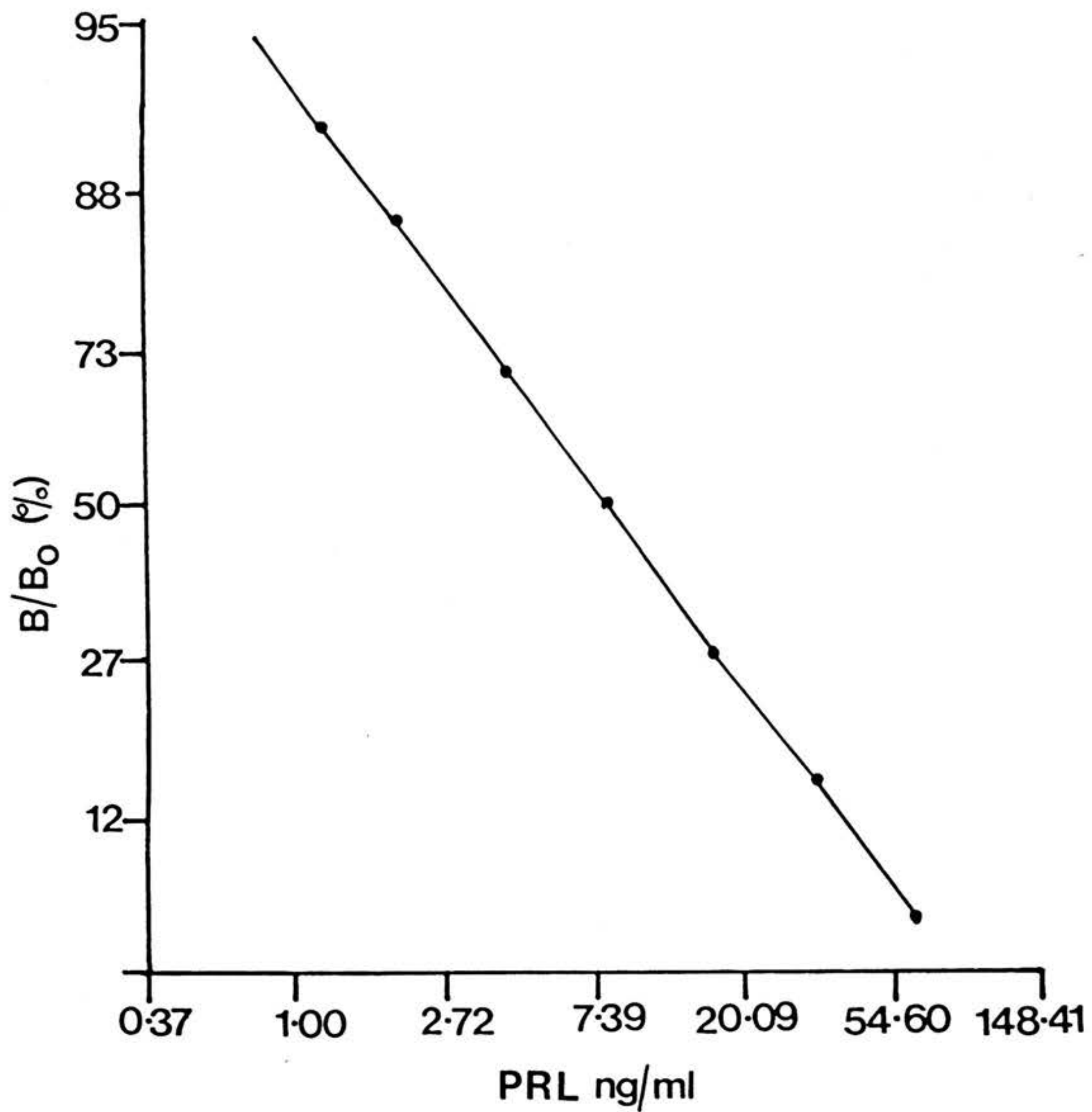


Fig. 2-B: standard curve of a representative assay for prolactin (PRL). Each point represents the mean of 3 replicate samples.

Table 2-1 Quality control data for prolactin radioimmunoassays

Assay	Total Counts (TC)	Blanks %TC	Total Bound %TC	Equivalent conc. (ng/ml) B/Bo		
				20%	50%	80%
1	9696.8	2.33	45.6	31.6	10.2	3.3
2	10961	2.40	40.7	25.3	8.2	2.6

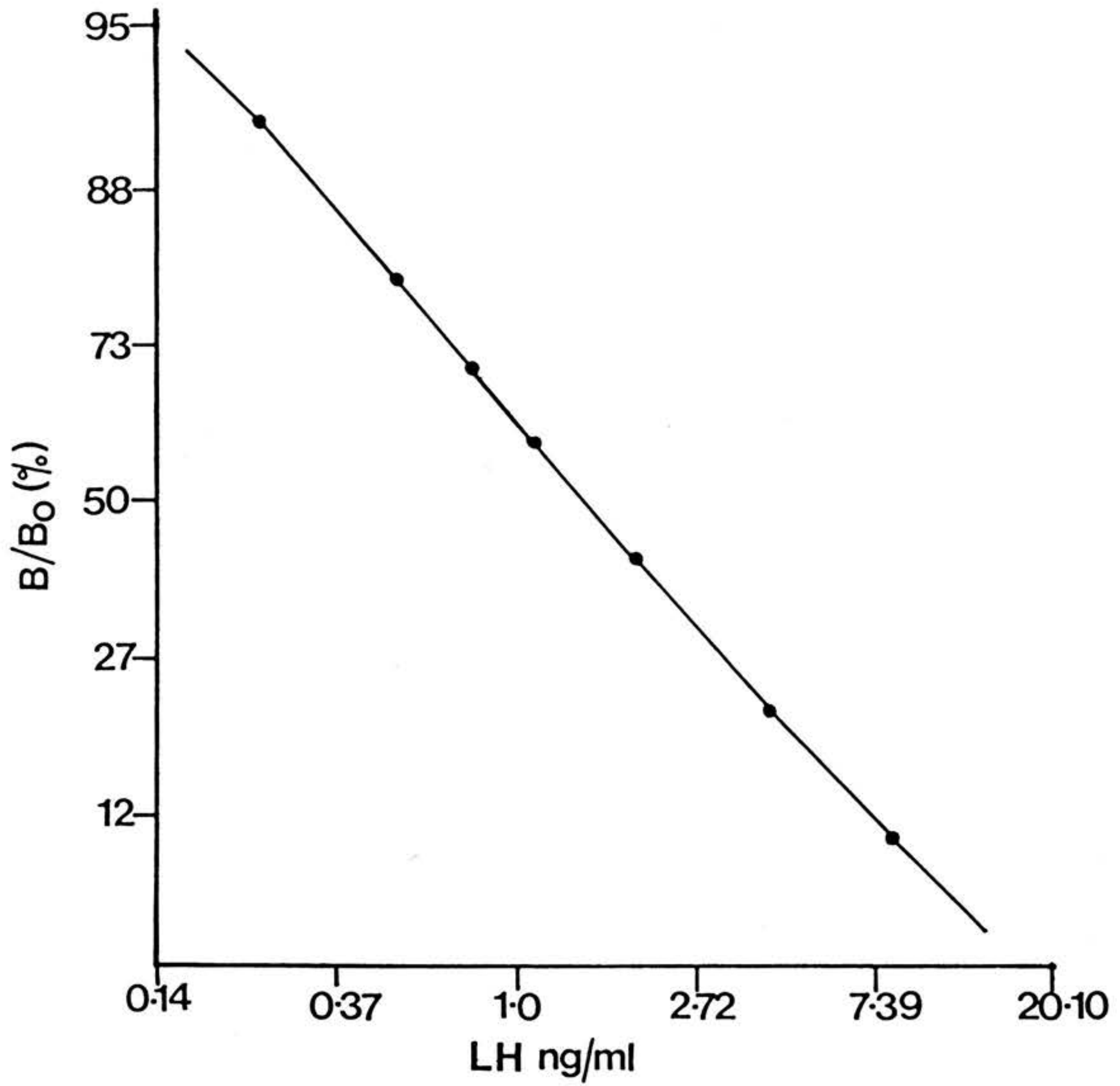


Fig. 2-C: standard curve of a representative assay for luteinising hormone (LH). Each point represents the mean of 3 replicate samples.

Table 2-2. Quality Control data for luteinising hormone radioimmunoassays

Assay	Total Counts (TC)	Blanks %TC	Total Bound %TC	Equivalent conc. (ng/ml) $\frac{B}{Bo}$		
				20%	50%	80%
1	9791	1.7	50.3	4.1	1.4	0.4
2	9711	1.6	41.2	4.0	1.3	0.4

in Appendix VI.

A representative standard curve and quality control data for LH RIA are shown in Fig. 2-C and Table 2-2 respectively. The lower limit of sensitivity of the assay for 20 µl plasma samples was 2.5 ng/ml.

2.3.3 Determination of arterial plasma [^{14}C] 2-deoxyglucose concentrations by liquid scintillation counting

Aliquots (20 µl) of arterial plasma were pipetted into scintillation vials containing 1 ml distilled water. The vials were shaken and 10 ml scintillation fluid (Aqualuma Plus, Lumac B.V., The Netherlands) were added. The vials were shaken vigorously and left in a scintillation counter (Beckman LS 6800) for a minimum of 1 h before counting. The counter was calibrated with an internal standard (Beckman H-3 95900 DPM) and the samples were counted for 4 min with an efficiency of 93% to obtain [^{14}C] disintegrations per minute(DPM).

2.3.4 Determination of arterial plasma glucose concentrations by the quantitative enzymatic method

The quantitative enzymatic method for the determination of glucose concentration is based on the conversion of glucose to glucose-6-phosphate by ATP in the presence of hexokinase, coupled with the subsequent reduction of NAD to NADP. As NADP has a high absorbance at 340 nM and NAD has no absorbance at this wavelength the reaction can be followed by measuring the increase in absorbance at 340 nM, which is directly proportional to the amount of glucose present.

A Sigma glucose assay kit was used (Stock No. 15-10). The

stock reagents were reconstituted with 31 ml distilled water (ATP 1 mmol/l, NADP 0.5 mmol/l, Mg^{++} 2 mmol/l, Hexokinase 800 U/l, G-6-PD 500 U/l, buffer salts). Plasma samples (10 μl) and 10 μl aliquots of glucose standards (Glucose, B.D.H.) were mixed with 1.5 ml of reconstituted reagents, and were left at room temperature for 10 min. The absorbance of each sample was then measured at 340 nM, using a spectrophotometer (Gilford 250).

A standard curve of glucose concentration and absorbance was then constructed from the glucose standards, using a linear regression, using the method of least squares (Fig. 2-D). The glucose concentration of each arterial plasma sample was then computed using the standard curve and converted to μmol/ml using a multiplication factor of 0.0556.

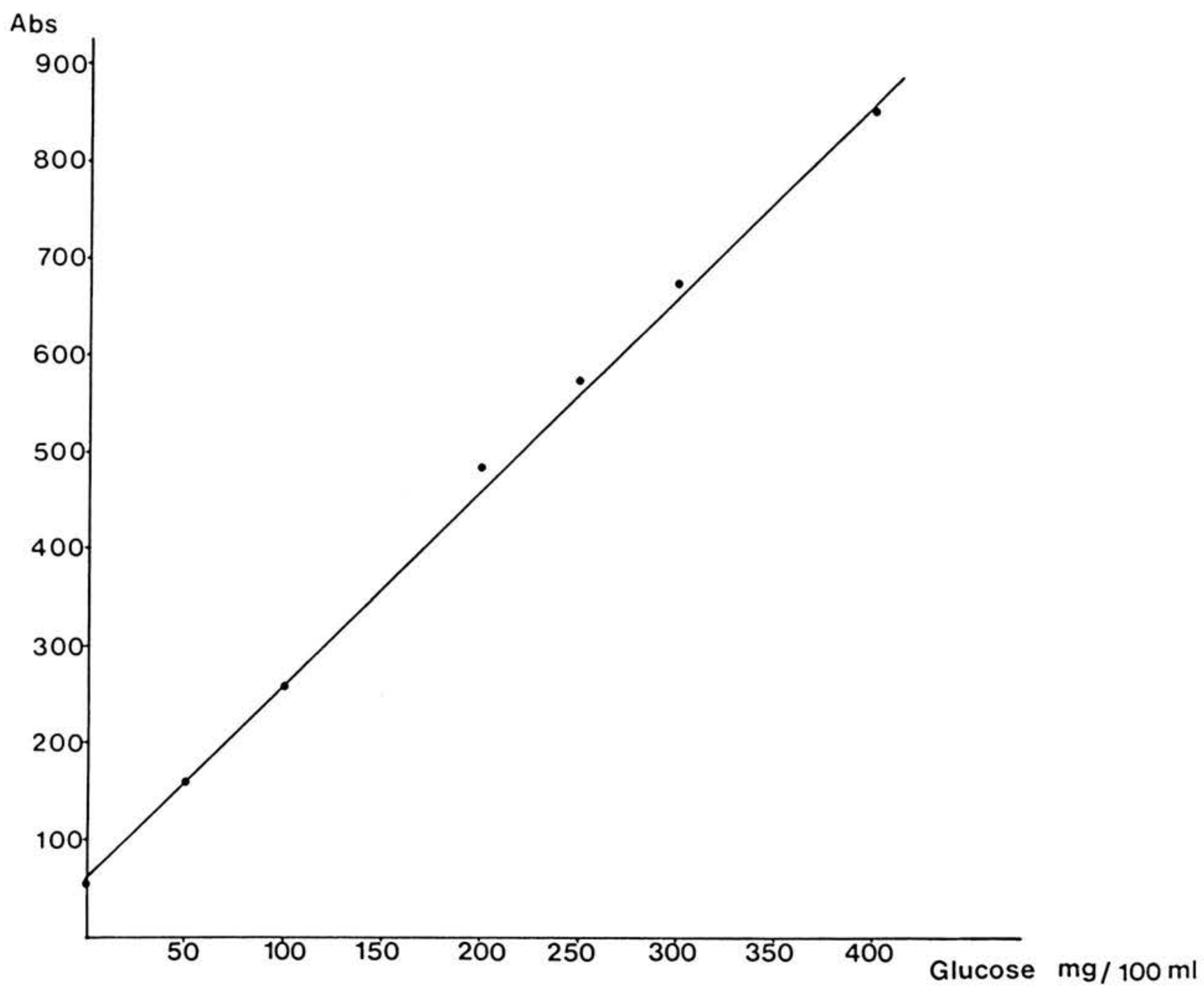


Fig. 2-D: standard curve for glucose concentration and absorbance.
Each point represents the mean of 3 replicate samples.

CHAPTER III

Effects of Water Deprivation and Desamino-D-Arginine^{8}-Vasopressin on the Hypothalamo-hypophysial System in Wistar and Brattleboro Rats

3.1 INTRODUCTION

Projections from the PVN and SON to the PN have been recognised for some time (Cajal, 1894; Rasmussen, 1940; Bodian and Maren, 1951). The role of these magnocellular neurones in the synthesis, transport and release of the posterior pituitary hormones, vasopressin and oxytocin and their respective neurophysins, has been well documented (e.g. Brownstein et al., 1980), and it has been shown that the peptides can be released from nerve terminals in the PN in response to different stimuli such as suckling and haemorrhage (Poulain et al., 1977). The PVN is now thought to consist of many distinct cellular subdivisions (Swanson and Kuypers, 1980), and the organisation of oxytocin- and vasopressin-containing cells within these subdivisions of the PVN and in the SON have been clarified (Swaab et al., 1975a,b; Vandesande and Dierickx, 1975; Swanson et al., 1981a). Although the electrical and synthetic activities of the neurosecretory neurones have been investigated in response to osmotic stimulation (Takabatake and Sachs, 1964; Dyball, 1971; Morris and Dyball, 1974), little is known about the metabolic activity associated with the neuroendocrine function of these neurones. Schwartz et al. (1979) used the 2DG technique to determine metabolic activity in the rat neurohypophysial system after osmotic stimulation. In rats maintained on two per cent sodium chloride in drinking water, the PN showed a large increase in 2DG uptake with no concurrent change in uptake by the PVN and SON. The aim of the present studies was to examine the effect of another osmotic stimulus, water deprivation, on metabolic activity in the rat hypothalamo-neurohypophysial system using [^{14}C]2DG and

$[^3H]$2DG. The study also included measurements of the metabolic activity of this system in the Brattleboro rat, which has hereditary diabetes insipidus due to a lack of vasopressin (Valtin, Sawyer and Sokol, 1965). In addition, the effects of water deprivation and the administration of desamino-D-arginine-^{8}-vasopressin (dDAVP) on activity in the hypothalamo-neurohypophysial system were studied in the Brattleboro rat.

3.2 MATERIALS AND METHODS AND EXPERIMENTAL DESIGN

3.2.1 Animals

The animals used were adult female rats of the Wistar strain, 180-250 g body weight, purchased from Charles River U.K. Ltd. (Margate, Kent), the Piebald Virol Glaxo (PVG) strain, 130-200 g body weight, bred in the department, and homozygous Brattleboro rats, 200-290 g body weight, also bred in the department. The rats were maintained as described in section 2.1.

3.2.2 Studies with $[^{14}C]$ 2-deoxyglucose

The following groups of animals were studied:

1. Wistar rats, normal and deprived of water but not food for 4 days before injecting 2DG.
2. Normal PVG rats (included as another control group for the Brattleboro rats).
3. Brattleboro rats, either untreated, or deprived of water but not food for 12-15 h before injecting 2DG, or injected with dDAVP (Desmopressin, Ferring Pharmaceuticals, Middlesex). The dDAVP was injected i.p. at a dose of 10 μg/100 g body weight, diluted with 0.9% saline solution (final volume 0.1 ml) at either 24h before, or at the same time as 2DG (45 min study). Animals

treated with dDAVP 24h before 2DG were housed in metabolic cages for the 24h before and after the injection of dDAVP, and the urine was collected and measured.

All animals were implanted with an intra-atrial cannula 24h before the 2DG was injected (2.2.4). The vaginal smear of each animal was examined before injection of 2DG; each group comprised at least one animal at each stage of the oestrous cycle. The 2DG was injected i.v. at a dose of 250 μCi/ kg body weight (2.2.4). In some animals, blood samples (0.4 ml) were collected by way of the cannula (2.2.4) immediately before and at 30 and 42 min after the injection of 2DG. Plasma was prepared from the blood samples (2.2.7) and the plasma osmolalities were determined using a Wescor Inc. 5100B vapour pressure osmometer. Measurements were made on at least two samples in duplicate from each animal.

The animals were killed by decapitation 45 min after the injection of 2DG. The brain and pituitary gland were removed _en bloc_ and were processed for autoradiography (2.2.1). The rma of selected brain and pituitary gland areas were then determined from the autoradiographs (2.3.1.1). Significance of differences in rma was assessed by the unpaired t-test, and in plasma osmolality by the unpaired and paired t-test as appropriate.

3.2.3 Studies with [^{3}H] 2-deoxyglucose

Brattleboro rats were implanted with an intra-atrial cannula (2.2.4) and allowed to recover for 10h. The animals were then deprived of water for 12-15h (overnight) before [^{3}H]2DG was injected i.v. (2.2.4). After 45 min the animals were anaesthetised with sodium pentobarbitone (2.2.3) and perfused through the heart

with saline and fixative (2.2.2). The brain was removed and a tissue block containing the hypothalamus was prepared for emulsion autoradiography (2.2.2). The autoradiographic slides were developed 14–18 weeks later, and the tissue sections were then stained (2.2.2). The slides were examined with the aid of a Zeiss microscope, under light and dark fields.

3.3 RESULTS

3.3.1 Results of studies with [^{14}C] 2-deoxyglucose

3.3.1.1 Use of an internal standard

There were no significant differences in rma of the IC between individual animals, strains and experimental groups (Table 3-1). It was therefore justified to assume that areas of white matter quite distinct topographically and in terms of blood supply were stable in terms of rma in this study, and so to use the CC as an internal standard.

3.3.1.2 Wistar rats

3.3.1.2.1 Comparison with PVG rats

There were no significant differences in rma between the two strains in any brain and pituitary gland area measured (Tables 3-1 and 3-2).

3.3.1.2.2 Effect of water deprivation

Table 3-1 and Fig. 3-C show that the rma of the PN was significantly ($P < 0.001$) greater in water-deprived than in normal Wistar rats. Water deprivation also increased significantly ($P < 0.02$) the rma of the PVN (Table 3-1) and in particular the anterior region of the nucleus ($P < 0.001$, Table 3-1 and Fig. 3-A). The rma of the SON was not significantly affected by water

Table 3-1. Relative metabolic activity of selected brain regions and the pituitary gland in rats

Values are expressed as a ratio (mean ± S.E.M.) of [^{14}C] concentration of the selected region to [^{14}C] concentration of the corpus callosum

Type of animal	No. of Animals	PN	PD	SON	APVN	PVN	IC
Wistar rats	6	1.00 ± 0.07	0.80 ± 0.05	1.07 ± 0.04	1.28 ± 0.04	1.29 ± 0.06	1.05 ± 0.04
Wistar rats, water-deprived	4	2.19 ± 0.26	0.58 ± 0.03	1.25 ± 0.09	1.68 ± 0.04	1.47 ± 0.02	1.09 ± 0.01
PVG rats	5	1.07 ± 0.06	0.89 ± 0.04	1.03 ± 0.05	1.36 ± 0.05	1.30 ± 0.05	1.00 ± 0.04
Brattleboro rats	5	1.40 ± 0.07	1.14 ± 0.05	1.05 ± 0.07	1.36 ± 0.03	1.43 ± 0.08	1.02 ± 0.02
Brattleboro rats, water-deprived	4	2.99 ± 0.34	0.87 ± 0.06	1.54 ± 0.27	1.63 ± 0.10	1.99 ± 0.18	1.04 ± 0.04
Brattleboro rats + dDAVP-24h	4	1.11 ± 0.08	0.87 ± 0.03	1.05 ± 0.02	1.41 ± 0.05	1.24 ± 0.04	1.11 ± 0.04
Brattleboro rats + dDAVP-45 min	4	1.01 ± 0.06	0.89 ± 0.07	1.00 ± 0.06	1.35 ± 0.07	1.26 ± 0.05	1.05 ± 0.04

SON, supraoptic nuclei; APVN, anterior portion of paraventricular nucleus; PVN, paraventricular nucleus; IC, internal capsule

Table 3-2. Relative metabolic activity of selected brain regions in rats

Values are expressed as a ratio (mean ± S.E.M.) of [^{14}C] concentration of the selected region to [^{14}C] concentration of the corpus callosum.

	Type and number of animal					
	Wistar (n=6)	Wistar, water-deprived (n=4)	PVG (n=5)	Brattleboro (n=5)	Brattleboro, water-deprived (n=4)	Brattleboro + dDAVP-24 h (n=4)
S	2.08 ± 0.14	2.30 ± 0.09	1.95 ± 0.12	1.79 ± 0.05	1.85 ± 0.07	2.03 ± 0.08
POA	1.46 ± 0.09	1.38 ± 0.04	1.28 ± 0.06	1.24 ± 0.03	1.27 ± 0.04	1.30 ± 0.02
SCN	2.12 ± 0.08	2.23 ± 0.12	1.82 ± 0.11	1.60 ± 0.06	1.78 ± 0.07	1.72 ± 0.06
AHA	1.63 ± 0.07	1.67 ± 0.07	1.34 ± 0.06	1.33 ± 0.06	1.38 ± 0.05	1.41 ± 0.03
VmArc	1.37 ± 0.08	1.28 ± 0.04	1.19 ± 0.05	1.24 ± 0.03	1.26 ± 0.05	1.30 ± 0.05
MFB	1.94 ± 0.06	2.13 ± 0.06	1.77 ± 0.07	1.82 ± 0.05	1.94 ± 0.07	1.98 ± 0.05
AMYG	1.45 ± 0.07	1.43 ± 0.02	1.21 ± 0.05	1.28 ± 0.04	1.24 ± 0.03	1.31 ± 0.03
MM	2.70 ± 0.14	3.11 ± 0.27	2.61 ± 0.11	2.77 ± 0.06	2.85 ± 0.13	2.99 ± 0.20
IP	2.46 ± 0.10	2.68 ± 0.16	2.54 ± 0.12	2.72 ± 0.08	2.59 ± 0.09	2.74 ± 0.14

S, septum; POA, preoptic area; SCN, suprachiasmatic nucleus; AHA, anterior hypothalamic area; VmArc, ventromedial-arcuate hypothalamic area; MFB, medial forebrain bundle; AMYG, amygdala; MM, mamillary body; IP, interpeduncular nucleus

Table 3-2 (cont). Relative metabolic activity of selected brain regions in rats

Values are expressed as a ratio (means ± S.E.M.) of [^{14}C] concentration of the selected region to [^{14}C] concentration of the corpus callosum

	Type and number of animal					
	Wistar (n=6)	Wistar, water-deprived (n=4)	PVG (n=5)	Brattleboro (n=5)	Brattleboro, water-deprived (n=4)	Brattleboro + dDAVP-24 h (n=4)
VT	2.20 ± 0.17	2.46 ± 0.16	2.24 ± 0.13	2.18 ± 0.08	2.31 ± 0.07	2.40 ± 0.16
LT	2.58 ± 0.13	2.76 ± 0.21	2.65 ± 0.18	2.99 ± 0.16	2.74 ± 0.19	3.20 ± 0.21
VHIPPM	2.30 ± 0.15	2.27 ± 0.14	2.05 ± 0.06	2.07 ± 0.06	2.12 ± 0.05	2.10 ± 0.09
LGEN	2.15 ± 0.13	2.46 ± 0.19	2.24 ± 0.12	2.21 ± 0.04	2.29 ± 0.08	2.39 ± 0.16
MGEN	2.60 ± 0.11	2.97 ± 0.20	2.21 ± 0.09	2.31 ± 0.09	2.34 ± 0.08	2.26 ± 0.09
CS	2.11 ± 0.06	2.44 ± 0.26	1.96 ± 0.07	2.11 ± 0.08	2.29 ± 0.17	2.20 ± 0.10
CI	3.81 ± 0.21	3.92 ± 0.31	2.69 ± 0.14	3.04 ± 0.18	2.92 ± 0.15	2.66 ± 0.23
CG	1.61 ± 0.03	1.66 ± 0.08	1.33 ± 0.04	1.51 ± 0.03	1.46 ± 0.04	1.51 ± 0.08

VT, ventral thalamic area; LT, lateral thalamic area; VHIPPM, ventral hippocampus (molecular layer); LGEN, lateral geniculate bodies; MGEN, medial geniculate bodies; CS, superior colliculus; CI, inferior colliculus; CG, central grey

deprivation, but that of the PD was significantly ($P < 0.01$) reduced compared with the rma of the PD in normal animals (Table 3-1).

3.3.1.3 Brattleboro rats

3.3.1.3.1 Relative metabolic activity in untreated and water-deprived animals

Table 3-1 and Fig. 3-C show that the rma of the PN in untreated Brattleboro rats was significantly greater than that in Wistar ($P < 0.005$) and PVG ($P < 0.01$) rats; however, there were no significant differences between these 3 groups of animals in the rma of the APVN, PVN or SON. In Brattleboro rats deprived of water the rma of the PN was more than 2-times greater ($P < 0.005$) than that in untreated Brattleboro rats, and nearly 3-times greater than the rma of the PN in untreated PVG and Wistar rats (Table 3-1 and Fig. 3-C). The rma of the APVN was increased significantly ($P < 0.025$) compared with that in untreated Brattleboro rats (Table 3-1), as was the rma of the PVN ($P < 0.02$, Table 3-1 and Fig. 3-B). In water-deprived Brattleboro rats the metabolic activity of a fibre tract extending ventrally from the PVN was also increased (Fig. 3-B). When the brain sections from which the autoradiograph was made were stained immunocytochemically with an antiserum against neurophysin (by Dr. John Morris, Department of Human Anatomy, University of Oxford) a fibre tract, which corresponded in position to that of the tract which showed increased rma, was stained for neurophysin (Fig. 3-B). The fibre tract showing increased rma in water-deprived Brattleboro rats was not present in water-deprived Wistar or untreated Brattleboro rats. There was no significant difference in the rma of the SON in water-deprived compared with

untreated Brattleboro rats.

The rma of the PD of untreated Brattleboro rats was greater than that in PVG ($P < 0.01$) and Wistar ($P < 0.005$) rats, and the rma of the PD in water-deprived Brattleboro rats was significantly ($P < 0.02$) less than that in untreated Brattleboro rats (Table 3-1).

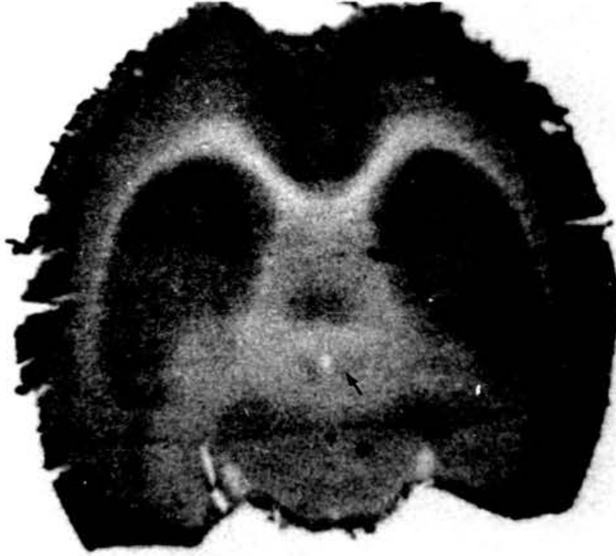
3.3.1.3.2 Effect of dDAVP

The injection of dDAVP markedly inhibited urine output in Brattleboro rats; the mean ± S.E.M. urinary output before dDAVP injection was 76.6 ± 4.0 ml/100 g/24h (n=4) compared with 3.6 ± 0.4 ml/100 g/24h (n=4) after the injection. The rma of the PN was significantly less (Table 3-1 and Fig. 3-C) in animals injected with dDAVP compared with the value in untreated Brattleboro rats, whether the injection was given 24 h before ($P < 0.05$) or at the same time as 2DG ($P < 0.005$). The rma of the PN in the animals injected with dDAVP was not significantly different from that in the PVG and Wistar rats. The rma of the APVN, PVN and SON in animals injected with dDAVP was not significantly different from that in either untreated Brattleboro, PVG or Wistar rats.

The rma of the PD was significantly less in animals injected with dDAVP compared with untreated Brattleboro rats, whether the injection was given 24h before ($P < 0.01$) or at the same time as 2DG ($P < 0.025$) (Table 3-1).

3.3.1.4 The relative metabolic activity of other brain areas

In addition to the brain and pituitary areas shown in Table 3-1, the rma of the following brain areas were determined in animals of all the experimental groups: S, POA, SCN, AHA, VmArc, MFB, AMYG, MM, VT, LT, VHIPPM, LGEN, MGEN, IP, CS, CI, CG. In none of these



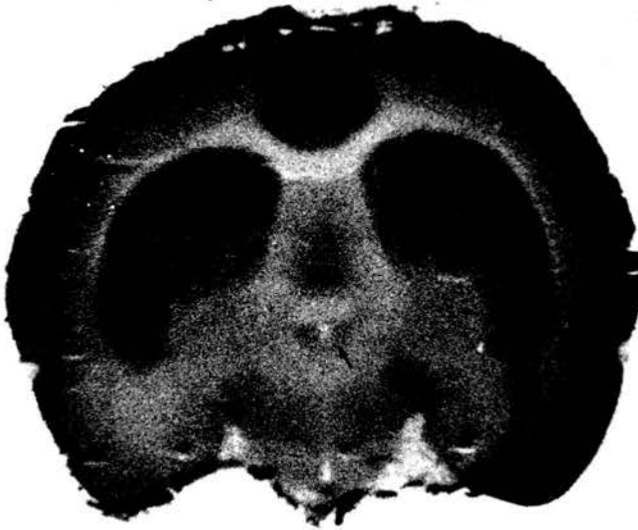


Fig. 3-A: autoradiographs of coronal sections of the brain at the level of the anterior region of the paraventricular nucleus (APVN; arrow) in Wistar rats injected with [^{14}C] 2-deoxyglucose: APVN in untreated (a) and water-deprived (b) animals.

Fig. 3-B: coronal sections of the brain at the level of the paraventricular nucleus (arrow) in Brattleboro rats injected with [^{14}C]2-deoxyglucose; autoradiographs from (a) untreated and (b) water-deprived animals, (c) immunocytochemical staining for neurophysin of the brain section used to produce (b) showing the position of the fibre tract (T; arrow).

c
T

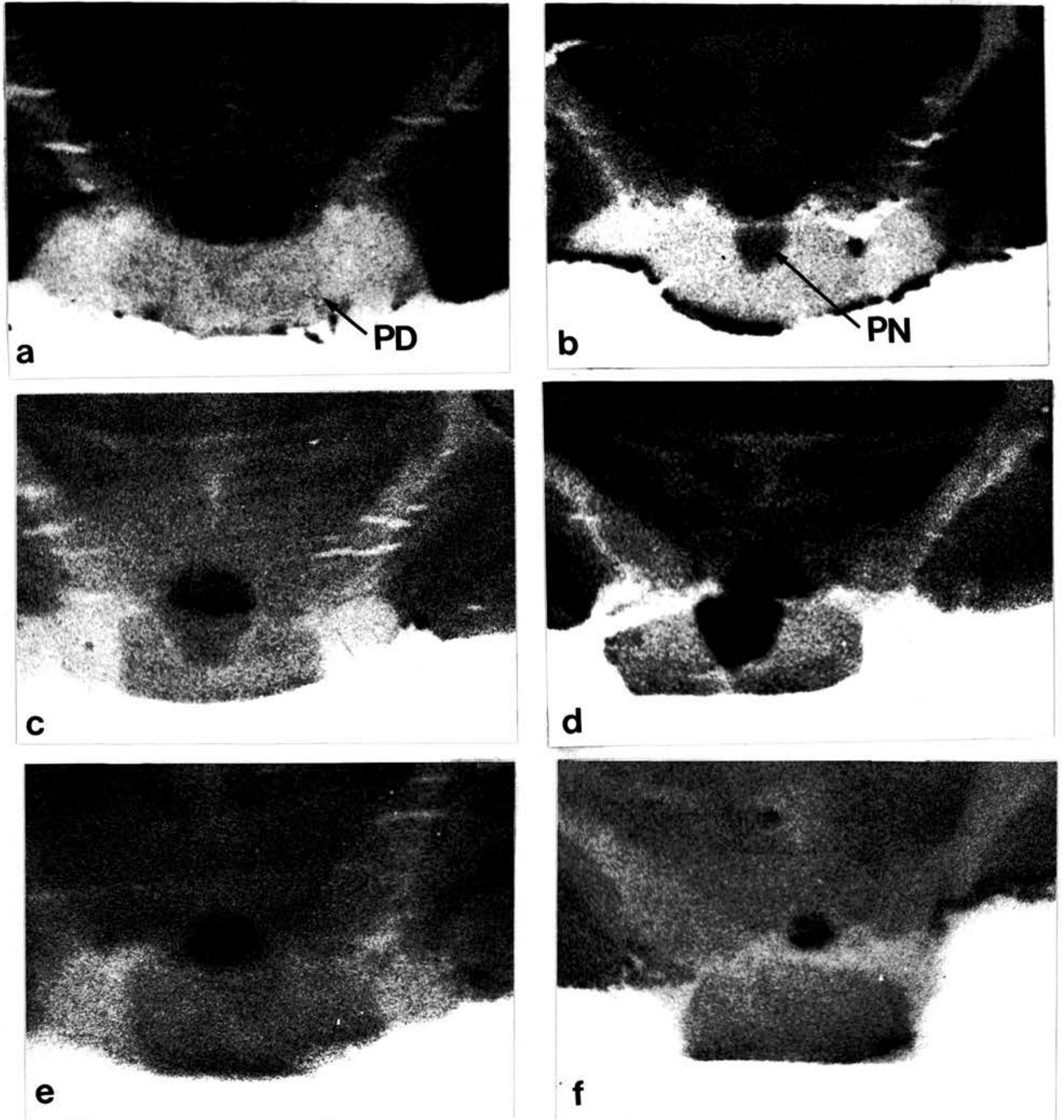


Fig. 3-C: autoradiographs of coronal sections of the pituitary gland in rats injected with [^{14}C]2-deoxyglucose, showing the pars nervosa (PN) and pars distalis (PD) in untreated (a) and water-deprived (b) Wistar rats, untreated (c) and water-deprived (d) Brattleboro rats, an untreated PVG rat (e) and a Brattleboro rat injected with desamino-D-arginine^{8}-vasopressin 45 min before decapitation (f).

areas was there a significant difference in rma either between strains or treatment groups (Table 3-2).

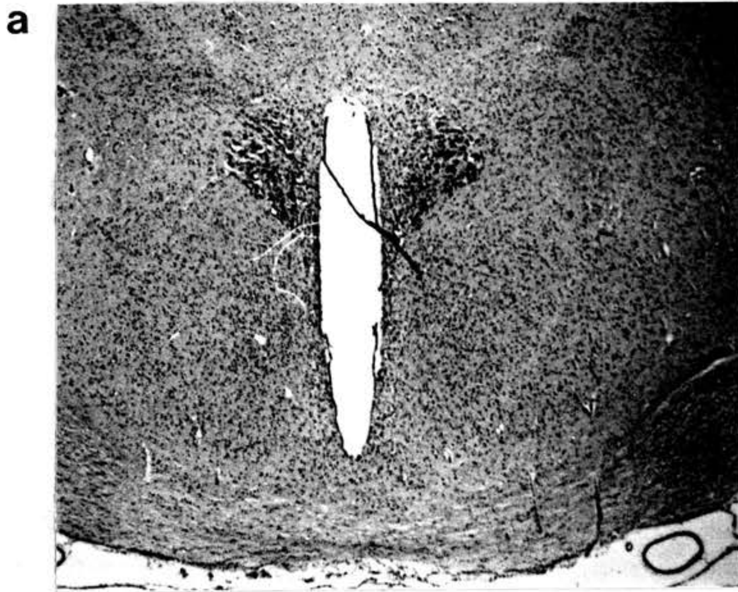
3.3.1.5 Plasma osmolality

In Brattleboro rats the mean (± S.E.M.) plasma osmolality was 289 ± 4 mosm/kg (n=5) compared with 280 ± 6 mosm/kg (n=4) in PVG rats. In the water-deprived Brattleboro rats the mean plasma osmolality was 335 mosm/kg (n=2). Twenty-four hours after the injection of dDAVP the plasma osmolality of Brattleboro rats had dropped significantly ($P < 0.05$) to 267 ± 7 mosm/kg (n=4). In animals injected with dDAVP at the same time as 2DG (45 min study) the plasma osmolality decreased from 292 ± 9 to 286 ± 10 mosm/kg (n = 3) after injection of the peptide. The difference, 6 ± 1 mosm/kg (mean ± S.E.M.), was significant ($P < 0.05$) as assessed by paired t-test.

3.3.2 Results of studies with [^{3}H] 2-deoxyglucose

Figs. 3-D - 3-F show the distribution of [^{3}H]2DG in the PVN and SON under light and dark fields of microscopy (X 80 and X 800). The [^{3}H]2DG was localised around the perikarya, but was seldom seen over the perikarya. The [^{3}H]2DG was found mainly in the magnocellular divisions of the PVN (Fig. 3-E). The anterior parvocellular (Fig. 3-F) and periventricular divisions of the PVN contained less [^{3}H]2DG, and it was distributed more diffusely throughout these areas; that is, in contrast to the magnocellular divisions, silver grains were not concentrated at the edges of the profiles of the perikarya. In the SON, the [^{3}H]2DG was found in both the dorsal and ventral compartments of the nucleus, although the amount of [^{3}H]2DG was less than in the magnocellular divisions

a



b

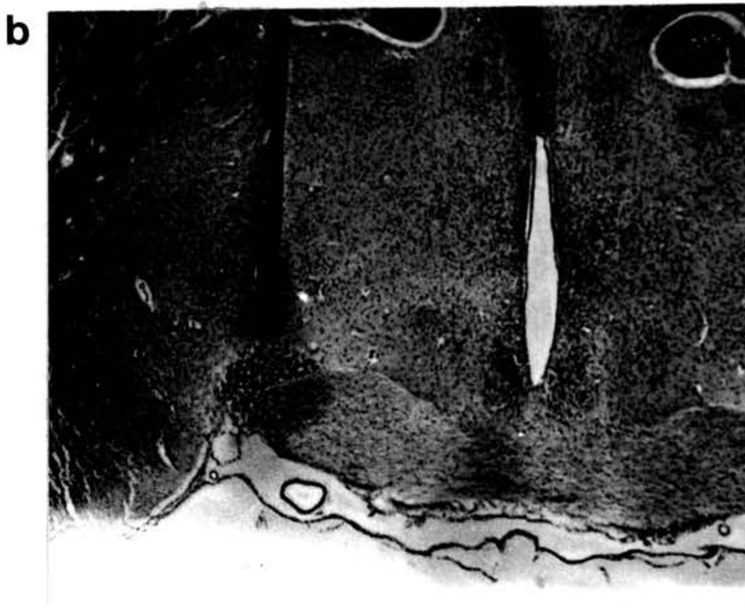
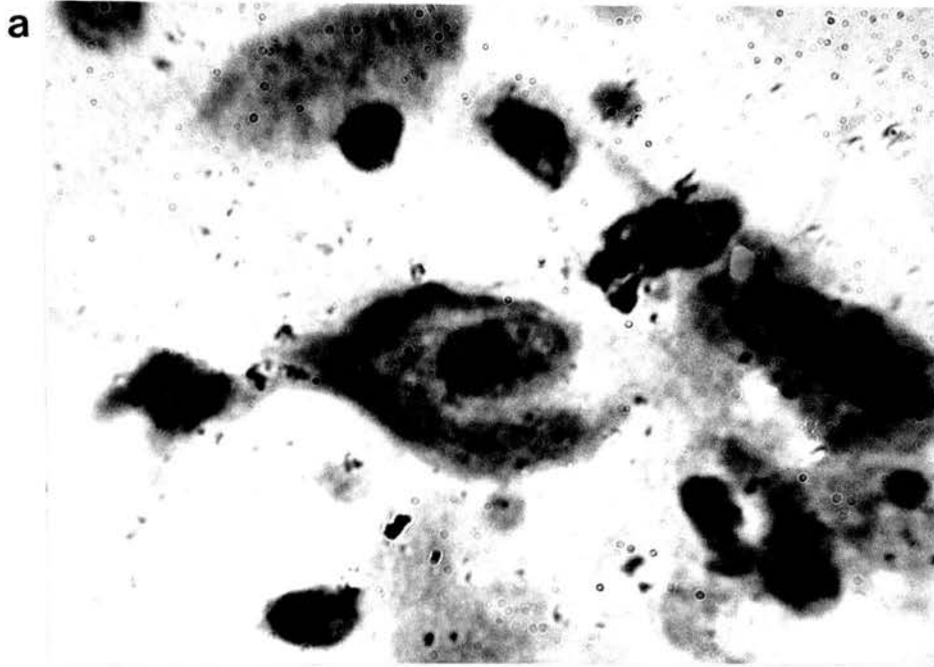


Fig. 3-D: low power (x 80) light field photomicrographs of the paraventricular (a) and supraoptic (b) nuclei in water-deprived Brattleboro rats injected with [^{3}H]2-deoxy-glucose.



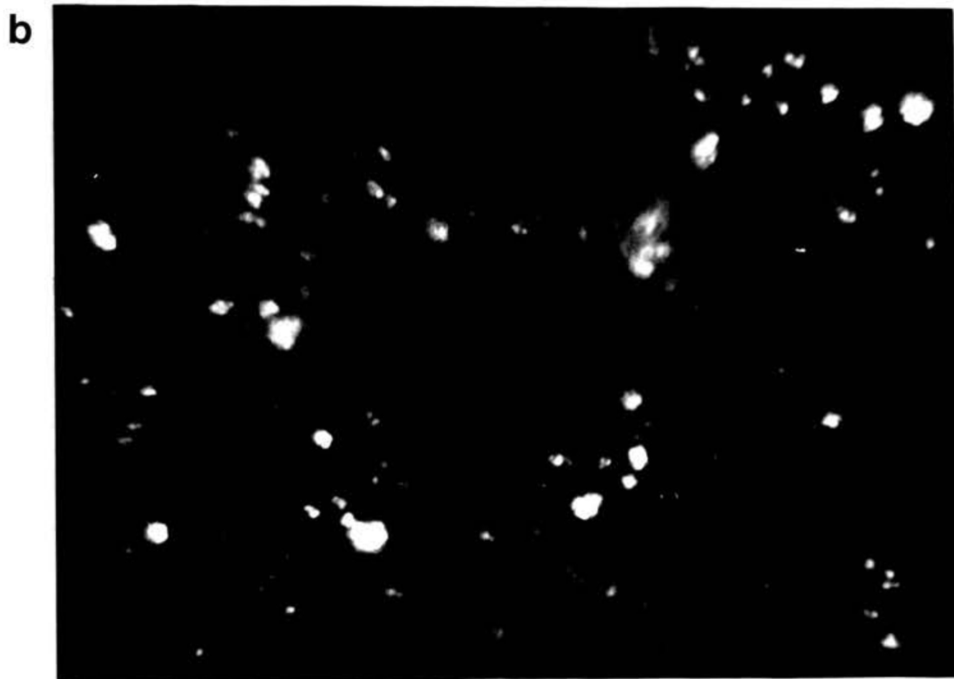


Fig. 3-E: photomicrographs of the magnocellular division of the paraventricular nucleus in water-deprived Brattleboro rats injected with [^{3}H]2-deoxyglucose; (a) light field and (b) dark field microscopy (x 800).

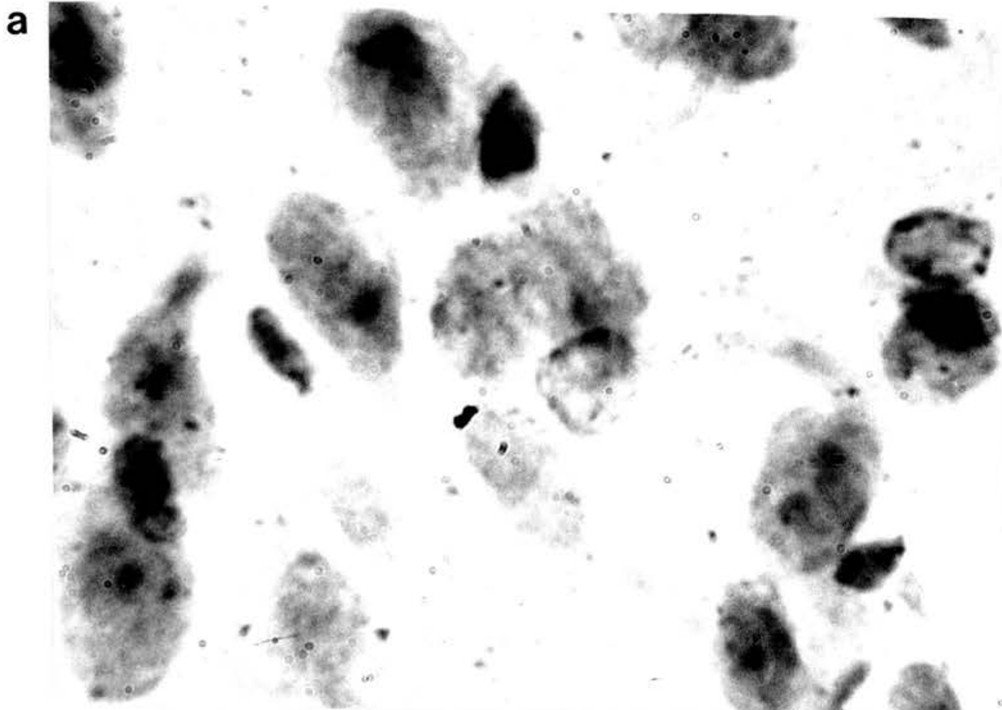


Fig. 3-F: photomicrographs of the parvocellular division of the paraventricular nucleus in water-deprived Brattleboro rats injected with [^{3}H]2-deoxyglucose; (a) light field and (b) dark field microscopy (x 800).

of the PVN.

DISCUSSION

The results using [^{14}C]2DG provide quantitative, as well as qualitative, confirmation of the data of Schwartz et al. (1979) that an osmotic stimulus increases the metabolic activity of the PN. However, in contrast to Schwartz et al. (1979), the rma of the PVN was also increased in these studies. The reason for this difference may be due to the different stimuli used, salt loading in the case of Schwartz et al. (1979) and dehydration in the present study. On the basis of the finding that 2DG uptake increased in the PN but not in the PVN after salt loading, Schwartz et al. (1979) suggested that increased metabolism correlated most closely with the functional activity that occurs at nerve terminals, and it has since been shown to reflect mainly sodium pump activity (Mata, Fink, Gainer, Smith, Davidsen, Savaki, Schwartz and Sokoloff, 1980). If, indeed, rma reflects terminal and not cell body activity, the increased 2DG uptake in the PVN in these studies could be due to increased activity in the terminals of afferent fibres to the PVN. The PVN receives projections from a variety of brain areas (see Introduction). These areas include the limbic system (Powell and Rorie, 1967; Silverman et al., 1981; Tribollet and Dreifuss, 1981), the hypothalamus (Kaelber and Leeson, 1967; Sawchenko and Swanson, 1983) and the catecholaminergic cell groups in the brain stem and medulla (Carlsson et al., 1962; Fuxe and Hökfelt, 1969; Ungerstedt, 1971; Swanson et al., 1981a). Some of the projections to the PVN are thought to exert a tonic excitatory input, since hypothalamic deafferentation reduced electrical activity in the PVN (Dyball and

Dyer, 1971). Whether the noradrenergic projection from the A_1 cell group in the medulla inhibits or stimulates activity in the PVN and SON is less certain. Studies using the placement of electrolytic and chemical lesions in the A_1 cell group showed that vasopressin release was increased, suggesting that a tonic inhibition had been removed (Blessing et al., 1982). Studies using the microinjection of monoamines into the SON, PVN and cerebral ventricles, however, have shown inhibition (Barker, Crayton and Nicoll, 1971; Moss, Dyball and Cross, 1971; Kimura, Share, Wang and Crofton, 1981) and stimulation (Kühn, 1974; Milton and Paterson, 1974) of vasopressin release. The resolution of the $[^{14}C]$2DG method of autoradiography is not sufficient to determine whether the 2DG was localised in the perikarya of magnocellular or parvocellular neurones, or in terminals in the PVN. Results from the high resolution studies using $[^{3}H]$2DG in the water-deprived Brattleboro rat, however, showed that the 2DG was localised around the perikarya of the neurones in the PVN and SON, mostly in the magnocellular divisions of the PVN (Swanson and Kuypers, 1980) where the vasopressin-staining neurones are located (Vandesande and Dierickx, 1975). It seems likely, therefore, that the increased rma of the PVN reflected increased activity in terminals of afferent neurones, probably from areas other than the A_1 cell group in the medulla, since this projects mainly to the parvocellular divisions of the PVN (Sawchenko and Swanson, 1982b) and is possibly inhibitory for vasopressin release (Blessing and Reis, 1982; Blessing et al. 1982). However, without ultrastructural confirmation the possibility cannot be excluded that silver grains around the

perikarya were due to [^{3}H] 2DG in structures (e.g. perikaryal membranes) other than the terminals of afferent neurones.

The electrophysiological responses of SON neurones to osmotic stimulation have been well studied (e.g. Koizumi, Ishikawa and Brooks, 1964; Dyball, 1971), and it is thought that most, if not all, of the large neurones in the SON contribute to the supraoptic-hypophysial tract (Cajal, 1894; Rasmussen, 1940). It is surprising therefore, that osmotic stimulation resulted in an increase in rma of the PVN but not the SON. It seems unlikely that the neurones of this nucleus are activated by water deprivation to a lesser degree than neurones of the PVN (Brimble and Dyball, 1977; Brimble, Dyball and Forsling, 1978), and, indeed, there was a slight trend towards increased rma of the SON in water-deprived Wistar and Brattleboro rats (Table 3-1). The studies using [^{3}H]2DG presented in this chapter showed that 2DG was present throughout the SON, although there appeared to be less 2DG than in the magnocellular divisions of the PVN. This suggests that the absence of a significant detectable change in the rma of the SON could reflect lower, or more diffuse activity in the terminals of afferent neurones to the SON, compared with the activity in those to the PVN. A recent study has shown that in the periphery, the rate of energy metabolism in an innervated neural structure (such as the superior cervical ganglion) is, at least in part, regulated by the impulse frequency of the electrical input to the structure (Yarowsky, Kadekaro and Sokoloff, 1983). If the same applies to the CNS, afferent activity to structures such as the SON and PVN would be important in regulating the metabolic activity in these nuclei,

and therefore differences in rma can be explained by differences in activity of afferent neurones. The neurones of the SON may act as sodium or osmoreceptors (Andersson, 1977; Mason, 1980; Abe and Ogata, 1982; Noble and Wakerley, 1982), but probably require some afferent input for the control and maintenance of neurosecretion (e.g. Leng et al., 1982). If the SON was less dependent than the PVN on afferent input for the initiation of increased activity, or if the afferent input to the SON was mainly inhibitory and therefore decreased following water deprivation, the rma of the SON would not necessarily change, since the increased activity would be apparent mainly at the nerve terminals in the PN (Schwartz et al., 1979; Mata et al., 1980) and not in the perikarya of the SON. The absence of a change in the rma of the SON cannot readily be attributed to technical difficulties in the measurement of the rma of the nucleus, since a significant increase in rma of the SON occurred after suckling and mammary nerve stimulation in the anaesthetised lactating rat (Table 5-1, Chapter V).

The homozygous Brattleboro rat is totally deficient in vasopressin and vasopressin neurophysin (Valtin et al., 1965; Pickering, Jones, Burford, McPherson, Swann, Heap and Morris, 1975; Sunde and Sokol, 1975). Even when given free access to water Brattleboro rats are mildly dehydrated in terms of plasma osmolality and sodium concentration (Valtin and Schroeder, 1964), and show marked hypertrophy of perikarya and axons in the hypothalamo-neurohypophysial system (Sokol and Valtin 1965; Orkand and Palay, 1967). The mild dehydration is possibly a sufficient stimulus to cause an higher rma of the PN but not PVN compared with

Wistar and PVG rats. The increased metabolic activity of the Brattleboro PN is consistent with the finding by Dyball (1974) that SON neurones in the Brattleboro rat have a higher mean firing rate than in non-diabetic rats.

In spite of the absence of vasopressin, the Brattleboro rat is capable of responding to an osmotic stimulus to the same extent as a normal rat, in that dehydration led to a more than two-fold increase in rma of the PN, a significant increase in rma of the PVN and an increase in metabolic activity of a fibre tract extending ventrally from the PVN. Dyball and Leng (1982) showed that in the Brattleboro rat an increase in firing rate of SON neurones occurred after an i.p. injection of hypertonic saline. However, the greater rma of the PN in Wistar and Brattleboro rats following water deprivation may also reflect increased activity in other, non vasopressin-containing neurones. The pituitary content of vasopressin and oxytocin is decreased by 70% after water deprivation and salt loading in normal rats (Jones and Pickering, 1969) which suggests that dehydration is not a selective stimulus for vasopressin-containing neurones, in that oxytocin-containing neurones also respond. Electrophysiological investigations of magnocellular activity using stimuli for vasopressin release, such as an i.p. injection of hypertonic saline and controlled haemorrhage, have also shown that the firing rate of oxytocin-containing neurones increased (Brimble and Dyball, 1977; Poulain et al., 1977; Brimble et al., 1978). It is likely, therefore, that increased activity in oxytocinergic neurones following water deprivation in Wistar and Brattleboro rats also contributes to the greater rma of the PN in

these animals. In the study using [^{3}H]2DG, the 2DG labelling which surrounded the perikarya was distributed throughout the SON in water-deprived Brattleboro rats. Since the topographical organisation of the oxytocin- and vasopressin-containing cells is quite distinct in the SON (Swaab et al., 1975a,b; Sokol et al., 1976; Swanson et al., 1981a), the distribution of [^{3}H]2DG reflected activity in afferents throughout the nucleus. This suggests further that the increase in rma of the PN following water deprivation in Brattleboro rats could be attributed to increased metabolic activity of both oxytocin- containing neurones and neurones that contained neither vasopressin nor oxytocin. Further evidence that the activity of oxytocinergic neurones is increased by water deprivation was provided by the fact that, 1) the rma of the anterior portion of the PVN, the region of the nucleus which contains mainly oxytocin-staining cells (Sawchenko and Swanson, 1982a), increased following water deprivation in Wistar and Brattleboro rats and 2) the fibre tract showing increased metabolic activity in the water deprived Brattleboro rats stained for neurophysin, indicative in this case of oxytocinergic neurones.

In the untreated Brattleboro rat the high rma of the PN cannot necessarily be attributed to activity in the oxytocin-containing neurones, since there has been no direct evidence to show that the oxytocinergic neurones are more active in Brattleboro than non-diabetic rats. The pituitary content of oxytocin has been shown to be lower (Valtin et al., 1965) and the plasma concentration of oxytocin has been shown to be greater in Brattleboro compared with Long Evans rats (Dogterom, Van Wimersma Greidanus and Swaab, 1977;

Balment, Brimble and Forsling, 1980; Edwards, La Rochelle and Gellai, 1982) which suggests indirectly that the oxytocinergic neurones are more active in the Brattleboro rat.

The PN also contains DA nerve fibres originating in the rostral part of the ARC and the ventral part of the PVH (Björklund et al., 1973a,b). The DA content of the PN (and attached PI) increased after water deprivation and salt loading in normal rats (Holzbauer, Sharman, Godden, Mann and Stephens, 1980), as did the accumulation of dihydroxyphenylalanine (DOPA) following the administration of an aromatic amino acid decarboxylase inhibitor (used as a method for estimating DA synthesis in vivo; Alper, Demarest and Moore, 1982). This suggests that dehydration stimulates the activity of tubero-hypophysial neurones, which may have contributed to the high rma of the PN. Dopaminergic nerve endings are found in close proximity to vasopressinergic nerve endings (Pelletier, 1983), but the effects of DA on neurohypophysial terminals are uncertain. Dopamine has been shown to inhibit the evoked release of oxytocin in vitro (Barnes and Dyball, 1982) and to stimulate firing in putative oxytocinergic neurones in hypothalamic slices (Mason, 1983). Dopamine has also been shown to facilitate milk ejection (Clarke et al., 1979; Moos and Richard, 1982) and diuresis (Moos and Richard, 1982) in vivo, and systemically administered bromocriptine and α ergocriptine did not inhibit the milk ejection reflex (Russell et al., 1981).

The high rma of the PN in Brattleboro rats may also involve the synthesis, transport and release of 'protein X' found in the magnocellular neurones of Brattleboro rats (Russell, Brownstein and

Gainer, 1980); in water deprived Wistar rats the high rma of the PN may involve the synthesis, transport and release of dynorphin and enkephalin, shown to coexist with vasopressin in magnocellular neurones (Martin and Voigt, 1981; Watson, Akil, Fischli, Goldstein, Zimmerman, Nilaver and Van Wimersma Greidanus, 1982b). However, the metabolic activity associated with the release of neuro-hormones is thought to be minimal compared with that associated with sodium pump activity (Mata et al., 1980), suggesting that the high rma of the PN in Brattleboro rats and following water deprivation in Wistar and Brattleboro rats was mainly due to increased electrical activity, although the metabolic activity associated with increased synthesis and transport of neuro-hormones in neurones projecting to the PN cannot be excluded.

The high fluid turnover in Brattleboro rats can be corrected by the administration of vasopressin (Valtin and Schroeder, 1964). The administration of dDAVP to Brattleboro rats in this study reduced the rma of the PN to the value seen in Wistar and PVG rats. This decreased metabolic activity was correlated with a decrease in plasma osmolality whether the dDAVP was given 24 h before or at the same time as 2DG, and with a fall in urinary output from 70% body weight/24 h to 3% body weight/24 h in the 24 h study. Desamino-D-arginine^{8} vasopressin acts predominantly as an antidiuretic and has negligible pressor activity (Sawyer, Grzonka and Manning, 1981). Taken together these data suggest that the dDAVP reduced the rma of the PN in the Brattleboro rat by reducing water loss, thereby removing the main stimulus (presumably high plasma osmolality) for increased activity of the PN. However, these results do not exclude

the possibility that dDAVP may have acted directly by reducing the firing rate of hypothalamo-neurohypophysial neurones. Vasopressin has been shown to reduce the firing rate of some hypothalamo-neurohypophysial neurones (Nicoll and Barker, 1971; Moss, Dyball and Cross, 1972) but has also been shown to increase the firing rate of SON (Abe, Inoue, Matsuo and Ogata, 1983) and hippocampal (Mühlethaler, Dreifuss and Gähwiler, 1982) neurones. It has been suggested that vasopressin itself may modify the firing of vasopressin-containing neurones (e.g. Leng and Dyball, 1983) to promote a discharge pattern that is optimal for the release of vasopressin (Dutton and Dyball, 1979), since sustained stimulation of the neural stalk results in more frequent bursts of activity in phasic cells in non-diabetic rats but not in Brattleboro rats (Leng, 1981; Leng and Wiersma, 1981). [^{3}H] Arginine vasopressin binding sites have been identified in the rat PVN and SON (Yamamura, Gee, Brinton, Davis, Hadley and Wamsley, 1983) which may represent receptors for vasopressin. In addition, possibly 43% of the total number of terminals that synapse on the magnocellular neurones in the PVN are intrinsic (Kiss, Palkovits, Záborszky, Tribollet, Szabó and Makara, 1983) which suggests that a high level of local integrative function occurs in the PVN. The interaction of PVN neurones can be increased by dehydration, which has been shown to increase the extent of membrane involved in soma-soma contacts (Tweedle and Hatton, 1977; Gregory, Tweedle and Hatton, 1980), and this may facilitate interactions between magnocellular neurones leading to an efficient discharge pattern of phasic cells (Hatton, 1982). Vasopressin and oxytocin administered i.v. and s.c. have

been detected in cerebro-spinal fluid (CSF) two minutes after administration (although the amount found in CSF was only a small (0.002%) proportion of the total dose administered peripherally), demonstrating that these hormones can cross the blood-brain barrier in the rat (Mens, Witter and Van Wimersma Greidanus, 1983). Desamino-D-arginine^{8}- vasopressin, however, was not detected in CSF when administered peripherally in the sheep (Stegner, Artman, Leake and Fisher, 1983). Therefore, it is possible, but unlikely, that in the present studies on the Brattleboro rat dDAVP had a central action to reduce the rma of the PN, as well as indirectly by reducing plasma osmolalities to normal values.

The rma of the PD in the Brattleboro rat was higher than in Wistar and PVG rats, possibly indicating increased metabolic activity associated with the synthesis and release of anterior pituitary hormones. However, it has been shown that the pituitary contents and plasma concentrations of growth hormone, ACTH and PRL are decreased in the Brattleboro rat (McCann, Antunes-Rodrigues, Naller and Valtin, 1966; Arimura, Sawano, Redding and Schally, 1968; Buckingham and Leach, 1980; Adler and Sokol, 1982; Fujimoto and Hedge, 1982b). Plasma concentrations of follicle stimulating hormone (FSH) (Oprescu, Simionescu and Coculescu, 1982) and thyroid stimulating hormone (TSH) are increased in the Brattleboro rat, and the pituitary content of TSH is also increased (Fujimoto and Hedge, 1982a,b). In the rat, the gonadotrophs and thyrotrophs make up only a small proportion of the cells in the PD (Denef, Hautekeete, Dewolf and Vanderschueren, 1978). It seems unlikely therefore, that the high rma of PD in the Brattleboro rat can be

attributed to a high rate of synthesis and release of FSH and TSH. The rma of the PD in the Brattleboro rat decreased after treatment with dDAVP, in both the 24h and 45 min studies. Desamino-D-arginine^{8}-vasopressin has been shown to be ineffective in reducing plasma concentration of TSH in the Brattleboro rat (Fujimoto and Hedge, 1982 a,b). If the high rma of the PD was due, at least in part, to metabolic activity associated with the synthesis and release of TSH, the rma of the PD should not have decreased after treatment with dDAVP. Water deprivation also decreased the rma of the PD in Wistar and Brattleboro rats. Vasopressin has been implicated in the control of ACTH release (e.g. Gillies and Lowry, 1980; Gillies, Linton and Lowry, 1982) through a direct input of vasopressinergic neurones to the external lamina of the ME (Rinne, 1960; Dierickx et al., 1976), which respond to manipulations of the pituitary-adrenal axis (Watkins, Schwabedal and Bock, 1974; Zimmerman, Stillman, Recht, Antunes, Carmel and Goldsmith, 1977). Stimuli resulting in vasopressin release such as water deprivation, might be expected, therefore, to increase adenohypophysial activity in Wistar rats, unless osmotic stimuli selectively activate only the magnocellular neurones that project to the PN, as suggested by Dyball and Pountney (1973). However, the rma of the PD was reduced by water deprivation in the Wistar rat, and by water deprivation and by treatment with dDAVP in the Brattleboro rat. In addition, the study using [^{3}H]2DG reported in this chapter, showed that in water deprived Brattleboro rats, most of the 2DG found in the PVN was localised in the magnocellular divisions of the nucleus, and not in the parvocellular and

periventricular divisions which project to the ME (Swanson, Sawchenko, Wiegand and Price, 1980; Wiegand and Price, 1980). A possible explanation for the effects on the rma of the PD of these two treatments is that blood flow to the PD was reduced, although dDAVP has only slight pressor activity (Sawyer et al., 1981).

In summary, the main findings in the studies reported in this chapter were that:

1) in contrast to studies in which the osmotic stimulus was a salt load, water deprivation led to a significant increase in rma of the PVN as well as the PN;

2) the increase in 2DG uptake by the PVN was localised around the perikarya, possibly in the terminals of afferent projections mainly to the magnocellular divisions of the PVN;

3) increased activity of the neurohypophysial neurones occurred when there was a genetic deficiency of vasopressin, and the activity could be increased further by an osmotic stimulus and reduced by the administration of dDAVP, and

4) the rma of the PD was increased when there was a genetic deficiency of vasopressin, and the activity could be reduced by water deprivation and the administration of dDAVP.

CHAPTER IV

Effects of Water Deprivation and Desamino-D-Arginine^{8}-Vasopressin on the Hypothalamo-hypophysial System in Mice with Hereditary Nephrogenic Diabetes Insipidus

4.1 INTRODUCTION

The aim of these studies was to investigate further the effects of water deprivation and the administration of dDAVP on the rma of the hypothalamo-neurohypophysial system, using a strain of mouse with hereditary diabetes insipidus. The mouse, DI +/+ Severe, is unable to concentrate urine, and this defect cannot be corrected with exogenous vasopressin (Falconer, Latyszewski and Isaacson, 1964; Naik and Valtin, 1969). The massive excretion of hypotonic urine (150% body weight in ml/24 h, Valtin, Sokol and Sunde, 1975) is due to the decreased water permeability of the collecting ducts in the kidney (Kettyle and Valtin, 1972). The reduced water permeability of the ducts is thought to be due to the inability of vasopressin to activate adenylate cyclase in the ducts (Dousa and Valtin, 1974; Jackson, Edwards, Valtin and Dousa, 1980), by which mechanism vasopressin exerts its antidiuretic action (Orloff and Handler, 1962; Dousa and Valtin, 1976).

Studies in the Brattleboro rat have shown that a decrease in the rma of the hypothalamo-neurohypophysial system occurred after treatment with dDAVP (Chapter III). This reduction in activity was probably due mainly to the resulting decrease in plasma osmolality, although a central action of dDAVP could not be excluded. Since dDAVP cannot restore urine osmolality to a normal value in the DI +/+ Severe mouse, any effect of dDAVP on the activity of the magnocellular system must be due to a central action of the peptide. The study also included experiments using DI +/+ Non-severe mice, a related but genetically different strain with a mild deficiency in the ability to concentrate urine.

4.2 MATERIALS METHODS AND EXPERIMENTAL DESIGN

The animals used were adult female and male DI +/+ Severe and DI +/+ Non-severe diabetes insipidus mice, 20-30 g body weight, obtained from a breeding colony at the Department of Human Anatomy, University of Oxford. Adult female Normal house mice, 20-25 g body weight, were used from the departmental breeding colony. The mice were maintained as described in section 2.1.

The following groups of animals were studied:

1) DI +/+ Severe, either deprived of water but not food for 4-5 h before the injection of 2DG, or injected with dDAVP at the same time as 2DG, or injected with dDAVP at the same time as 2DG following 4-5 h water deprivation, or not subjected to any of these treatments (untreated).
2) DI +/+ Non-severe, either untreated, deprived of water but not food for 4-5 h before the injection of 2DG, or injected with dDAVP at the same time as 2DG following 4-5 h water deprivation.
3) Normal house mice.

The dDAVP was injected i.p. at a dose of 10 μg/100 g body weight, diluted with 0.9% saline solution (final volume 0.25 ml). The 2DG was injected i.p. at a dose of 400-500 μCi/kg body weight (2.2.1). The animals were killed 45 min after the injection of 2DG. The brain and pituitary gland were removed en-bloc and were processed for autoradiography (2.2.1). The rma of selected areas of the brain and pituitary gland were determined from the autoradiographs (2.3.1.1). The rma of the following brain and pituitary gland areas were determined; SON, PVN, IC, S, POA, MFB, SCN, FX, AHA, VmArc, AMYG, MM, IP, VHIPP, VT, LT, LGEN, MGEN, CG, CS, CI, PN

and PD. Significance of differences in rma was assessed by analysis of variance and Duncan's multiple range test.

4.3 RESULTS

4.3.1 Use of an internal standard

There were no differences in rma of the IC between individual animals, strains and experimental groups (Table 4-1). Therefore, it was justified to assume that areas of white matter were stable in terms of rma in this study, and so to use the CC as an internal standard.

4.3.2 Strain differences

The rma of the PD was significantly greater ($P < 0.01$) in Severe mice than in Non-severe and Normal mice (Table 4-1). There were no differences between strains in the rma of any other areas of the brain and pituitary gland (Tables 4-1 and 4-2).

4.3.3 Effect of water deprivation

Table 4-1 and Fig. 4-A show that the rma of the PN increased ($P < 0.05$) in Severe mice after 4-5 h of water deprivation. Water deprivation also caused a decrease ($P < 0.001$) in the rma of the PD in Severe mice to a value similar to that in the other groups of animals (Table 4-1 and Fig. 4-A). The same period of water deprivation (4-5 h) had no effect on the rma of any brain and pituitary gland area measured in Non-severe mice (Tables 4-1, 4-2). The rma of the PVN and PN were increased ($P < 0.01$ and $P < 0.005$, respectively) in Severe, water-deprived mice compared with those in Non-severe, water-deprived mice (Table 4-1 and Fig. 4-B).

4.3.4 Effect of desamino-D-arginine^{8}-vasopressin

Desamino-D-arginine^{8}-vasopressin administered at the same

Table 4-1. Relative metabolic activity of selected brain regions and the pituitary gland in mice

Values are expressed as a ratio (mean ± S.E.M.) of [^{14}C] concentration of the selected region to [^{14}C] concentration of the corpus callosum.

Type of animal	No. of animals	SON	PVN	PN	PD	IC
Severe	5	1.00±0.04	1.09±0.04	1.00±0.12	0.82±0.12	1.02±0.02
Severe, water-deprived	8	0.91±0.05	1.21±0.06	1.46±0.21	0.43±0.04	0.94±0.03
Severe, water-deprived +dDAVP	7	0.96±0.04	1.29±0.06	1.77±0.16	0.41±0.03	0.92±0.02
Severe + dDAVP	5	0.85±0.03	1.06±0.03	1.00±0.10	0.52±0.05	0.90±0.02
Non-severe	6	1.02±0.05	0.99±0.03	0.79±0.14	0.61±0.06	0.95±0.06
Non-severe, water-deprived	5	0.88±0.03	0.99±0.03	0.75±0.05	0.48±0.04	0.94±0.05
Non-severe, water-deprived +dDAVP	6	0.90±0.05	1.03±0.02	1.02±0.10	0.60±0.04	0.88±0.03
Normal	5	0.88±0.02	1.00±0.07	0.77±0.06	0.61±0.04	1.04±0.07

SON, supraoptic nuclei; PVN, paraventricular nucleus; IC, internal capsule

Table 4-1 (cont.). Significance of differences of the relative metabolic activity (rma) of areas of the brain and the pituitary gland in Table 4-1 (Duncan's multiple range test):

$P < 0.05$

Severe, water-deprived (PVN) v Normal (PVN) Non-severe, water-deprived + dDAVP (PVN)

Severe, water-deprived + dDAVP (PVN) v Severe (PVN)

Severe, water-deprived (PN) v Severe (PN), Severe + dDAVP (PN), Non-severe, water-deprived + dDAVP (PN)

Severe, water-deprived (PD) v Non-severe (PD), Non-severe, water-deprived + dDAVP (PD), Normal (PD)

Severe, water-deprived + dDAVP (PD) v Non-severe (PD), Non-severe, water-deprived + dDAVP (PD), Normal (PD)

$P < 0.01$

Severe, water-deprived (PVN) v Non-severe (PVN), Non-severe, water-deprived (PVN)

Severe, water-deprived + dDAVP (PVN) v Severe + dDAVP (PVN)

Severe (PD) v Non-severe (PD), Non-severe, water-deprived + dDAVP (PD), Normal (PD)

$P < 0.005$

Severe, water-deprived + dDAVP (PVN) v Non-severe, water-deprived + dDAVP (PVN)

Severe, water-deprived (PN) v Non-severe (PN), Non-severe, water-deprived (PN), Normal (PN)

$P < 0.001$

Severe, water-deprived + dDAVP (PVN) v Non-severe (PVN), Non-severe, water-deprived (PVN), Normal (PVN)

Severe, water-deprived + dDAVP (PN) v Severe (PN), Severe + dDAVP (PN), Non-severe (PN), Non-severe, water-deprived (PN), Non-severe, water-deprived + dDAVP (PN), Normal (PN)

Severe (PD) v Severe + dDAVP (PD), Severe, water-deprived (PD),
Severe, water-deprived + dDAVP (PD), Non-severe,
water-deprived (PD)

Table 4-2. Relative metabolic activity of selected brain regions in mice

Values are expressed as a ratio (mean ± S.E.M.) of [^{14}C] concentration of the selected region to [^{14}C] concentration of the corpus callosum.

	Type and number of animal							
	Severe (n=5)	Severe, water-deprived (n=6)	Severe, water-deprived + dDAVP (n=5)	Severe + dDAVP (n=4)	Non-severe (n=5)	Non-severe, water-deprived (n=4)	Non-severe, water-deprived + dDAVP (n=3)	Normal (n=5)
S	1.52±0.02	1.72±0.10	1.69±0.12	1.55±0.09	1.45±0.04	1.43±0.07	1.67±0.26	1.62±0.08
POA	1.01±0.03	1.07±0.02	1.06±0.04	0.96±0.05	1.00±0.05	0.99±0.03	1.07±0.09	1.09±0.06
MFB	1.26±0.03	1.26±0.03	1.26±0.07	1.24±0.08	1.24±0.07	1.21±0.03	1.41±0.15	1.41±0.06
SCN	1.29±0.04	1.47±0.10	1.45±0.06	1.32±0.16	1.33±0.09	1.28±0.05	1.73±0.35	1.56±0.21
FX	1.42±0.02	1.48±0.11	1.46±0.16	1.33±0.05	1.26±0.03	1.23±0.04	1.37±0.05	1.41±0.03
AHA	1.15±0.02	1.14±0.04	1.20±0.10	1.06±0.08	1.04±0.05	1.09±0.03	1.12±0.04	1.17±0.05
VmArc	1.02±0.05	0.96±0.04	0.95±0.06	0.96±0.05	0.97±0.10	0.95±0.02	0.93±0.04	0.94±0.03
AMYG	1.08±0.03	1.11±0.03	1.09±0.06	1.07±0.06	1.09±0.09	1.05±0.04	1.04±0.04	1.11±0.07
MM	2.48±0.08	2.92±0.19	2.78±0.31	2.95±0.18	2.23±0.09	2.18±0.08	3.24±0.78	2.74±0.19
IP	2.00±0.10	2.41±0.15	2.60±0.26	2.43±0.19	1.82±0.07	1.98±0.12	2.33±0.42	2.13±0.13
VHIPPM	1.56±0.04	1.62±0.06	1.57±0.09	1.70±0.10	1.55±0.05	1.47±0.03	1.61±0.15	1.69±0.06
VT	1.85±0.07	2.07±0.16	1.91±0.15	2.07±0.11	1.82±0.02	1.83±0.14	2.18±0.09	1.90±0.12

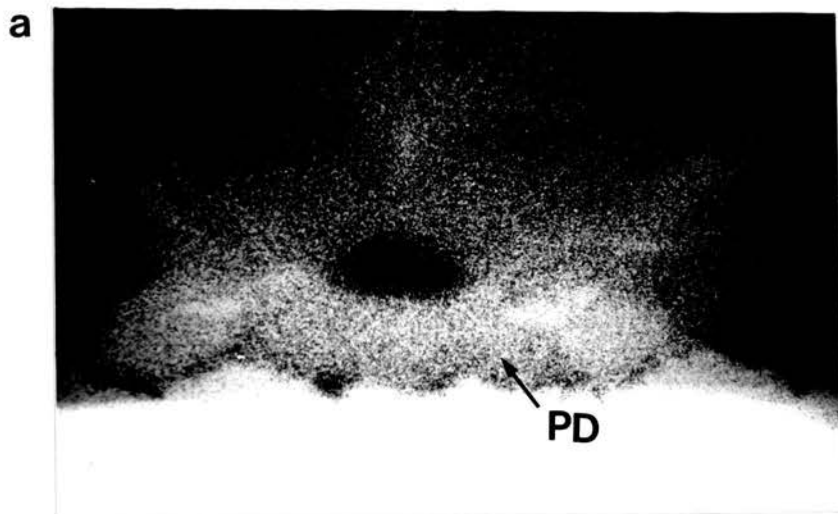
S, septum; POA, preoptic area; MFB, medial forebrain bundle; SCN, suprachiasmatic nucleus; FX, fornix; AHA, anterior hypothalamic area; VmArc, ventromedial-arcuate hypothalamic area; AMYG, amygdala; MM, mamillary body; IP, interpeduncular nucleus; VHIPPM, ventral hippocampus (molecular layer); VT, ventral thalamic area

Table 4-2 (cont). Relative metabolic activity of selected brain regions in mice

Values are expressed as a ratio (mean ± S.E.M.) of [^{14}C] concentration of the selected region to [^{14}C] concentration of the corpus callosum.

	Type and number of animal							
	Severe (n=5)	Severe, water-deprived (n=6)	Severe, water-deprived + dDAVP (n=5)	Severe + dDAVP (n=4)	Non-severe (n=5)	Non-severe, water-deprived (n=4)	Non-severe, water-deprived + dDAVP (n=3)	Normal (n=5)
LT	2.18±0.06	2.29±0.10	2.55±0.39	2.55±0.18	2.10±0.05	1.99±0.09	2.64±0.29	2.26±0.08
LGEN	1.62±0.05	1.83±0.09	1.73±0.15	1.84±0.07	1.58±0.01	1.60±0.03	1.70±0.24	1.71±0.08
MGEN	1.85±0.04	2.16±0.16	2.09±0.20	2.22±0.12	1.82±0.05	1.83±0.06	2.19±0.27	1.93±0.08
CS	1.84±0.06	1.97±0.10	1.90±0.15	1.82±0.04	1.64±0.03	1.55±0.10	1.83±0.23	2.05±0.16
CG	1.23±0.04	1.27±0.03	1.18±0.04	1.24±0.04	1.16±0.03	1.14±0.03	1.22±0.07	1.26±0.07
CI	2.13±0.14	2.49±0.36	2.78±0.33	2.97±0.18	1.99±0.14	2.36±0.19	3.58±0.94	2.66±0.23

LT, lateral thalamic area; LGEN, lateral geniculate bodies; MGEN, medial geniculate bodies; CS, superior colliculus; CG, central grey; CI, inferior colliculus



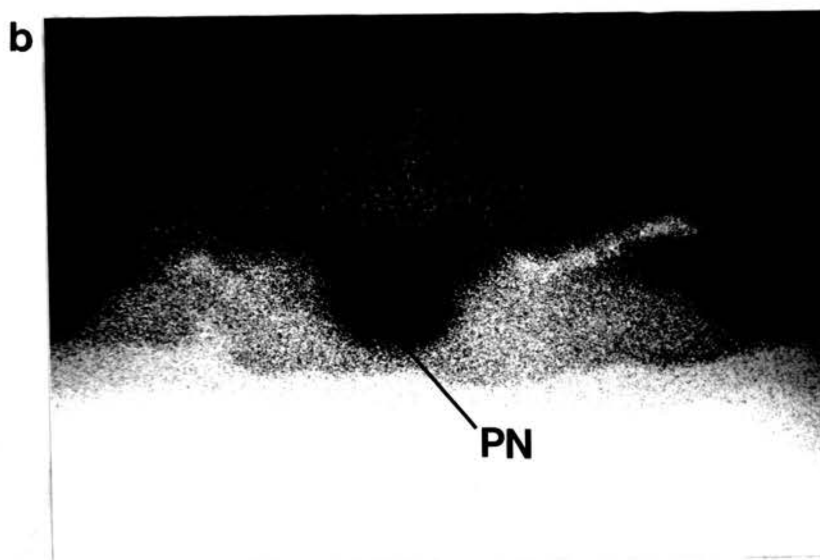
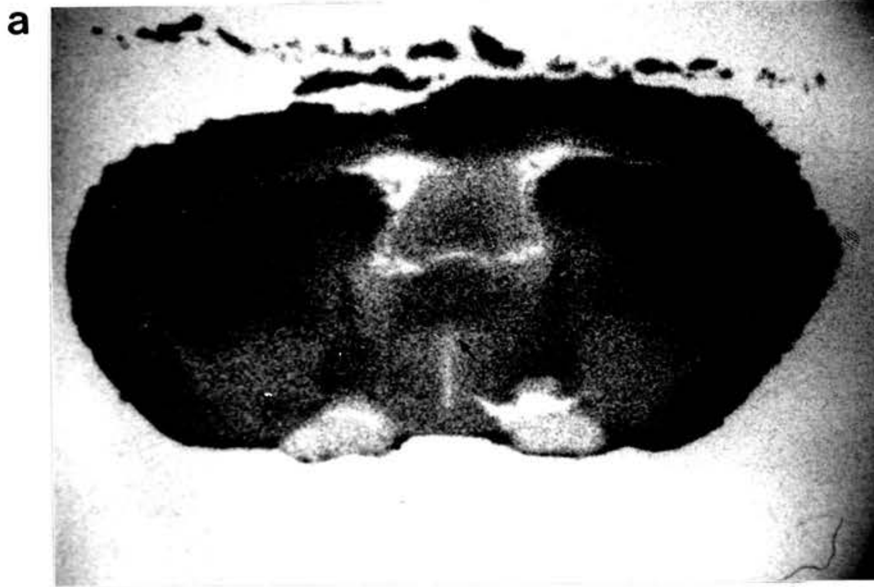


Fig. 4-A: autoradiographs of coronal sections of the pituitary gland showing the pars nervosa (PN) and pars distalis (PD) in DI +/+ Severe mice injected with [^{14}C]2-deoxyglucose; (a) untreated and (b) water-deprived mice.



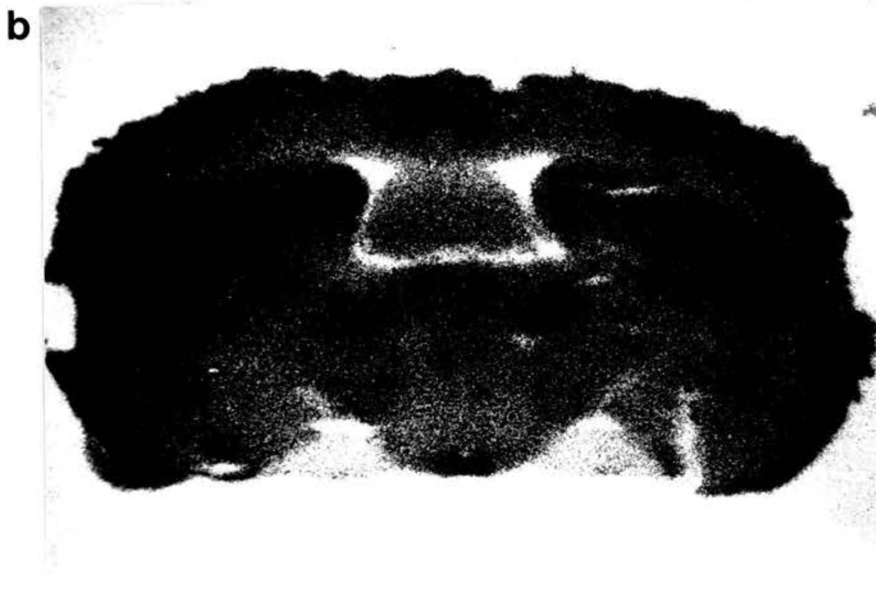
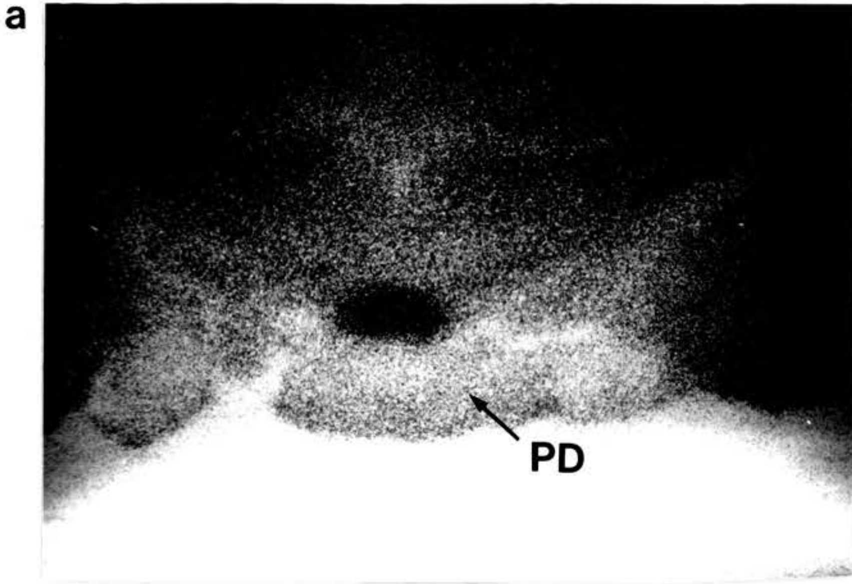


Fig. 4-B: autoradiographs of coronal sections of the brain at the level of the paraventricular nucleus (arrow) in water-deprived DI +/+ Non-severe (a) and water-deprived DI +/+ Severe (b) mice injected with [^{14}C]2-deoxyglucose.



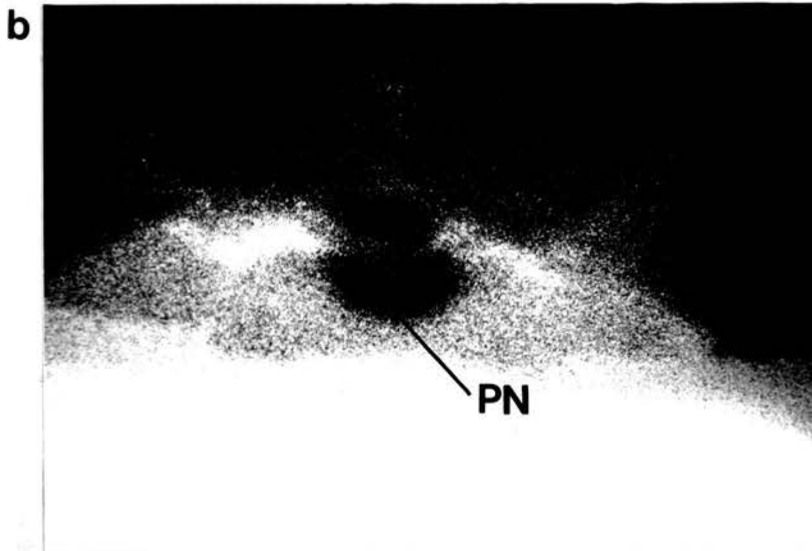


Fig. 4-C: autoradiographs of coronal sections of the pituitary gland showing the pars nervosa (PN) and pars distalis (PD) in DI +/+ Severe mice injected with [^{14}C]2-deoxyglucose; (a) untreated and (b) water-deprived Severe mouse injected with desamino-D-arginine^{8}-vasopressin 45 min before decapitation.

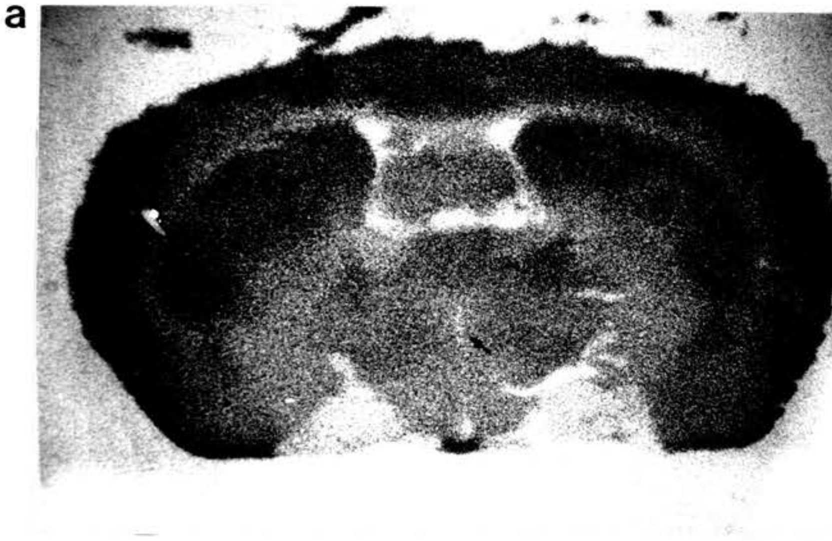


Fig. 4-D: autoradiographs of coronal sections of the brain at the level of the paraventricular nucleus (arrow) in water-deprived mice injected with $[^{14}C]$2-deoxyglucose and desamino-D-arginine^{8}-vasopressin 45 min before decapitation; (a) DI +/+ Non-severe and (b) DI +/+ Severe mice.

time as 2DG significantly decreased ($P < 0.001$) the rma of the PD in Severe mice (Table 4-1), but had no effect on the rma of any other brain and pituitary gland area measured (Tables 4-1 and 4-2). When the injection of dDAVP was given to Severe mice following 4-5 h of water deprivation, the rma of the PVN and PN were greater ($P < 0.05$ and $P < 0.001$, respectively) than those in untreated Severe mice (Table 4-1 and Fig. 4-C) and not significantly different from those in Severe, water-deprived mice (Table 4-1). The rma of the PD was decreased ($P < 0.001$) in Severe, water-deprived mice treated with dDAVP compared with Severe mice (Table 4-1 and Fig. 4-C) and not significantly different from that in Severe, water-deprived mice (Table 4-1).

Desamino-D-arginine^{8}-vasopressin had no effect on the rma of any brain and pituitary gland area measured in Non-severe water-deprived mice (Tables 4-1 and 4-2). Severe, water-deprived mice treated with dDAVP showed a greater rma of the PVN and PN ($P < 0.005$, $P < 0.001$), and a lower ($P < 0.05$) rma of the PD, compared with Non-severe, water-deprived mice treated with dDAVP (Table 4-1 and Fig. 4-D).

4.4 DISCUSSION

The main results in this study were that in DI +/+ Severe mice the rma of the PVN and PN were not different from those in DI +/+ Non-severe and Normal house mice, and could be increased by water deprivation. The increased rma induced by water deprivation could not be reversed by dDAVP. The fact that the rma of the PN in DI +/+ Severe mice was not significantly greater than that in the other two

strains suggests that, in contrast to the Brattleboro rat, the metabolic activity in the hypothalamo-neurohypophysial system is no greater than in control mice. This is not surprising since plasma osmolalities measured in Severe mice were not significantly different from those of control VII +/+ mice (Naik and Sokol, 1970). Unpublished data from P. Stern, F.T. La Rochelle and H. Valtin (Department of Physiology, Dartmouth Medical School, Hanover, New Hampshire) also indicated that DI +/+ Severe mice can have plasma osmolalities in the normal range. The rma of the PVN and PN in Severe mice could, however, be increased by water deprivation. The hypothalamus and PN contain abundant neurosecretory material, and the release mechanism for vasopressin is not impaired in Severe mice (Naik and Valtin, 1969). Therefore, the increase in rma of the PVN and PN following water deprivation can probably be interpreted as a measure of increased neuronal metabolism that accompanies the release of vasopressin. The same period of water deprivation had no effect on the rma of any brain and pituitary gland area measured in Non-severe mice, in which, because of a semi-intact urine concentrating mechanism, the tolerance to an osmotic stimulus (such as water deprivation) is greater than in Severe mice. In this respect the Severe strain of mouse resembles the Brattleboro rat, where a short period of water deprivation (12-15 h) caused a large increase in rma of the PVN and the PN (Chapter III).

The reduction in rma of the hypothalamo-neurohypophysial system in the Brattleboro rat following treatment with dDAVP (Chapter III) can be interpreted as being secondary to the resulting reduction in plasma osmolality. However, a central action of dDAVP could not be

excluded. Vasopressin has been shown to reduce the firing rate of some hypothalamo-neurohypophysial neurones (Nicoll and Barker, 1971; Moss et al., 1972) but has also been shown to increase the firing rate of SON (Abe et al., 1983) and hippocampal (Mühlethaler et al., 1982) neurones. Any central action of dDAVP resulting in changes in rma should therefore be apparent in Severe mice, since the kidney is unable to respond to the antidiuretic action of vasopressin and its analogues. Desamino-D-arginine^{8}- vasopressin alone had no effect on the rma of the magnocellular system in Severe mice. When dDAVP was administered to Severe mice deprived of water for 4-5 h the rma of the PVN and PN remained high, and were not significantly different from those of water-deprived Severe animals. These results show that at the dose administered in this study, dDAVP had no central effects on the rma of the hypothalamo-neurohypophysial system. Since rma has been shown to reflect sodium pump activity (Mata et al., 1980), dDAVP probably did not alter the firing rate of the magnocellular neurones in this study by a sufficient degree to be detected by the 2DG method, possibly due to an inability to cross the blood-CSF barrier (Stegner et al., 1983).

The rma of the SON did not change in Severe mice following water deprivation. This suggests that, as in the Brattleboro rat (Chapter III), either the neurones of the SON are not activated by water deprivation to the same degree as neurones of the PVN, which seems unlikely (Brimble and Dyball, 1977; Brimble et al., 1978), or the neurones of the SON are less dependent on afferent input for increased firing.

The condition of diabetes insipidus appears to be accompanied

by an higher rma of the PD, as shown in this study and in Chapter III. The higher rma of the PD could indicate an higher rate of synthesis and release of adenohypophysial hormones compared with control animals, as discussed in Chapter III. The decrease in the rma of the PD in Severe mice following water deprivation, administration of dDAVP and the combined treatments cannot easily be explained. It is interesting to note, however, that these results are again identical to those found in the Brattleboro rat, and may be due to altered blood flow to the PD (Chapter III).

CHAPTER V

Pathways Involved in Milk Ejection and Prolactin Release in the Lactating Rat

5.1 INTRODUCTION

Milk ejection is produced by a neurohormonal reflex. Suckling generates a neural signal which is conveyed by the mammary nerve and an ascending pathway to the forebrain where, by activating the magnocellular neurones of the PVN and SON, the signal triggers the release of oxytocin (Cross et al., 1975). Milk ejection in the rat is an intermittent response to a maintained stimulus, in that the young suckle continuously but obtain milk only at intervals of about 5 min (Lincoln et al., 1973). Although each milk ejection is known to result from a transient (2-6 s) accelerated discharge of magnocellular neurones occurring 10-12 s before milk ejection (Wakerley and Lincoln, 1973; Summerlee and Lincoln, 1981), neither the anatomy of the ascending pathway nor the mechanism by which the pathway activates the magnocellular neurones are fully understood. Studies involving electrical stimulation and the placement of surgical lesions in the guinea pig, rabbit and goat, showed that the afferent pathway through the midbrain was discrete and coincided with the TSTH (Tindal et al., 1967; Mena and Beyer, 1968; Tindal et al., 1969; Knaggs et al., 1972; Tindal and Knaggs, 1975). However, this was not confirmed by other studies on the rabbit and the rat which suggested that the pathway was diffuse and scattered across the TM and TL (Urban et al., 1971; Voloschin and Dottaviano, 1976; Juss and Wakerley, 1981).

The aim of the present investigation was to determine whether these questions could be answered by the 2DG method for tracing metabolically active pathways. In addition to using the natural stimulus of suckling in conscious and anaesthetised rats, the effect

of mammary nerve stimulation (which is an effective stimulus for milk ejection in the anaesthetised rat, Mena et al., 1978) was also studied. The LCGU were also measured in a separate group of anaesthetised suckled animals, and were compared with measurements of rma in the same animals.

5.2 MATERIALS AND METHODS AND EXPERIMENTAL DESIGN

5.2.1 Animals

The animals used in these studies were adult female lactating rats of the Wistar strain, 250–415 g body weight, with litters of 10–15 pups. The animals were purchased from Charles River U.K. Ltd. (Margate, Kent) and were maintained as described in section 2.1. Experiments were carried out on 9–13 days post-partum after overnight separation of the young from the doe, apart from 1 pup from each litter which was left with the doe until the start of the experiment. Experiments using conscious animals were carried out in the animal house, in the same room as that in which the animals were housed.

5.2.2 Experiments on conscious animals

Does were implanted with an intra-atrial cannula (2.2.4) and were allowed to recover for 48 h. A blood sample (0.4 ml) was collected by way of the cannula (2.2.4) and the separated litters were weighed. The young were then replaced with the doe, and allowed to suckle for 20 min before another blood sample (0.4 ml) was collected. The 2DG was then injected as described in section 2.2.4, at a dose of 150 uCi/kg body weight, and the doe was left to nurse the pups undisturbed for 45 min. A final blood sample was collected and the animal was killed by decapitation. Litters that

had been returned to does to suckle were weighed again, killed and the stomachs inspected for the presence of fresh milk. Control animals were treated similarly except that the pups were not returned to the doe.

5.2.3 Experiments on anaesthetised animals

The design of these experiments was based on the finding of Burnet and Wakerley (1976) that suckling elicited a good PRL response in anaesthetised animals, provided that the does were left undisturbed for 3 h after induction of anaesthesia and before the pups were replaced.

5.2.3.1 Experiments using the measurement of relative metabolic activity

Does were injected with urethane (2.2.3) and the external jugular vein was exposed. The animal was then left undisturbed under a warming lamp for 3 h, after which a blood sample (0.4 ml) was withdrawn as described in section 2.2.5. The pups were then replaced and allowed to suckle for 1h before 2DG was injected i.v. (section 2.2.5) at a dose of 150uCi/kg body weight. The pups were monitored for the 'stretch response' as an indication of milk ejection by the doe, as described by Vorherr, Kleeman and Lehman (1967) and Lincoln et al. (1973). After 45 min a blood sample (0.4 ml) was taken and the doe was killed by decapitation. Control animals were treated in a similar manner except that the pups were not replaced.

5.2.3.2 Experiments using the measurement of rates of local cerebral glucose utilisation

In a separate group of anaesthetised animals the LCGU was also

measured, using a modification of the method described by Sokoloff et al. (1977). Animals were anaesthetised with urethane (2.2.3) and the left femoral vein and both femoral arteries were cannulated (2.2.5, 2.2.6). Three hours after anaesthesia had been induced a venous blood sample (0.3 ml) was collected (2.2.5) and the pups were returned to the doe and allowed to suckle for 1 h. At zero time the animal was injected with 2DG (150 μCi/kg body weight) over 30 s, by way of the venous cannula (2.2.5). Arterial blood samples (50–100 μl) were collected (2.2.6) at 0 s, 15 s, 30 s, 45 s, 1 min, 2 min, 3 min, 5 min, 7.5 min, 10 min, 15 min, 25 min, 35 min and 45 min, with respect to the time of the injection of 2DG. A venous blood sample was collected at 40 min (2.2.5) and all the blood samples were placed on ice. The pups were again monitored for the 'stretch response', indicative of milk ejection by the doe. The animal was decapitated after the final blood sample had been collected, and the time of death was noted. The pups were killed and the stomachs were inspected for the presence of fresh milk. Control animals were treated in a similar manner except that the pups were not replaced. Plasma was prepared from the arterial blood samples (2.2.7) and the plasma [^{14}C] 2DG concentrations were determined by measuring the [^{14}C] content of the plasma samples in a liquid scintillation counter (2.3.3). Arterial plasma glucose concentrations were also measured, by an enzymatic method using hexokinase-glucose-6-phosphate dehydrogenase (2.3.4).

5.2.4 Mammary nerve stimulation.

Animals were injected with urethane (section 2.2.3) and a mammary nerve was exposed and prepared for stimulation according to

the method of Mena et al. (1978), described in section 2.2.11. The external jugular vein was also exposed. After a 45-60 min rest period a blood sample (0.4ml) was taken and 2DG was injected i.v. at a dose of 150uCi/kg body weight as described in section 2.2.5. The nerve was then stimulated (section 2.2.11) for a period of 45 min at the end of which a blood sample (0.4 ml) was taken and the animal killed by decapitation. Control animals were anaesthetised with urethane and an incision of the same length as that required to expose the mammary nerve was made in the skin of the abdomen; blood samples were collected and 2DG was injected in the same manner as in the experimental animals, but the mammary nerve was not stimulated.

5.2.5 Autoradiography and Calculations

The brain and pituitary gland were removed *en bloc* from each animal and were processed for autoradiography as described in section 2.2.1. The rma of selected areas of the brain and pituitary gland were determined (2.3.1.1). In the experiment measuring LCGU, the calculated [^{14}C] content for each structure and the arterial plasma concentrations of glucose and [^{14}C] 2DG were used in the operational equation described by Sokoloff et al. (1977) to determine LCGU (2.3.1.2).

The significance of differences between groups in either rma or LCGU was assessed using the unpaired t-test and the Wilcoxon test. The plasma was separated from the venous blood samples (section 2.2.7) and was assayed for PRL by RIA (section 2.3.2.2).

5.3 RESULTS

5.3.1 Conscious animals

In the conscious animals there was no significant difference in

rma of any of the brain areas measured in the suckled compared with the unsuckled control animals (Tables 5-1,5-2). The concentration of PRL in plasma however, increased after the pups were replaced (Fig. 5-A). The litters increased in weight by 10 ± 1 g (for 9 pups) (mean ± S.E.M. n=8), and inspection of the stomachs of the pups showed that fresh milk was present.

5.3.2 Anaesthetised animals

5.3.2.1 Experiments using the measurement of relative metabolic activity

In the anaesthetised suckled animals there was a significant increase in rma of the SON and PVN ($P < 0.005$) compared with the rma of these nuclei in the anaesthetised, unsuckled animals (Table 5-1). No significant change in rma occurred in the PN or PD, or in any other brain area measured (Tables 5-1 and 5-2). The plasma PRL concentration in the anaesthetised suckled animals did not increase after the pups had suckled for 105 min (paired t-test, n=5, Table 5-4).

5.3.2.2 Experiments using the measurement of local cerebral glucose utilisation

Table 5-3 shows the rma and corresponding LCGU of selected areas of the brain and pituitary gland, measured in a separate group of anaesthetised suckled rats. Inspection of the stomachs of the pups showed that some litters that had suckled did not receive any milk, although they had shown the 'stretch response'. The suckled does were therefore placed in two separate groups; those that had milk ejected, as assessed by the presence of fresh milk in the stomachs of most of the pups, and those that had not. There were no

significant differences in either the rma or the LCGU between the 3 groups in any of the areas of the brain and pituitary gland measured (Table 5-3; t- and Wilcoxon tests). If the results from the 2 groups of suckled animals were combined irrespective of milk yield, there were again no significant differences either in rma or in LCGU between suckled and unsuckled control animals in any of the areas of the brain and pituitary gland measured (Table 5-3).

The plasma PRL concentration did not increase after suckling in either the does that had milk ejected (n=4), or those that had not (n=4, Table 5-5, paired t-test). Plasma PRL concentrations did not increase significantly (as assessed by the paired t-test) when the group results in Table 5-5 were analysed either separately or combined (suckled, good plus suckled, poor milk ejections). However, the percentage increments in plasma PRL concentrations for the 2 groups of suckled animals when combined were significant ($P < 0.01$, n=8; paired t-test). If the percentage increments in PRL concentrations for the 2 groups of suckled animals were analysed separately, only the animals that had shown poor milk ejection showed an increase in plasma PRL concentration after suckling ($P < 0.01$, n=4).

5.3.3 Mammary nerve stimulation

Table 5-1 and Figs. 5-B and 5-C show that mammary nerve stimulation resulted in a significant increase in rma of the SON ($P < 0.02$) and PVN ($P < 0.05$) compared with those in sham-operated animals. The rma of the PN increased ($P < 0.01$; Fig. 5-D), as did the rma of the PD and of the TSTH in the region of the pons ($P < 0.05$ and $P < 0.025$, respectively) compared with those in

Table 5-1. Relative metabolic activity of selected brain regions and the pituitary gland in conscious and anaesthetised rats

Values are expressed as a ratio (mean ± S.E.M.) of [^{14}C] concentration of the selected region to [^{14}C] concentration of the corpus callosum.

Treatment	No. of Animals	SON	PVN	PN	PD	TSTH	VMT	MDT	ZI	NR	LLM
Conscious suckled	8	0.91 ± 0.02	1.12 ± 0.04	1.01 ± 0.06	0.73 ± 0.04	1.05 ± 0.03	2.47 ± 0.12	2.17 ± 0.07	1.78 ± 0.05	1.77 ± 0.09	2.37 ± 0.11
Conscious unsuckled	5	0.98 ± 0.04	1.17 ± 0.03	0.99 ± 0.03	0.68 ± 0.03	1.05 ± 0.04	2.37 ± 0.12	2.15 ± 0.10	1.78 ± 0.06	1.73 ± 0.07	1.99 ± 0.11
Anaesthetised and Suckled	5	1.53***** ± 0.04	1.62***** ± 0.03	1.62 ± 0.20	0.76 ± 0.08	1.10 ± 0.06	1.94 ± 0.08	1.89 ± 0.11	1.74 ± 0.06	1.59 ± 0.03	2.21 ± 0.10
Anaesthetised and unsuckled	4	1.16 ± 0.05	1.31 ± 0.07	1.33 ± 0.18	0.70 ± 0.08	1.12 ± 0.06	2.00 ± 0.18	1.90 ± 0.18	1.76 ± 0.02	1.81 ± 0.21	2.24 ± 0.24
Mammary nerve Stimulation	5	1.69** ± 0.11	1.89* ± 0.17	1.68**** ± 0.12	0.83* ± 0.04	1.25*** ± 0.01	2.14* ± 0.07	2.02* ± 0.09	1.80* ± 0.03	1.87* ± 0.04	2.16***** ± 0.07
Sham-operated control	4	1.25 ± 0.07	1.41 ± 0.03	1.11 ± 0.09	0.66 ± 0.04	1.17 ± 0.03	2.48 ± 0.12	2.42 ± 0.19	1.99 ± 0.07	2.20 ± 0.14	2.87 ± 0.15

Significantly different from corresponding control group: * $p < 0.05$; ** $P < 0.025$; *** $p < 0.02$; **** $p < 0.01$; ***** $p < 0.005$.

SON, supraoptic nuclei; PVN, paraventricular nucleus; TSTH, spinothalamic tract; VMT, ventromedial thalamic area; MDT, mediodorsal thalamic area; ZI, zona incerta; NR, red nucleus; LLM, lateral lemniscus

Table 5-2. Relative metabolic activity of selected brain regions in conscious and anaesthetised rats

Values are expressed as a ratio (mean ± S.E.M.) of [^{14}C] concentration of selected region to [^{14}C] concentration of the corpus callosum

	Type and number of animal					
	Conscious suckled (n=8)	Conscious unsuckled (n=5)	Anaesthetised and suckled (n=5)	Anaesthetised unsuckled (n=4)	Mammary nerve stimulated (n=5)	Sham-operated control (n=4)
S	1.58±0.09	1.68±0.07	1.39±0.01	1.52±0.11	1.61±0.07	1.45±0.04
SCN	1.51±0.06	1.75±0.11	1.77±0.09	1.83±0.24	1.81±0.08	2.16±0.28
VMH	1.06±0.02	1.10±0.03	1.35±0.05	1.35±0.06	1.40±0.05	1.33±0.04
ARC	1.01±0.03	1.15±0.05	1.42±0.04	1.35±0.09	1.38±0.04	1.38±0.07
MFB	1.47±0.06	1.51±0.04	1.41±0.02	1.43±0.08	1.61±0.03	1.77±0.13
AMYG	1.32±0.05	1.30±0.05	1.36±0.04	1.40±0.05	1.42±0.04	1.50±0.06
VT	2.10±0.11	1.83±0.08	1.51±0.03	1.62±0.08	1.64±0.03	1.79±0.09
IC	0.96±0.03	0.97±0.02	0.98±0.02	0.99±0.04	0.96±0.01	0.91±0.02
DHIPPM	2.10±0.10	1.98±0.06	1.68±0.08	1.81±0.17	1.74±0.10	1.86±0.05
DHIPPG	1.59±0.08	1.48±0.06	1.36±0.03	1.47±0.11	1.29±0.06	1.35±0.07

S, septum; SCN, suprachiasmatic nucleus; VMH, ventromedial hypothalamic nucleus; ARC, arcuate nucleus; MFB, medial forebrain bundle; AMYG, amygdala; VT, ventral thalamic area; IC, internal capsule; DHIPPM, dorsal hippocampus (molecular layer); DHIPPG, dorsal hippocampus (granular layer)

Table 5-2 (cont). Relative metabolic activity of selected brain regions in conscious and anaesthetised rats

Values are expressed as a ratio (mean ± S.E.M.) of [^{14}C] concentration of selected region to [^{14}C] concentration of the corpus callosum

	Type and number of animal					
	Conscious suckled (n=8)	Conscious unsuckled (n=5)	Anaesthetised and suckled (n=5)	Anaesthetised unsuckled (n=4)	Mammary nerve stimulated (n=5)	Sham-operated control (n=4)
VHIPPM	1.90±0.09	1.91±0.04	1.82±0.09	2.10±0.23	1.68±0.11	1.79±0.15
VHIPPG	1.50±0.08	1.50±0.04	1.48±0.05	1.76±0.09	1.46±0.05	1.52±0.09
SN	1.32±0.05	1.36±0.04	1.51±0.03	1.54±0.02	1.57±0.06	1.57±0.09
HAB	1.85±0.07	1.94±0.09	1.85±0.11	1.96±0.11	2.01±0.10	2.19±0.13
RF	1.50±0.08	1.47±0.07	1.36±0.04	1.42±0.08	1.54±0.06	1.56±0.08
TL	1.40±0.06	1.39±0.06	1.39±0.02	1.41±0.06	1.47±0.02	1.45±0.04
RA	1.60±0.06	1.61±0.05	2.23±0.18	2.11±0.10	2.47±0.17	2.45±0.26
CG	1.42±0.06	1.49±0.06	1.76±0.07	1.65±0.06	1.82±0.08	1.81±0.06

VHIPPM, ventral hippocampus (molecular layer); VHIPPG, ventral hippocampus (granular layer); SN, substantia nigra; HAB, habenula nuclei; RF, midbrain reticular formation; TL, lateral tegmentum; RA, raphe nucleus; CG, central grey

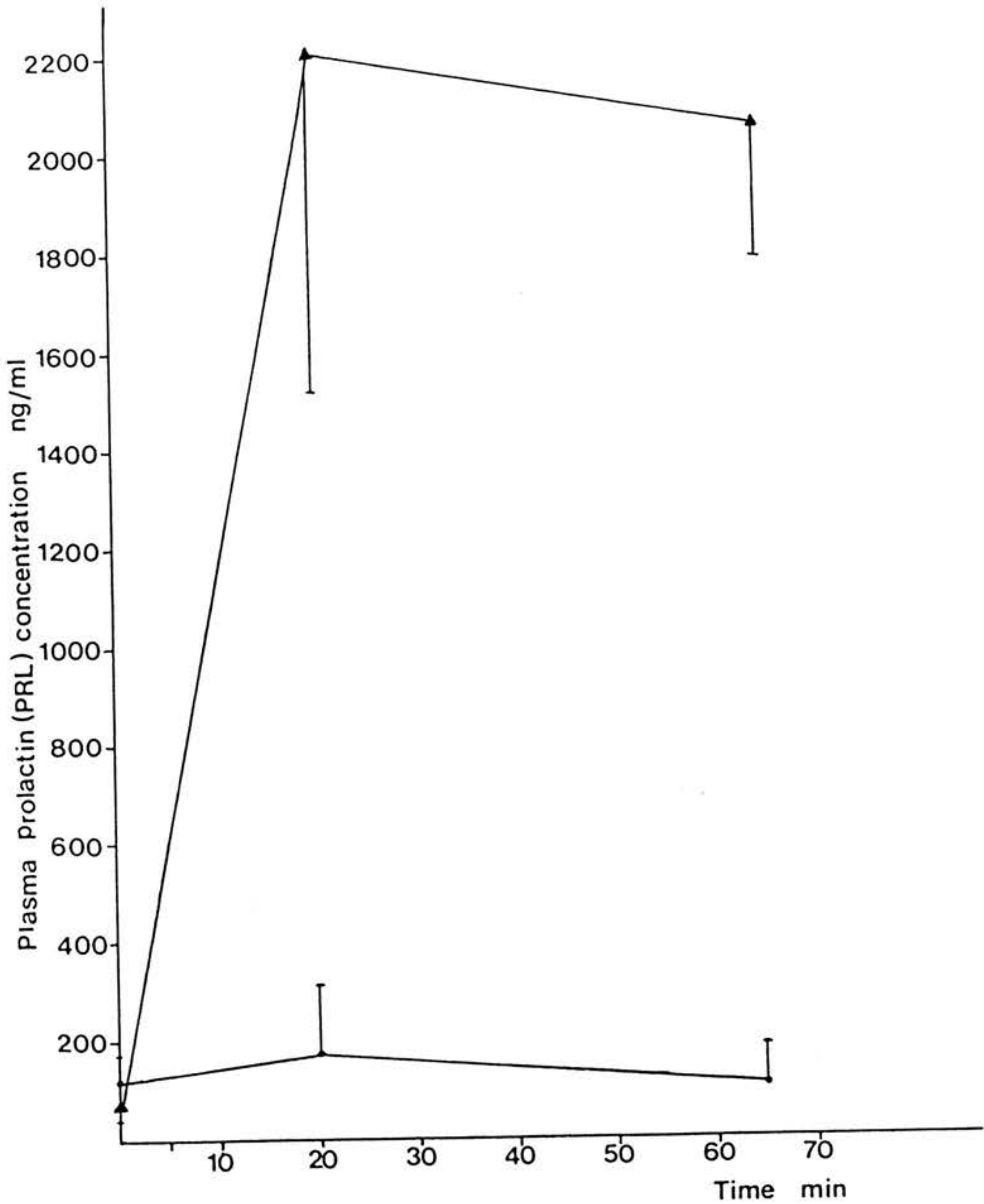


Fig. 5-A: plasma prolactin (PRL) concentration in conscious suckled animals. Blood samples were collected by way of an intra-atrial cannula inserted 48 h previously under halothane anaesthesia. Pups (apart from 1) were separated from the doe overnight, and returned to the doe after the first blood sample had been collected. (●-●) control unsuckled (n=5), (▲-▲) suckled (n=8).

Table 5-3. Local cerebral glucose utilisation (LCGU; mean ± S.E.M., μmol/100 g/min) and relative metabolic activity (rma; ratio (mean ± S.E.M.) of [^{14}C] concentration of selected region to [^{14}C] concentration of the corpus callosum) of brain and pituitary gland areas in rats

	LCGU			rma		
	Anaesthetised and suckled, good milk ejection (n=4)	Anaesthetised and suckled, poor milk ejection (n=4)	Anaesthetised unsuckled (n=4)	Anaesthetised and suckled, good milk ejection (n=4)	Anaesthetised and suckled, poor milk ejection (n=4)	Anaesthetised unsuckled (n=4)
SON	42 ± 3	43 ± 6	41 ± 1	1.24 ± 0.10	1.14 ± 0.02	1.13 ± 0.03
PVN	59 ± 6	60 ± 6	56 ± 3	1.58 ± 0.20	1.44 ± 0.02	1.36 ± 0.05
PN	71 ± 19	57 ± 9	62 ± 3	1.73 ± 0.35	1.49 ± 0.13	1.49 ± 0.07
PD	17 ± 1	22 ± 4	21 ± 2	0.71 ± 0.04	0.79 ± 0.10	0.76 ± 0.06
TSTH	37 ± 3	39 ± 3	44 ± 4	1.05 ± 0.05	1.07 ± 0.03	1.10 ± 0.07
VMT	85 ± 4	89 ± 9	83 ± 4	2.11 ± 0.14	2.00 ± 0.11	1.99 ± 0.15
MDT	74 ± 4	77 ± 7	72 ± 3	1.88 ± 0.14	1.78 ± 0.08	1.72 ± 0.08
ZI	67 ± 3	68 ± 7	64 ± 4	1.73 ± 0.13	1.59 ± 0.05	1.54 ± 0.04
NR	66 ± 2	67 ± 7	66 ± 4	1.70 ± 0.08	1.58 ± 0.08	1.59 ± 0.06
LLM	61 ± 2	63 ± 5	59 ± 3	1.61 ± 0.07	1.52 ± 0.05	1.44 ± 0.04
S	57 ± 2	65 ± 6	61 ± 3	1.52 ± 0.07	1.54 ± 0.03	1.47 ± 0.03
SCN	69 ± 5	75 ± 3	74 ± 4	1.77 ± 0.10	1.75 ± 0.12	1.71 ± 0.03
VMH	42 ± 3	47 ± 5	45 ± 2	1.24 ± 0.11	1.21 ± 0.01	1.19 ± 0.02
ARC	48 ± 6	56 ± 6	51 ± 2	1.37 ± 0.16	1.37 ± 0.03	1.30 ± 0.02

SON, supraoptic nuclei; PVN, paraventricular nucleus; PN, pars nervosa; PD, pars distalis; TSTH, spinothalamic tract; VMT, ventromedial thalamic area; MDT, mediodorsal thalamic area; ZI, zona incerta; NR, red nucleus; LLM, lateral lemniscus; S, septum; SCN, suprachiasmatic nucleus; VMH, ventromedial hypothalamic nucleus; ARC, arcuate nucleus

Table 5-3 (cont). Local cerebral glucose utilisation (LCGU; mean ± S.E.M., μmol/100 g/min) and relative metabolic activity (rma; ratio (mean ± S.E.M. of [^{14}C] concentration of selected region to [^{14}C] concentration of the corpus callosum) of brain and pituitary gland areas in rats

	LCGU			rma		
	Anaesthetised and suckled, good milk ejection (n=4)	Anaesthetised and suckled, poor milk ejection (n=4)	Anaesthetised unsuckled (n=4)	Anaesthetised and suckled, good milk ejection (n=4)	Anaesthetised and suckled, poor milk ejection (n=4)	Anaesthetised unsuckled (n=4)
MFB	51 ± 3	56 ± 5	53 ± 2	1.41 ± 0.10	1.36 ± 0.02	1.35 ± 0.03
AMYG	55 ± 2	59 ± 5	57 ± 5	1.49 ± 0.07	1.44 ± 0.04	1.41 ± 0.07
VT	55 ± 2	59 ± 6	58 ± 5	1.50 ± 0.08	1.44 ± 0.05	1.42 ± 0.07
IC	28 ± 1	32 ± 4	30 ± 2	0.87 ± 0.02	0.88 ± 0.02	0.87 ± 0.02
DHIPPM	76 ± 4	83 ± 4	78 ± 3	1.91 ± 0.10	1.89 ± 0.10	1.81 ± 0.07
DHIPPG	45 ± 3	53 ± 4	51 ± 2	1.29 ± 0.06	1.33 ± 0.03	1.30 ± 0.02
VHIPPM	81 ± 5	87 ± 4	84 ± 2	2.05 ± 0.11	1.96 ± 0.10	1.93 ± 0.04
VHIPPG	54 ± 4	60 ± 6	59 ± 3	1.45 ± 0.07	1.44 ± 0.01	1.44 ± 0.04
SN	44 ± 2	53 ± 6	49 ± 2	1.26 ± 0.03	1.32 ± 0.02	1.27 ± 0.03
HAB	68 ± 5	70 ± 6	64 ± 2	1.76 ± 0.15	1.64 ± 0.05	1.53 ± 0.02
RF	52 ± 2	53 ± 4	51 ± 2	1.42 ± 0.07	1.33 ± 0.03	1.30 ± 0.02
TL	45 ± 2	48 ± 3	47 ± 1	1.29 ± 0.06	1.24 ± 0.03	1.24 ± 0.01
RA	68 ± 2	69 ± 5	64 ± 3	1.74 ± 0.09	1.61 ± 0.05	1.55 ± 0.03
CG	57 ± 3	58 ± 3	57 ± 3	1.53 ± 0.11	1.42 ± 0.05	1.41 ± 0.04
CC	35 ± 1	39 ± 4	37 ± 2			

MFB, medial forebrain bundle; AMGY, amygdala; VT, ventral thalamic area; IC, internal capsule; DHIPPM, dorsal hippocampus (molecular layer); DHIPPG, dorsal hippocampus (granular layer); VHIPPM, ventral hippocampus (molecular layer); VHIPPG, ventral hippocampus (granular layer); SN, substantia nigra; HAB, habenular nuclei; RF, midbrain reticular formation; TL, lateral tegmentum; RA, raphe nucleus; CG, central grey; CC, corpus callosum

Table 5-4. Plasma prolactin concentrations (ng/ml) in anaesthetised animals and animals in which the mammary nerve was stimulated. All does were separated from their litter (except for 1 pup) overnight. The first sample was collected 3 h after urethane was administered. The second sample was collected after the pups had suckled for 105 min and the mammary nerve was stimulated for 45 min. Non-detectable values were attributed the lowest detectable value in the assay for 20 μl samples, 20 ng/ml.

	Animal	1st sample	2nd sample	Increment	%Increment
Anaesthetised and suckled	1	64.0	23	-41	-64
	2	< 20	< 20		
	3	190	128	-61	-32
	4	< 20	< 20		
	5	32	< 20	-12	-37
Anaesthetised unsuckled	1	< 20	< 20		
	2	55	52	-3	-6
	3	153	211	58	38
	4	< 20	< 20		
Mammary nerve stimulation	1	67	62	-5	-8
	2	< 20	< 20		
	3	43	< 20	-23	-54
	4	55	< 20	-35	-63
	5	< 20	< 20		
Sham-operated control	1	< 20	20		
	2	< 20	< 20		
	3	58	63	5	8
	4	100	183	83	83

Table 5-5. Plasma prolactin concentrations (ng/ml) in anaesthetised animals. Does were separated from their litter (except for 1 pup) overnight. The first sample was collected 3 h after urethane was administered, and the second sample was collected after the pups had suckled for 105 min. The lower limit of detection of the assay for a 20 μl sample was 12 ng/ml.

	Animals	1st sample	2nd sample	Increment	% Increment
Anaesthetised and suckled, good milk ejection	1	1188	963	-225	-19
	2	20	71	51	256
	3	44	107	63	142
	4	25	34	9	35
Anaesthetised and suckled, poor milk ejection	1	80	476	396	493
	2	19	68	50	262
	3	26	89	64	249
	4	15	66	51	339
Anaesthetised unsuckled	1	34	50	15	44
	2	14	81	67	481
	3	17	13	-4	-29
	4	22	22		

Table 5-6. Comparison of the effects of different anaesthetics on local cerebral glucose utilisation of selected brain areas in the rat (µmoles/100 g/min)

	Type of Anaesthetic				
Grey matter	Thiopental (n=8) (Sokoloff et al., 1977)	Chloral hydrate (n=6) (Grome and McCulloch, 1983)	Halothane (n=5) (Savaki et al., 1983)	Urethane (n=4) (present study)	Control, conscious (n=7) (Savaki et al., 1983)
S		32 ± 2	56 ± 4	61 ± 3	55 ± 2
SCN			69 ± 1	74 ± 4	62 ± 4
AMYG	41 ± 2	34 ± 1	59 ± 3	57 ± 5	70 ± 4
HIPP		59 ± 3	79 ± 2	78 ± 3	68 ± 3
VMT			82 ± 4	83 ± 4	90 ± 5
MDT		51 ± 2	62 ± 2	72 ± 3	97 ± 5
VT	55 ± 1	40 ± 1	84 ± 6	58 ± 5	109 ± 4
HAB		54 ± 3	71 ± 4	64 ± 2	93 ± 7
SN		35 ± 1	56 ± 5	49 ± 2	43 ± 1
NR		39 ± 1	57 ± 2	66 ± 4	60 ± 3
RA		46 ± 3	65 ± 3	64 ± 3	77 ± 4
LLM	75 ± 4	57 ± 2		59 ± 3	
White matter					
IC	29 ± 2	22 ± 1	21 ± 1	30 ± 2	23 ± 2
CC	30 ± 2	30 ± 2	37 ± 3	37 ± 2	32 ± 2

S, septum; SCN, suprachiasmatic nucleus; AMYG, amygdala; HIPP, hippocampus; VMT, ventromedial thalamic area; MDT, mediodorsal thalamic area; VT, ventral thalamic area; HAB, habenula nuclei; SN, substantia nigra; NR, red nucleus; RA, raphe nucleus; LLM, lateral lemniscus; IC, internal capsule; CC, corpus callosum

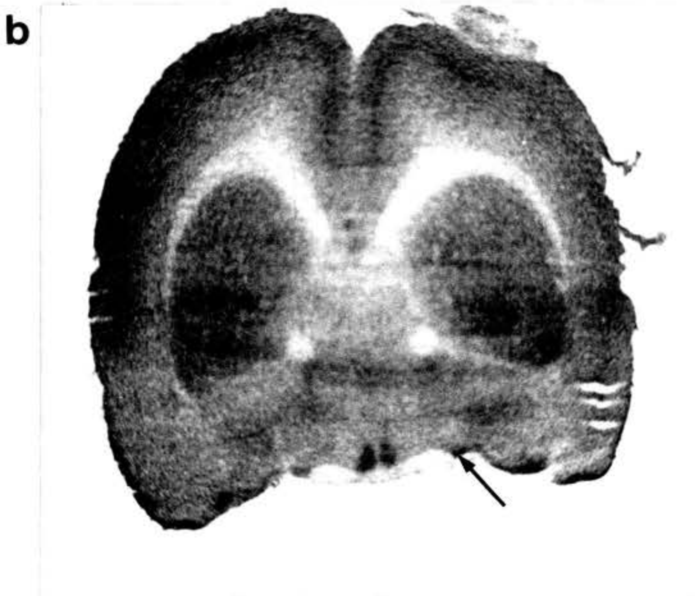


Fig. 5-B: autoradiographs of coronal sections of the brain at the level of the supraoptic nucleus (arrow) in rats anaesthetised with urethane and injected with [^{14}C]2-deoxyglucose; (a) sham operated and (b) mammary nerve-stimulated animals.

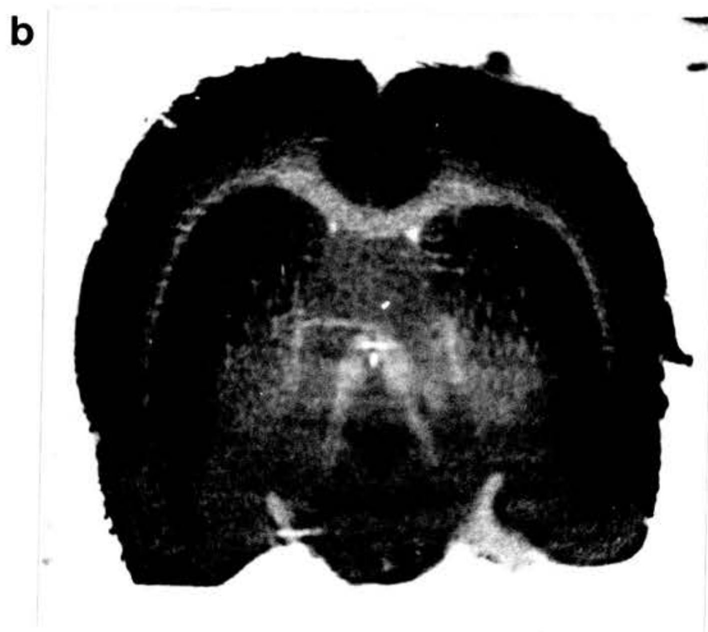
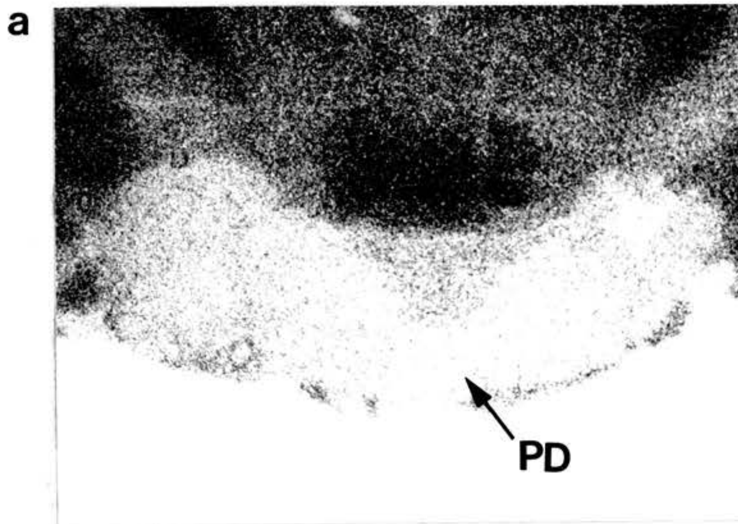


Fig. 5-C: autoradiographs of coronal sections of the brain at the level of the paraventricular nucleus (arrow) in rats anaesthetised with urethane and injected with [^{14}C]2-deoxyglucose; (a) sham-operated and (b) mammary nerve-stimulated animals.



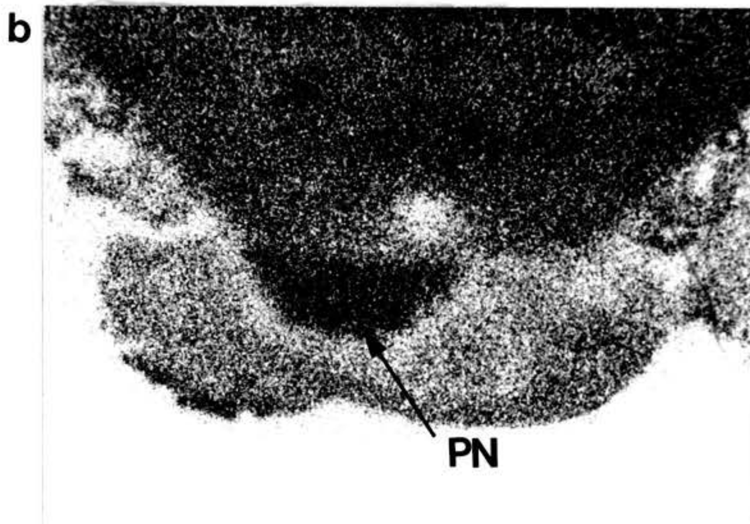


Fig. 5-D: autoradiographs of coronal sections of the pituitary gland in rats anaesthetised with urethane and injected with [^{14}C]2-deoxyglucose, showing the pars nervosa (PN) and pars distalis (PD) in sham-operated (a) and mammary nerve-stimulated (b) animals.

sham-operated animals. Mammary nerve stimulation was also followed by a significant decrease in rma in the VMT and MDT, ZI and NR ($P < 0.05$) and in the LLM ($P < 0.005$) compared with sham-operated animals. Mammary nerve stimulation did not alter the plasma PRL concentration (paired t-test, n=5, Table 5-4).

5.4 DISCUSSION

An increase in 2DG uptake is known to reflect an increase in metabolism (Sokoloff et al., 1977), notably that associated with sodium pump activity (Mata et al., 1980). The absence of a detectable increase in rma in the hypothalamo-neurohypophysial system in the conscious suckled rat was therefore surprising, since substantial increases in electrical activity (Summerlee and Lincoln, 1981) and synthetic activity (as assessed by increased nucleolar dry mass, Russell, 1980) of the magnocellular neurones have been shown to occur in response to suckling in the conscious rat. Since the 2DG method is biased towards the glucose utilisation which occurs in the first 15 min after the injection of the 2DG (McCulloch, 1982; see Introduction), only neural activity related to the milk ejection reflex occurring during this critical time period was likely to produce a significant change in rma. Conscious does that had been suckled had shown milk ejection during the 65 min experimental period, as assessed by the presence of fresh milk in the stomachs and the change in body weight of the pups. Presumably milk ejection occurred in these animals either before the 2DG was injected, or in the later part of the experiment when most of the 2DG was already trapped in the cells (Introduction).

Auditory and other external stimuli can inhibit the milk

ejection reflex (Grosvenor and Mena, 1976), presumably by preventing slow wave sleep which, in the rat, is a prerequisite for milk ejection (Voloschin and Tramezzani, 1979; Lincoln, Hentzen, Hin, Van der Schoot, Clarke and Summerlee, 1980). In order to minimise the effects of external stimuli on the doe, and to promote a period of slow wave sleep, two groups of anaesthetised suckled animals were studied (5.3.2.1 and 5.3.2.2). Anaesthesia itself does not abolish the milk ejection reflex (Lincoln et al., 1973). In the first group of anaesthetised animals (5.3.2.1)the rma of the SON and PVN increased significantly above control values but no change in rma was found in any other fore-, mid- or hind-brain structure measured, or in the pituitary gland. The increase in the rma of the PVN and SON could be due to an increase in the metabolism of the cell bodies as well as of the nerve terminals of afferent projections to the PVN and SON. Whether the increased rma in the hypothalamo-neurohypophysial system reflects only terminal activity, as suggested by Schwartz et al. (1979), or whether activity in the cell bodies was also increased cannot be resolved using this method of autoradiography, due to the limits of resolution. High resolution autoradiographic studies measuring [^{3}H] 2DG uptake in response to an osmotic stimulus (3.3.2) showed that the increase in 2DG uptake in the PVN was localised mainly around the perikarya, presumably in afferent nerve terminals, and not in the cell bodies of the nucleus. That the 2DG technique measured terminal and not cell body activity in this study is suggested further by the increase in rma of the PVN and SON with no concomitant increase in the rma of the PN. However, the increase in rma of the PVN and SON could also be

due to local synaptic activity or dentritic activity caused by local depolarisations of the magnocellular perikarya.

In the second group of anaesthetised suckled animals (5.3.2.2), individual animals showed high rma and LCGU in the SON, PVN and PN after suckling. However, no significant changes in rma or LCGU were found in any area of the brain and pituitary gland measured in the suckled animals compared with the values in unsuckled control animals by either t- or Wilcoxon tests. The LCGU of brain areas in this study were more similar to the values obtained under thiopental, chloral hydrate and halothane anaesthesia (Sokoloff et al., 1977; Grome and McCulloch, 1983; Savaki, Desban, Glowinski and Besson, 1983) than to the values obtained in conscious animals (Savaki et al., 1983) (Table 5-6). It is unlikely, therefore, that any change in LCGU of brain and pituitary gland areas in the suckled animals was masked by high values in the unsuckled, control animals. The most likely explanation is that in the majority of these animals the milk ejections cannot have occurred in the critical initial 15 min following the injection of 2DG, whereas they probably occurred during this critical period in the first group of anaesthetised animals (5.3.2.1).

Mammary nerve stimulation resulted in an increase in rma of the PVN, SON, PN and PD. This is consistent with the fact that stimulation of the mammary nerve with similar parameters produced an increase in intra-mammary pressure indicative of oxytocin release (Mena et al., 1978). Activation of the magnocellular neurones sufficient to be detected by the 2DG method, therefore, did occur after mammary nerve stimulation which, presumably, is a more intense

stimulus than suckling. The rma of the TSTH in the region of the pons was also increased after mammary nerve stimulation, and this is consistent with the ascending milk ejection reflex path running coincident with that tract in a variety of species (Tindal et al., 1967; Mena and Beyer, 1968; Tindal et al., 1969; Knaggs et al., 1972; Tindal and Knaggs, 1975). Studies in which the techniques of electrophysiological recording or the placement of lesions in the nervous system have been used to study the milk ejection reflex in either conscious or anaesthetised animals, and have provided conflicting evidence for the precise anatomical location of the neural pathway in the midbrain for milk ejection; depending on the method and the animal species used the pathway has been found to be either discrete (Tindal et al., 1969) or diffuse (Urban et al., 1971; Voloschin and Dottaviano, 1976; Juss and Wakerley, 1981). The conflicting data for a discrete *versus* a diffuse afferent pathway in the rabbit could be explained by the simultaneous activation by the stimulus of a central inhibitory mechanism that overrides the milk ejection reflex. The inhibitory mechanism, postulated by Cross (1955a,b) and Tindal and Knaggs (1975), is thought to be associated with the septo-hippocampal pathway (Tindal and Blake, 1980), but its activity in the rat is relatively slight (Tindal and Blake, 1980; Lebrun, Poulain and Theodosis, 1983), and no change in the rma of either the S or the HIPP occurred in the present study. No increase in rma of the TL, RF or MFB was found, suggesting that perhaps the pathway through the midbrain for milk ejection is too diffuse for any increased activity to be detected by the 2DG method. The 'gating' mechanism which operates to cause intermittent

magnocellular activity could be located where afferents from the nipple enter the spinal cord, since stimulation of the cord was always effective in causing milk ejection (Dyer and Poulain, 1982). The site and mechanism of the 'gating' of the suckling stimulus cannot be easily investigated further using the 2DG method, since an increase in metabolic activity, however transient, results in the trapping of [^{14}C]2DG-6-P within a structure (see Introduction).

The reason for the decrease in rma of the VMT, MDT, NR, LLM and the ZI in the animals in which the mammary nerve was stimulated is not clear, but this decrease may reflect inhibition of metabolism related to the central effects of peripheral nerve stimulation.

Studies on conscious animals and animals anaesthetised with urethane have shown that the plasma concentration of PRL increased after suckling (e.g. Amenomori et al., 1970; Burnet and Wakerley, 1976) and after mammary nerve stimulation (Mena, Pacheco and Grosvenor, 1980). The absence of a change in plasma PRL concentration in the first group of anaesthetised, suckled animals (5.3.2.1), and in the animals in which the mammary nerve was stimulated (5.3.3), and only a trend towards an increase in plasma PRL concentration in the second group of anaesthetised, suckled animals (5.3.2.2) can be explained by the existence of different thresholds for the reflex release of oxytocin and of PRL (McNeilly and Hart, 1973; Wakerley, O'Neill and Ter Haar, 1978). These thresholds may be set by the preceding suckling experience of the mother (Wakerley et al., 1978). The release of PRL in anaesthetised suckled animals has never been recorded without the concomitant release of oxytocin (Wakerley et al., 1978). This interaction

between the two neuroendocrine reflexes may reflect the existence of common afferent pathways, as in the MFB and DLF, which are thought to be involved in milk ejection (Tindal et al., 1969) and PRL release (Tindal and Knaggs, 1972; 1977). However, since the two neuroendocrine events can be separated pharmacologically (e.g. ergot alkaloids suppress the release of PRL but not oxytocin, Russell et al., 1981), additional pathways are likely to be involved in PRL release compared with those involved in the milk ejection reflex.

In conscious suckled animals, where plasma PRL concentration increased above control values, no change in rma was found in any brain area or the pituitary gland (Tables 5-1, 5-2).This suggests that the release of PRL does not involve a detectable increase in metabolic activity of any brain area measured, or of the PD.

CHAPTER VI

Brain Electrical Stimulation and Lesioning Studies

6.1 INTRODUCTION

The preovulatory rise in plasma LH concentration is known to depend on the integrity of the neural connections between the POA-m and MBH (e.g. Halász and Pupp, 1965; Halász and Gorski, 1967; Tejasen and Everett, 1967; Köves and Halász, 1970; Butler and Donovan, 1971; Brown-Grant and Raisman, 1972; Van Rees, 1972; Blake, Scaramuzzi, Hilliard and Sawyer, 1973). The effects of electrical and electrochemical stimulation of the POA on ovulation and gonadotrophin release have been studied extensively in the rat. Stimulation has been shown to be followed by ovulation (Everett, 1965), an increase in LH concentration in peripheral plasma (Cramer and Barraclough, 1971; Kalra, Ajika, Krulich, Fawcett, Quijada and McCann, 1971; Velasco and Rothchild, 1973; Fink and Aiyer, 1974; Jamieson and Fink, 1976) and by an increase in pituitary stalk plasma LHRH concentration (Burger, Fink and Lee, 1972; Fink and Jamieson, 1976) which is modulated by sex steroids (Sherwood, Chiappa and Fink, 1976; Chiappa et al., 1977).

Other brain areas, such as the SCN, AHA, AMYG and HIPP may also be important in the control of gonadotrophin release (e.g. Hillarp, 1949; Taleisnik and McCann, 1961; Critchlow, 1963; Kennedy, 1964; Velasco and Taleisnik, 1969a,b; Kalra et al., 1971; Brown Grant and Raisman, 1972; Sawyer, 1972; Van Rees, 1972; Kawakami, Terasawa, Kimura and Wakabayashi, 1973; Jamieson and Fink, 1976; Brown Grant and Raisman, 1977; Chiappa et al., 1977; Wiegand et al., 1980).

The aim of these studies was to investigate the effects of electrical stimulation of various hypothalamic and limbic brain areas on metabolic activity throughout the brain, and so demonstrate

active neural connections between areas known to be important in neuroendocrine function. In addition, the effects of short-term ovariectomy and steroid replacement treatment on the changes in metabolic activity following POA stimulation have been studied, based on the model of Sherwood et al. (1976) where the LHRH response to electrical stimulation of the POA was augmented by the administration of OB. The effects of stimulation and lesioning of the VLGEN, and of lesioning immediately rostral to the RA on the rma throughout the brain have also been investigated, since these areas have been shown to project to the SCN (Dahlström and Fuxe, 1964; Ungerstedt, 1971; Björklund et al., 1973a; Swanson, Cowan and Jones, 1974; Ribak and Peters, 1975; Parent et al., 1981). The plasma concentration of LH was measured in the present experiments as a functional marker, since electrical stimulation of some hypothalamic and limbic brain areas has been shown to increase LH concentration in peripheral plasma, and LHRH release into hypophysial portal plasma (e.g. Fink and Jamieson, 1976; Sherwood et al., 1976; Chiappa et al., 1977).

6.2 MATERIALS AND METHODS AND EXPERIMENTAL DESIGN

6.2.1 Animals

The animals used in these studies were adult female Wistar rats, 200-300g body weight, purchased from Charles River U.K. Ltd. (Margate, Kent) and maintained as described in section 2.1.

6.2.2 Implantation of electrodes into brain areas excluding the median eminence

Electrodes were implanted under Avertin anaesthesia supplemented with ether (2.2.3) as described in section 2.2.9.1.

For stimulation of the POA, AHA, PVN and DHIPP bipolar electrodes separated by 2 mm and 11 mm long were used, except for stimulation of DHIPP where the electrodes used were 6 mm long. For stimulation of the SCN, AMYG and VHIPP bipolar electrodes separated by 1 mm and 11 mm long were used, and for stimulation of the VLGEN an unipolar electrode 11 mm long was used. The co-ordinates (De Groot, 1959) were as follows (A = anterior, V = vertical and L = lateral co-ordinates): POA, A 7.6, V- 1.5, 1 mm either side of mid-line; SCN, A 7.6, V- 2.3, 0.5 mm either side of mid-line; AHA, A 6.8, V- 2.2, 1 mm either side of mid-line; PVN, A 6.6, V- 1.4, 1 mm either side of mid-line; AMYG, A 6.4, V-2.6, L 4.5 (unilateral); VHIPP, A 3.4, V- 3.3, L 4.0 (unilateral); DHIPP, A 4.0, V + 2.0, L 2.5 (unilateral); VLGEN, A 3.2, V. - 1.3, L 3.2 (unilateral).

Animals were allowed to recover for a minimum of 5 days before experimentation.

6.2.3 Placement of electrodes into the median eminence

An unipolar electrode was placed in the ME under Althesin anaesthesia (2.2.3) as described in section 2.2.9.2, immediately before stimulation started.

6.2.4 Electrothermal lesioning and knife cut lesioning

Electrothermal lesions were placed in the VLGEN under Avertin anaesthesia supplemented with ether (2.2.3) as described in section 2.2.10.1, using a unipolar electrode connected to a Grass LM 4 lesion maker. The co-ordinates (De Groot, 1959) were A 3.2, V-1.3, L 3.2 (unilateral). For the lesion, a 3-5 mA current was passed with a frequency of 100 KHz for 20 sec.

Knife cut lesions were placed in the midbrain under Avertin

anaesthesia supplemented with ether (2.2.3), immediately rostral to the RA as described in section 2.2.10.2, using a rectangular knife 12 mm long and 0.6 mm wide fashioned from a razor blade. The co-ordinates (De Groot, 1959) were A 0.6, V-5.0, 0.3 mm either side of mid-line.

Lesioned animals were allowed to recover for a minimum of 3 days and a maximum of 7 days before experimentation.

6.2.5 Effect of short-term ovariectomy followed by treatment with either oestradiol benzoate or oil

Vaginal smears from animals with electrodes implanted into the POA (section 2.2.9.1) were examined daily, and after 2 consecutive 4-day oestrous cycles the animals were ovariectomised on dioestrus under ether anaesthesia (2.2.3) as described in section 2.2.12. Animals were then injected s.c. with either 10 μg OB (2.2.13) or 0.2 ml arachis oil. The POA stimulation experiments were then carried out the following day. After the animal was decapitated, the uterus was exposed by a midline abdominal incision, and was carefully removed by cutting the uterine horns free from the oviducts, and cutting the neck of the uterus just above the cervix. The uterus was then quickly transferred onto a piece of weighed filter paper and trimmed free of fat and mesentery. The paper and uterus were then weighed. The uterus was cut open to remove the fluid, and was weighed again on a fresh piece of filter paper ('dry' weight).

Experimental design

The vaginal smears of each animal was examined immediately before the start of the experiment. Each group comprised at least

one animal at each stage of the oestrous cycle. Stimulation studies and experiments using lesioned animals were carried out under Althesin anaesthesia (2.2.3). A blood sample (0.4 ml) was withdrawn from the external jugular vein (2.2.5) before stimulation started, and again after 15 min and 45 min of stimulation. The stimulation parameters were trains (30 sec on, 30 sec off) of accurately balanced rectangular wave pulses with a frequency of 50 Hz, pulse duration 1 msec and pulse amplitude of 500 μA (1 mA peak to peak). The 2DG was injected i.v. at a dose of 250 μCi/kg body weight (2.2.1) 1 min after stimulation had started. Control animals were implanted with electrodes and were anaesthetised, but were not stimulated. The animals were killed 45 min after the injection of 2DG, and the brain and pituitary gland were removed en-bloc and were processed for autoradiography (2.2.1). Some tissue sections throughout the area of electrode and lesion placement were stained for identification of the location of the electrode tips and lesions (2.2.9.1). The rma of selected areas of the brain and pituitary gland were then determined from the autoradiographs (2.3.1.1). The rma of a catalogue of 32 areas was always determined for each brain, consisting of areas known to be important for neuroendocrine function and white matter areas for use as internal standards; these areas were CC, S-m, S-l, DBB, POA-m, POA-l, SCN, SON, AHA, FX, PVN, MFB, ST, ARC, VMH, DMH, SM, AMYG CO+M, AMYG CE+L, IC, MM, IP, PN, PD, DHIPPM, DHIPPG, VHIPPM, VHIPPG, HAB-m, HAB-l, PYRCX, CG and RF. In addition, the rma of other brain areas were determined if seen to have changed dramatically or to have a well established neural connection with the area being stimulated. In experiments involving

stimulation on either side of the mid-line (POA, SCN, AHA, PVN and ME) the rma of each area was determined from a mean OD of 'right' and 'left' sides of the brain. In experiments involving unilateral stimulation of brain areas (AMYG, VHIPP, DHIPP, and VLGEN) and lesions of the VLGEN, the rma of areas on the two sides of brain were determined separately and termed ipsilateral (i) if on the same side as the implanted electrode or electrothermal lesion, and contralateral (c) if on the opposite side. Although the lesions made by the knife cuts were in the mid-line, the rma of areas on both sides of the brain were determined separately, and also termed i if on the side corresponding to the side in which the electrode or electrothermal lesion was placed in the other groups of animals, and c if on the opposite side. The rma of areas of brain and pituitary gland in control animals with electrode implants in either the SCN or the AHA, but unstimulated, were combined, and were also used as the control group for comparison with the rma of areas of the brain and pituitary gland of PVN stimulated animals. Similarly, the rma in animals bearing electrode implants in either the VHIPP or AMYG, but not stimulated, were combined.

The concentrations of LH in the plasma from the external jugular venous blood samples (2.2.7) were determined by RIA (2.3.2.3).

Significance of differences in rma was assessed by the unpaired t-test (for studies involving stimulation of SCN, AHA, PVN and ME) and by analysis of variance and Duncan's multiple range test (for studies involving stimulation of POA, AMYG, VHIPP, DHIPP, VLGEN and lesions of the VLGEN and immediately rostral to the RA).

Significance of differences between plasma LH concentrations was determined by the Mann-Whitney U test and paired t-test as appropriate.

6.3 RESULTS

6.3.1 Electrical stimulation of the preoptic area in intact rats and in short-term ovariectomised rats treated with oestradiol benzoate or oil

6.3.1.1 Position of electrode tips

A representative photomicrograph showing the position of the electrode tips in the POA is shown in Fig. 6-A (a). The anterior-posterior and vertical positions of all the electrode tips from the 6 groups of animals in the study are shown in Fig. 6-A (b).

6.3.1.2 Effect of electrode implants in the preoptic area without electrical stimulation; effects of short-term ovariectomy and treatment with oestradiol benzoate or oil

There were no significant differences in rma between POA unstimulated animals, ovariectomised animals treated with OB and unstimulated (OVX+OB-POA unstimulated) and ovariectomised animals treated with oil and unstimulated (OVX+oil-POA unstimulated) in any brain area measured, or in the PN. The rma of the PD however, was significantly decreased in OVX+oil-POA unstimulated animals compared with that in the POA unstimulated ($P < 0.05$) and the OVX+OB-POA unstimulated ($P < 0.005$) animals (Table 6-1).

6.3.1.3 Effect of electrical stimulation in the intact rat

Electrical stimulation caused a significant increase in the rma of the following areas of brain and pituitary gland: S-m, S-l, DBB, POA-m, POA-l, SCN, SON, AHA, FX, PVN, MFB, ST, ARC, VMH, DMH, SM,

AMYG CO+M, AMYG CE+L, PN, HAB-1, PYRCX, CG, PVT, LGEN and AVT (Table 6-1). The increases in rma were dramatic in the majority of these areas (more than 3-fold in some areas), and are clearly demonstrated in the autoradiographs shown in Figs. 6-B and 6-C.

6.3.1.4 Effect of electrical stimulation on the short-term ovariectomised rat treated with oestradiol benzoate

Table 6-1 shows that the rma of the same areas of brain and pituitary gland increased in the ovariectomised animals treated with OB and stimulated (OVX-OB-POA stimulated) compared with OVX+OB-POA unstimulated animals, as in the POA stimulated animals, with the exception of the LGEN. In addition, the rma of the MGEN decreased in OVX-OB-POA stimulated animals compared with that in OVX-OB-POA unstimulated animals (Table 6-1).

6.3.1.5 Effect of electrical stimulation on the short-term ovariectomised rat treated with oil

The rma of the same areas of brain increased in ovariectomised animals treated with oil and stimulated (OVX+oil-POA stimulated) compared with OVX+oil-POA unstimulated animals, as in OVX+OB-POA stimulated animals, apart from the AVT and PN (Table 6-1). The rma of the MGEN and DHIPPM decreased in OVX+oil-POA stimulated animals compared with OVX+oil-POA unstimulated animals (Table 6-1).

6.3.1.6 Comparison of the effects of short-term ovariectomy with treatment with oestradiol benzoate or oil on the effects of electrical stimulation

As described in sections 6.3.1.3, 6.3.1.4 and 6.3.1.5, electrical stimulation caused an increase in rma of many common areas of brain and pituitary gland in POA stimulated, OVX+OB-POA

stimulated and OVX+oil-POA stimulated animals. However, there were differences between the 3 groups, in that the rma of the PN and AVT were increased in POA stimulated and OVX+OB-POA stimulated animals, but did not change in OVX+oil-POA stimulated animals (Table 6-1), and the rma of the LGEN was increased in only the POA stimulated animals. Decreases in the rma of the MGEN occurred in OVX+OB-POA stimulated and OVX+oil-POA stimulated animals, and a decrease in rma of the DHIPPM was also found in OVX+oil-POA stimulated animals compared with OVX + oil-POA unstimulated animals (Table 6-1).

Although the rma increased in 22 common areas in the 3 groups of electrically stimulated animals, there were significant differences in the magnitude of the increases in some areas. The rma of the S-m, DBB and SON were significantly greater in OVX+oil-POA stimulated animals than in POA stimulated or OVX+OB-POA stimulated animals, and the rma of the MFB was increased in OVX+oil-POA stimulated animals compared with that in POA stimulated animals (Table 6-1). The OVX+OB-POA stimulated animals showed a significantly greater increase in rma of the PD compared with POA stimulated and OVX+oil-POA stimulated animals, and a significantly greater increase in rma of the AMYG CE+L compared with POA stimulated animals (Table 6-1). In addition, the OVX+OB-POA stimulated animals showed smaller increases in rma of the HAB-1 and PVT compared with POA stimulated animals.

6.3.1.7 Comparison of the effects of electrical stimulation on the plasma concentration of luteinising hormone in intact rats and short-term ovariectomised rats treated with oestradiol benzoate or oil

Table 6-2 shows that stimulation of the POA produced an

Table 6-1. The relative metabolic activity of selected brain areas and the pituitary gland in anaesthetised rats after electrical stimulation of the preoptic area (POA), short-term ovariectomy (OVX) with treatment with oestradiol benzoate (OB)or oil, and combined treatments.

Values are expressed as a ratio (mean ± S.E.M.) of [^{14}C] concentration of the selected region to [^{14}C] concentration of the corpus callosum.

	Type and number of animal					
	POA control, unstimulated (n=4)	POA stimulated (n=4)	OVX+OB-POA unstimulated (n=4)	OVX+OB-POA stimulated (n=4)	OVX+oil-POA unstimulated (n=4)	OVX+oil-POA stimulated (n=4)
S-m	1.35±0.04	4.61±0.74******	1.41±0.02	4.14±0.45******	1.36±0.01	6.07±0.17******
S-l	1.21±0.05	4.56±0.27******	1.25±0.02	4.14±0.03******	1.19±0.02	4.36±0.50******
DBB	1.32±0.02	4.25±0.41******	1.39±0.04	4.58±0.37******	1.38±0.01	6.00±0.42******
POA-m	1.18±0.04	4.50±0.58******	1.29±0.05	3.96±0.82******	1.28±0.01	4.93±0.74******
POA-l	1.25±0.09	5.21±0.47******	1.29±0.07	5.42±0.61******	1.25±0.03	6.25±0.51******
SCN	1.79±0.12	2.95±0.45*	2.06±0.23	3.05±0.26*	1.80±0.07	3.30±0.55*****
SON	1.13±0.03	1.77±0.10******	1.13±0.06	1.71±0.17******	1.15±0.02	2.28±0.12******
AHA	1.22±0.04	3.75±0.84*****	1.28±0.02	3.75±0.66*****	1.22±0.02	4.65±0.59******
FX	1.07±0.01	1.87±0.32******	1.13±0.03	1.85±0.09******	1.09±0.03	1.96±0.15******
PVN	1.28±0.06	3.24±0.43******	1.41±0.04	3.13±0.46******	1.38±0.02	3.53±0.39******

Significantly different from corresponding control group: * $P<0.05$; ** $P<0.025$; *** $P<0.02$; **** $P<0.01$; ***** $P<0.005$; ****** $P<0.001$

S-m, medial septum; S-l, lateral septum; DBB, diagonal band of Broca; POA-m, medial preoptic area; POA-l, lateral preoptic area; SCN, suprachiasmatic nucleus; SON, supraoptic nucleus; AHA, anterior hypothalamic area; FX, fornix; PVN, paraventricular nucleus

Table 6-1 (cont). The relative metabolic activity of selected brain areas and the pituitary gland in anaesthetised rats after electrical stimulation of the preoptic area (POA), short-term ovariectomy (OVX) with treatment with oestradiol benzoate (OB) or oil, and combined treatments.

Values are expressed as a ratio (mean ± S.E.M.) of [^{14}C] concentration of the selected region to [^{14}C] concentration of the corpus callosum.

	Type and number of animal					
	POA control, unstimulated (n=4)	POA stimulated (n=4)	OVX+OB-POA unstimulated (n=4)	OVX+OB-POA stimulated (n=4)	OVX+oil-POA unstimulated (n=4)	OVX+oil-POA stimulated (n=4)
MFB	1.29±0.02	3.55±0.41******	1.34±0.04	3.77±0.23******	1.32±0.01	4.18±0.28******
ST	1.10±0.03	4.20±0.25******	1.14±0.03	3.63±0.64******	1.11±0.02	4.14±0.30******
ARC	1.17±0.05	2.65±0.40*****	1.29±0.05	2.48±0.48****	1.30±0.02	2.90±0.34******
VMH	1.19±0.03	3.82±0.73******	1.31±0.06	3.14±0.57*****	1.32±0.01	3.61±0.58*****
DMH	1.28±0.06	4.39±0.67******	1.33±0.03	3.49±0.48******	1.31±0.02	4.42±0.49******
SM	1.28±0.01	2.46±0.27******	1.36±0.05	2.22±0.17******	1.35±0.02	2.24±0.19******
AMYG CO+M	1.17±0.02	3.39±0.53******	1.23±0.02	3.31±0.66******	1.25±0.02	3.41±0.26******
AMYG CE+L	1.17±0.02	1.68±0.16*	1.25±0.02	2.36±0.33******	1.27±0.01	2.03±0.13*****
IC	0.98±0.04	1.04±0.03	1.02±0.01	1.04±0.02	1.04±0.02	1.03±0.03
MM	1.69±0.05	3.19±0.81	1.66±0.06	2.72±0.49	1.71±0.05	2.87±0.57

Significantly different from corresponding control group: * P<0.05; ** P<0.025; *** P<0.02; **** P<0.01; ***** P<0.005; ****** P<0.001

MFB, medial forebrain bundle; ST, stria terminalis; ARC, arcuate nucleus; VMH, ventromedial hypothalamic nucleus, DMH, dorsomedial hypothalamic nucleus; SM, stria medullaris; AMYG CO+M, cortical and medial amygdaloid nuclei; AMYG CE+L, central and lateral amygdaloid nuclei; IC, internal capsule; MM, mamillary body

Table 6-1 (cont). The relative metabolic activity of selected brain areas and the pituitary gland in anaesthetised rats after electrical stimulation of the preoptic area (POA), short-term ovariectomy (OVX) with treatment with oestradiol benzoate (OB) or oil, and combined treatments.

Values are expressed as a ratio (mean ± S.E.M.) of [^{14}C] concentration of the selected region to [^{14}C] concentration of the corpus callosum.

	Type and number of animal					
	POA control, unstimulated (n=4)	POA stimulated (n=4)	OVX+OB-POA unstimulated (n=4)	OVX+OB-POA stimulated (n=4)	OVX+oil-POA unstimulated (n=4)	OVX+oil-POA stimulated (n=4)
IP	2.13±0.06	2.61±0.27	2.29±0.12	2.58±0.28	2.11±0.03	2.36±0.18
PN	1.33±0.01	1.80±0.15*****	1.33±0.02	1.66±0.12*	1.32±0.04	1.60±0.12
PD	2.05±0.23	1.60±0.18	2.33±0.15	2.23±0.25	1.46±0.09	1.46±0.05
DHIPPM	1.43±0.02	1.38±0.05	1.51±0.03	1.40±0.07	1.51±0.01	1.31±0.02*****
DHIPPG	1.24±0.02	1.23±0.05	1.29±0.02	1.28±0.05	1.30±0.01	1.24±0.04
VHIPPM	1.49±0.07	1.59±0.16	1.56±0.04	1.64±0.05	1.58±0.01	1.58±0.04
VHIPPG	1.26±0.03	1.33±0.09	1.32±0.02	1.34±0.03	1.28±0.02	1.35±0.01
HAB-m	1.95±0.06	2.32±0.15	2.21±0.11	2.17±0.23	2.19±0.09	2.12±0.13
HAB-l	1.77±0.03	3.91±0.36******	1.90±0.09	3.24±0.37******	1.88±0.03	3.64±0.26******
PYRCX	1.18±0.01	2.81±0.50*	1.23±0.01	3.47±0.58******	1.23±0.01	3.53±0.41******

Significantly different from corresponding control group: * $P<0.05$; ** $P<0.025$; *** $P<0.02$; **** $P<0.01$; ***** $P<0.005$; ****** $P<0.001$

IP, interpeduncular nucleus; PN, pars nervosa; PD, pars distalis; DHIPPM, dorsal hippocampus (molecular layer); DHIPPG, dorsal hippocampus (granular layer); VHIPPM, ventral hippocampus (molecular layer);
VHIPPG, ventral hippocampus (granular layer); HAB-m, medial habenular nucleus; HAB-l, lateral habenular nucleus; PYRCX, pyriform cortex

Table 6-1 (cont). The relative metabolic activity of selected brain areas and the pituitary gland in anaesthetised rats after electrical stimulation of the preoptic area (POA), short-term ovariectomy (OVX) with treatment with oestradiol benzoate (OB) or oil, and combined treatments.

Values are expressed as a ratio (mean ± S.E.M.) of [^{14}C] concentration of the selected region to [^{14}C] concentration of the corpus callosum.

	Type and number of animal					
	POA control, unstimulated (n=4)	POA stimulated (n=4)	OVX+OB-POA unstimulated (n=4)	OVX+OB-POA stimulated (n=4)	OVX+oil-POA unstimulated (n=4)	OVX+oil-POA stimulated (n=4)
CG	1.37±0.04	3.40±0.40	1.44±0.02	2.76±0.36*****	1.44±0.03	3.22±0.39
RF	1.42±0.03	1.65±0.08	1.53±0.02	1.57±0.08	1.54±0.02	1.61±0.07
PVT	1.35±0.02	4.60±0.47******	1.39±0.02	3.41±0.46******	1.47±0.04	4.24±0.49******
AVT	1.49±0.03	2.27±0.06*****	1.50±0.06	1.92±0.30*	1.54±0.02	1.79±0.15
VT	1.75±0.01	1.67±0.03	1.72±0.03	1.75±0.09	1.82±0.07	1.79±0.09
LGEN	1.48±0.05	2.07±0.18******	1.55±0.03	1.63±0.08	1.44±0.02	1.71±0.11
MGEN	1.64±0.01	1.56±0.04	1.73±0.05	1.54±0.06****	1.64±0.03	1.51±0.04*

Significantly different from corresponding control group: * P<0.05; ** P<0.025; *** P<0.02; **** P<0.01; ***** P<0.005; ****** P<0.001

CG, central grey; RF, reticular formation; PVT, paraventricular thalamic area; AVT, anteroventral thalamic area; VT, ventral thalamic area; LGEN, lateral geniculate bodies; MGEN, medial geniculate bodies

Table 6-1 (cont). Significance of differences of relative metabolic activity of brain and pituitary gland regions in Table 6-1 (Duncan's multiple range test):

$P < 0.05$

Region	Comparisons
SCN	POA stimulated v POA unstimulated, OVX+OB-POA unstimulated, OVX+oil-POA unstimulated
	OVX+OB-POA stimulated v POA unstimulated, OVX+OB-POA unstimulated, OVX+oil-POA unstimulated
	OVX+oil-POA stimulated v OVX+OB-POA unstimulated
MFB	POA stimulated v OVX+oil-POA stimulated
AMYG CE+L	POA stimulated v POA unstimulated, OVX+oil-POA unstimulated
PN	OVX+OB-POA stimulated v POA unstimulated, OVX+OB-POA unstimulated, OVX+oil-POA unstimulated
PD	POA stimulated v OVX+OB-POA stimulated
	POA unstimulated v OVX+oil-POA stimulated, OVX+oil-POA unstimulated
DHIPPM	POA stimulated v OVX+OB-POA unstimulated, OVX+oil-POA unstimulated
HAB-1	POA stimulated v OVX+OB-POA stimulated
PYRCX	POA stimulated v POA unstimulated, OVX+OB-POA unstimulated, OVX+oil-POA unstimulated
PVT	POA stimulated v OVX+OB-POA stimulated
AVT	POA stimulated v OVX+oil-POA stimulated
	OVX+OB-POA stimulated v POA unstimulated, OVX+OB-POA unstimulated
LGEN	POA stimulated v OVX+oil-POA stimulated
MGEN	POA stimulated v OVX+OB-POA unstimulated
	OVX+oil-POA stimulated v POA unstimulated, OVX+oil-POA unstimulated

Table 6-1 (cont). Significance of differences of relative metabolic activity of brain and pituitary gland regions in Table 6-1 (Duncan's multiple range test):

$P < 0.01$

ARC   OVX+OB-POA stimulated v OVX+OB-POA unstimulated

PD   POA stimulated v OVX+OB-POA unstimulated

MGEN   OVX+OB-POA stimulated v OVX+OB-POA unstimulated

$P < 0.005$

S-m   POA stimulated v OVX+oil-POA stimulated

DBB   OVX+oil-POA stimulated v OVX+OB-POA stimulated

SCN   OVX+oil-POA stimulated v POA unstimulated, OVX+oil-POA unstimulated

AHA   POA stimulated v POA unstimulated, OVX+OB-POA unstimulated, OVX+oil-POA unstimulated
OVX+OB-POA stimulated v POA unstimulated, OVX+OB-POA unstimulated OVX+oil-POA unstimulated

FX   POA stimulated v OVX+OB-POA unstimulated

ARC   POA stimulated v POA unstimulated, OVX+OB-POA unstimulated, OVX+oil-POA unstimulated
OVX+OB-POA stimulated v POA unstimulated, OVX+oil-POA unstimulated

VMH   OVX+OB-POA stimulated v POA unstimulated, OVX+OB-POA unstimulated, OVX+oil-POA unstimulated
OVX+oil-POA stimulated v POA unstimulated, OVX+OB-POA unstimulated, OVX+oil-POA unstimulated

AMGY CE+L   POA stimulated v OVX+OB-POA stimulated
OVX+oil-POA stimulated v POA unstimulated, OVX+OB-POA unstimulated, OVX+oil-POA unstimulated

PN   POA stimulated v POA unstimulated, OVX+OB-POA unstimulated, OVX+oil-POA unstimulated

Table 6-1 (cont). Significance of differences of relative metabolic activity of brain and pituitary gland regions in Table 6-1 (Duncan's multiple range test):

PD
OVX+OB-POA stimulated v OVX+oil-POA stimulated,
OVX+oil-POA unstimulated
OVX+OB-POA unstimulated v OVX+oil-POA stimulated,
OVX+oil-POA unstimulated

DHIPPM
OVX+oil-POA stimulated v OVX+OB-POA unstimulated,
OVX+oil-POA unstimulated

CG
OVX+OB-POA stimulated v OVX+OB-POA unstimulated,
OVX+oil-POA unstimulated

AVT
POA stimulated v POA unstimulated, OVX+OB-POA unstimulated,
OVX+oil-POA unstimulated

LGEN
POA stimulated v OVX+OB-POA stimulated

MGEN
OVX+oil-POA stimulated v OVX+OB-POA unstimulated

$P < 0.001$

S-m
POA stimulated v POA unstimulated, OVX+OB-POA unstimulated,
OVX+oil-POA unstimulated
OVX+OB-POA stimulated v OVX+oil-POA stimulated, POA unstimulated
OVX+OB-POA unstimulated,
OVX+oil-POA unstimulated
OVX+oil-POA stimulated v OVX+OB-POA stimulated, POA unstimulated,
OVX+OB-POA unstimulated,
OVX+oil-POA unstimulated

S-1
POA stimulated v POA unstimulated, OVX+OB-POA unstimulated,
OVX+oil-POA unstimulated
OVX+OB-POA stimulated v POA unstimulated, OVX+OB-POA unstimulated,
OVX+oil-POA unstimulated
OVX+oil-POA stimulated v POA unstimulated, OVX+OB-POA unstimulated,
OVX+oil-POA unstimulated

DBB
POA stimulated v OVX+oil-POA stimulated, POA unstimulated,
OVX+OB-POA unstimulated, OVX+oil-POA unstimulated
OVX+OB-POA stimulated v POA unstimulated, OVX+OB-POA unstimulated
OVX+oil-POA unstimulated
OVX+oil-POA stimulated v POA stimulated, POA unstimulated
OVX+OB-POA unstimulated,
OVX+oil-POA unstimulated

Table 6-1 (cont). Significance of differences of relative metabolic activity of brain and pituitary gland regions in Table 6-1 (Duncan's multiple range test):

POA-m

POA stimulated v POA unstimulated, OVX+OB-POA unstimulated, OVX+oil-POA unstimulated
OVX+OB-POA stimulated v POA unstimulated, OVX+OB-POA unstimulated, OVX+oil-POA unstimulated
OVX+oil-POA stimulated v POA unstimulated, OVX+OB-POA unstimulated, OVX+oil-POA unstimulated

POA-l

POA stimulated v POA unstimulated, OVX+OB-POA unstimulated, OVX+oil-POA unstimulated
OVX+OB-POA stimulated v POA unstimulated, OVX+OB-POA unstimulated, OVX+oil-POA unstimulated
OVX+oil-POA stimulated v POA unstimulated, OVX+OB-POA unstimulated, OVX+oil-POA unstimulated

SON

POA stimulated v OVX+oil-POA stimulated, POA unstimulated, OVX+OB-POA unstimulated, OVX+oil-POA unstimulated
OVX+OB-POA stimulated v OVX+oil-POA stimulated, POA unstimulated, OVX+OB-POA unstimulated, OVX+oil-POA unstimulated
OVX+oil-POA stimulated v POA stimulated, OVX+OB-POA stimulated, POA unstimulated, OVX+OB-POA unstimulated, OVX+oil-POA unstimulated

AHA

OVX+oil-POA stimulated v POA unstimulated, OVX+OB-POA unstimulated, OVX+oil-POA unstimulated

FX

POA stimulated v POA unstimulated, OVX+oil-POA unstimulated
OVX+OB-POA stimulated v POA unstimulated, OVX+OB-POA unstimulated, OVX+oil-POA unstimulated
OVX+oil-POA stimulated v POA unstimulated, OVX+OB-POA unstimulated, OVX+oil-POA unstimulated

PVN

POA stimulated v POA unstimulated, OVX+OB-POA unstimulated OVX+oil-POA unstimulated
OVX+OB-POA stimulated v POA unstimulated, OVX+OB-POA unstimulated, OVX+oil-POA unstimulated
OVX+oil-POA stimulated v POA unstimulated, OVX+OB-POA unstimulated, OVX+oil-POA unstimulated

MFB

POA stimulated v POA unstimulated, OVX+OB-POA unstimulated OVX+oil-POA unstimulated
OVX+OB-POA stimulated v POA unstimulated, OVX+OB-POA unstimulated, OVX+oil-POA unstimulated
OVX+oil-POA stimulated v POA unstimulated, OVX+OB-POA unstimulated, OVX+oil-POA unstimulated

Table 6-1 (cont). Significance of differences of relative metabolic activity of brain and pituitary gland regions in Table 6-1 (Duncan's multiple range test):

ST
POA stimulated v POA unstimulated, OVX+OB-POA unstimulated OVX+oil-POA unstimulated
OVX+OB-POA stimulated v POA unstimulated, OVX+OB-POA unstimulated, OVX+oil-POA unstimulated
OVX+oil-POA stimulated v POA unstimulated, OVX+OB-POA unstimulated, OVX+oil-POA unstimulated

ARC
OVX+oil-POA stimulated v POA unstimulated, OVX+OB-POA unstimulated, OVX+oil-POA unstimulated

VMH
POA stimulated v POA unstimulated, OVX+OB-POA unstimulated, OVX+oil-POA unstimulated

DMH
POA stimulated v POA unstimulated, OVX+OB-POA unstimulated OVX+oil-POA unstimulated
OVX+OB-POA stimulated v POA unstimulated, OVX+OB-POA unstimulated, OVX+oil-POA unstimulated
OVX+oil-POA stimulated v POA unstimulated, OVX+OB-POA unstimulated, OVX+oil-POA unstimulated

SM
POA stimulated v POA unstimulated, OVX+OB-POA unstimulated OVX+oil-POA unstimulated
OVX+OB-POA stimulated v POA unstimulated, OVX+OB-POA unstimulated, OVX+oil-POA unstimulated
OVX+oil-POA stimulated v POA unstimulated, OVX+OB-POA unstimulated, OVX+oil-POA unstimulated.

AMYG CO+M
POA stimulated v POA unstimulated, OVX+OB-POA unstimulated OVX+oil-POA unstimulated
OVX+OB-POA stimulated v POA unstimulated, OVX+OB-POA unstimulated, OVX+oil-POA unstimulated
OVX+oil-POA stimulated v POA unstimulated, OVX+OB-POA unstimulated, OVX+oil-POA unstimulated

AMYG CE+L
OVX+OB-POA stimulated v POA unstimulated, OVX+OB-POA unstimulated, OVX+oil-POA unstimulated

HAB-1
POA stimulated v POA unstimulated, OVX+OB-POA unstimulated OVX+oil-POA unstimulated
OVX+OB-POA stimulated v POA unstimulated, OVX+OB-POA unstimulated, OVX+oil-POA unstimulated
OVX+oil-POA stimulated v POA unstimulated, OVX+OB-POA unstimulated, OVX+oil-POA unstimulated

Table 6-1 (cont). Significance of differences of relative metabolic activity of brain and pituitary gland regions in Table 6-1 (Duncan's multiple range test):

PYRCX
OVX+OB-POA stimulated v POA unstimulated, OVX+OB-POA unstimulated, OVX+oil-POA unstimulated
OVX+oil-POA stimulated v POA unstimulated, OVX+OB-POA unstimulated, OVX+oil-POA unstimulated

CG
POA stimulated v POA unstimulated, OVX+OB-POA unstimulated OVX+oil-POA unstimulated
OVX+OB-POA stimulated v POA unstimulated, OVX+OB-POA unstimulated, OVX+oil-POA unstimulated
OVX+oil-POA stimulated v POA unstimulated, OVX+OB-POA unstimulated, OVX+oil-POA unstimulated

PVT
POA stimulated v POA unstimulated, OVX+OB-POA unstimulated OVX+oil-POA unstimulated
OVX+OB-POA stimulated v POA unstimulated, OVX+OB-POA unstimulated, OVX+oil-POA unstimulated
OVX+oil-POA stimulated v POA unstimulated, OVX+OB-POA unstimulated, OVX+oil-POA unstimulated

LGEN
POA stimulated v POA unstimulated, OVX+OB-POA unstimulated, OVX+oil-POA unstimulated

Table 6-2. Plasma luteinising hormone concentrations (ng/ml) in anaesthetised animals. Electrodes were implanted in the preoptic area (POA), and after 2 consecutive 4-day oestrous cycles animals were ovariectomised on the day of dioestrus and treated with 10 μg oestradiol benzoate or 0.2 ml arachis oil administered s.c. 24 h later the animal was electrically stimulated for 45 min with trains (30 sec on, 30 sec off) of accurately balanced biphasic square wave pulses with a frequency of 50 Hz, pulse duration of 1 msec and pulse amplitude of 500 μA (1 mA peak to peak). The blood samples (0.4 ml) were collected from the external jugular vein before stimulation started and again after 15 min and 45 min of stimulation . For statistical analysis samples below the lower limit of detection of the assay were attributed the lowest detectable value (2.5 ng/ml for a 20 μl sample). Mean ± S.E.M. are also shown.

	Animals	1st sample	2nd sample	3 sample
POA unstimulated	1	< 2.5	< 2.5	< 2.5
	2	< 2.5	< 2.5	< 2.5
	3	< 2.5	< 2.5	< 2.5
	4	< 2.5	< 2.5	< 2.5
		< 2.5	< 2.5	< 2.5
POA stimulated	1	< 2.5	< 2.5	3.4
	2	< 2.5	6.2	7.0
	3	< 2.5	4.2	21.6
	4	< 2.5	3.1	5.3
		< 2.5	4.0	9.3±4.2
OVX+OB-POA unstimulated	1	< 2.5	< 2.5	< 2.5
	2	< 2.5	< 2.5	< 2.5
	3	< 2.5	< 2.5	< 2.5
	4	< 2.5	< 2.5	< 2.5
		< 2.5	< 2.5	< 2.5
OVX+OB-POA stimulated	1	< 2.5	10.2	9.4
	2	< 2.5	10.3	8.4
	3	< 2.5	9.6	11.2
	4	< 2.5	5.4	5.9
		< 2.5	8.9±1.2	8.7±1.1
OVX+oil-POA unstimulated	1	< 2.5	< 2.5	< 2.5
	2	< 2.5	< 2.5	< 2.5
	3	< 2.5	< 2.5	< 2.5
	4	< 2.5	< 2.5	< 2.5
		< 2.5	< 2.5	< 2.5
OVX+oil-POA stimulated	1	< 2.5	6.7	5.4
	2	8.8	4.9	6.0
	3	< 2.5	5.6	3.4
	4	< 2.5	3.5	< 2.5
			5.2±0.7	4.3

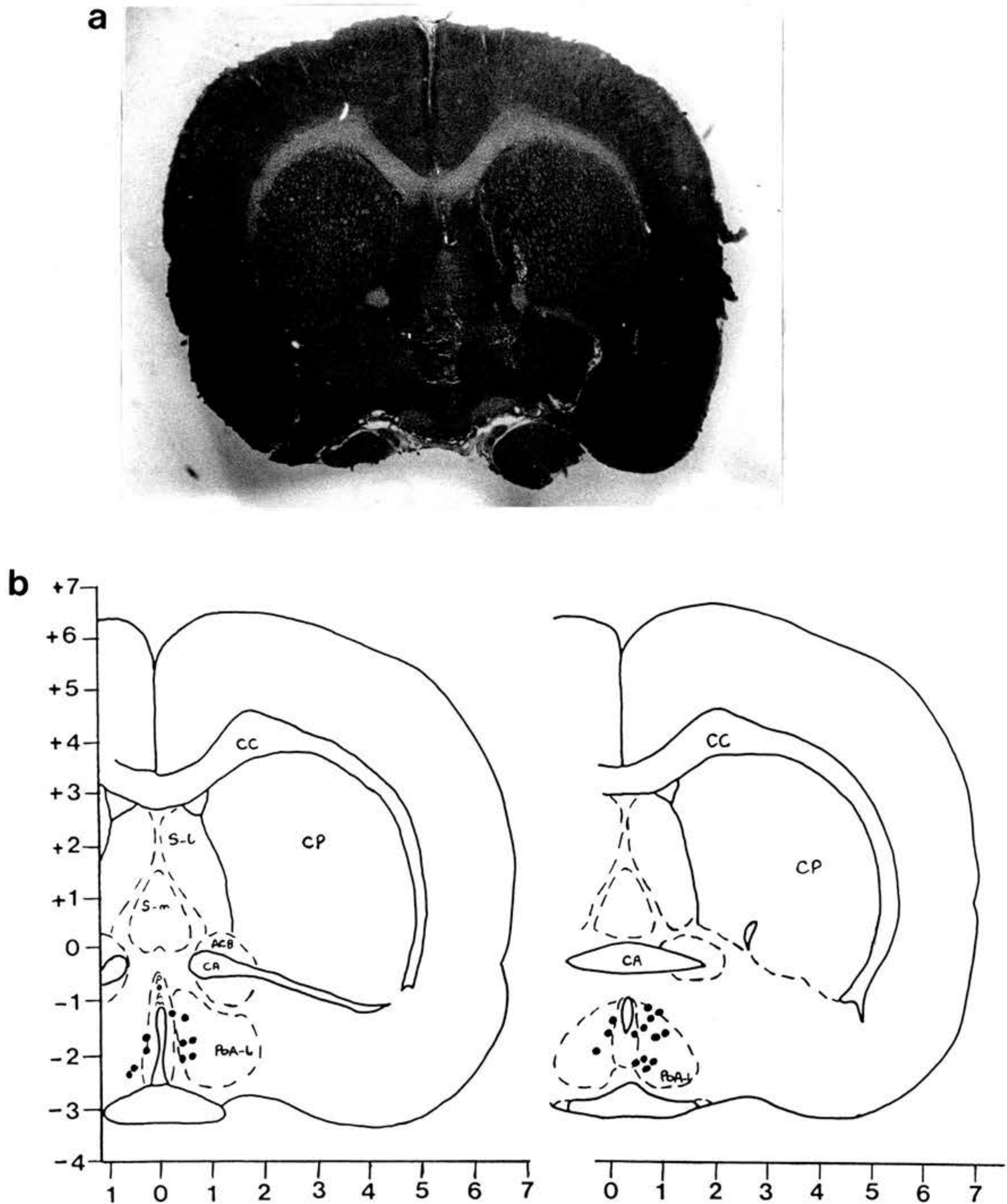
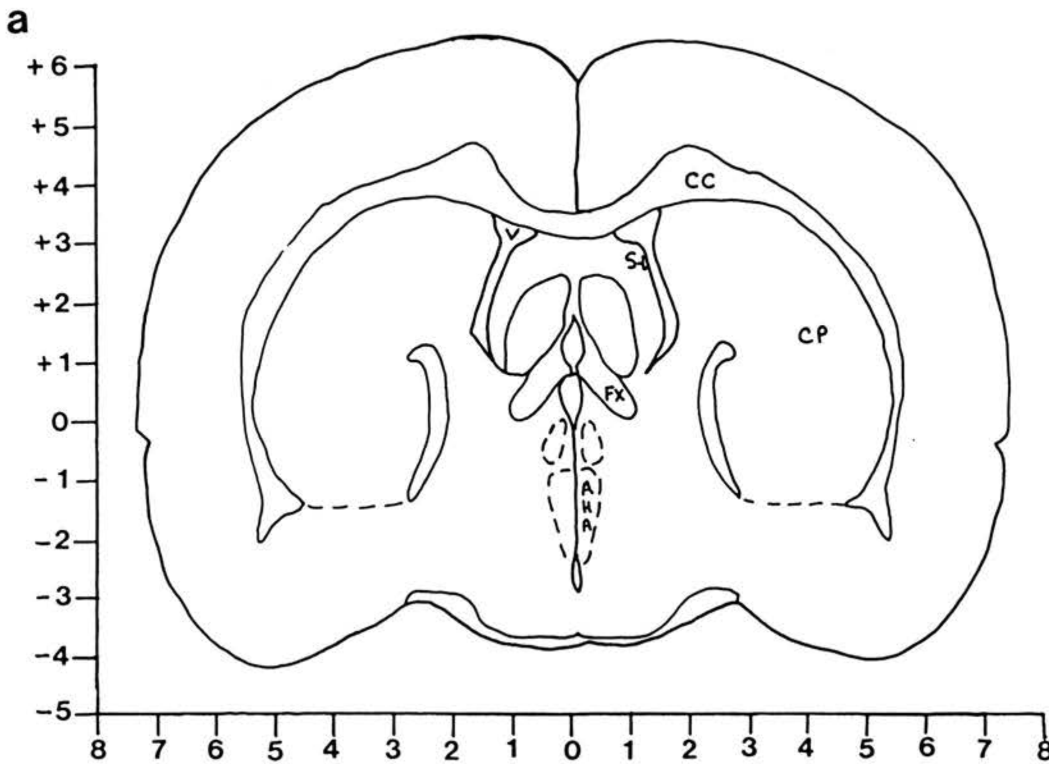


Fig. 6-A: (a) representative low power photomicrograph of a coronal section of the brain showing the position of the electrode tips in the preoptic area; (b) schematic diagram of coronal sections of the brain at A 7.8 and 7.6 (Pellegrino et al., 1967) showing the anterior-posterior and vertical positions of all the electrodes. Abbreviations: ACB, nucleus accumbens; CA, anterior commissure; CC, corpus callosum; CP, caudate putamen; POA-l, lateral preoptic area; POA-m, medial preoptic area; S-l, lateral septum, S-m, medial septum.

Fig. 6-B: (a) schematic diagram of a coronal section of the brain at A 6.8 (Pellegrino et al., 1967); autoradiographs of coronal sections of the brain at the same level in rats injected with [^{14}C]2-deoxyglucose: control, unstimulated (b) and preoptic area-stimulated (c) animals. Abbreviations: AHA, anterior hypothalamic area; CC, corpus callosum; CP, caudate putamen; FX, fornix; S-1, lateral septum; V, ventricle.



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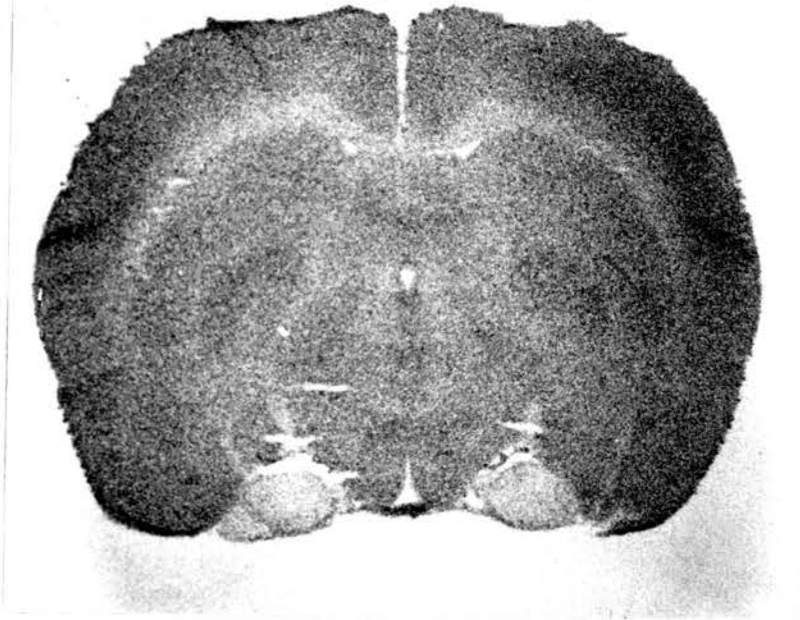

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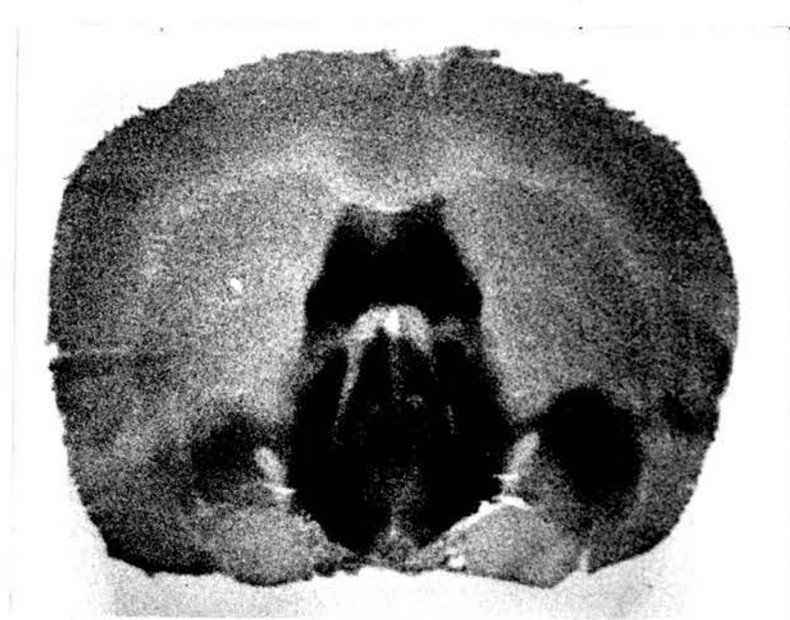
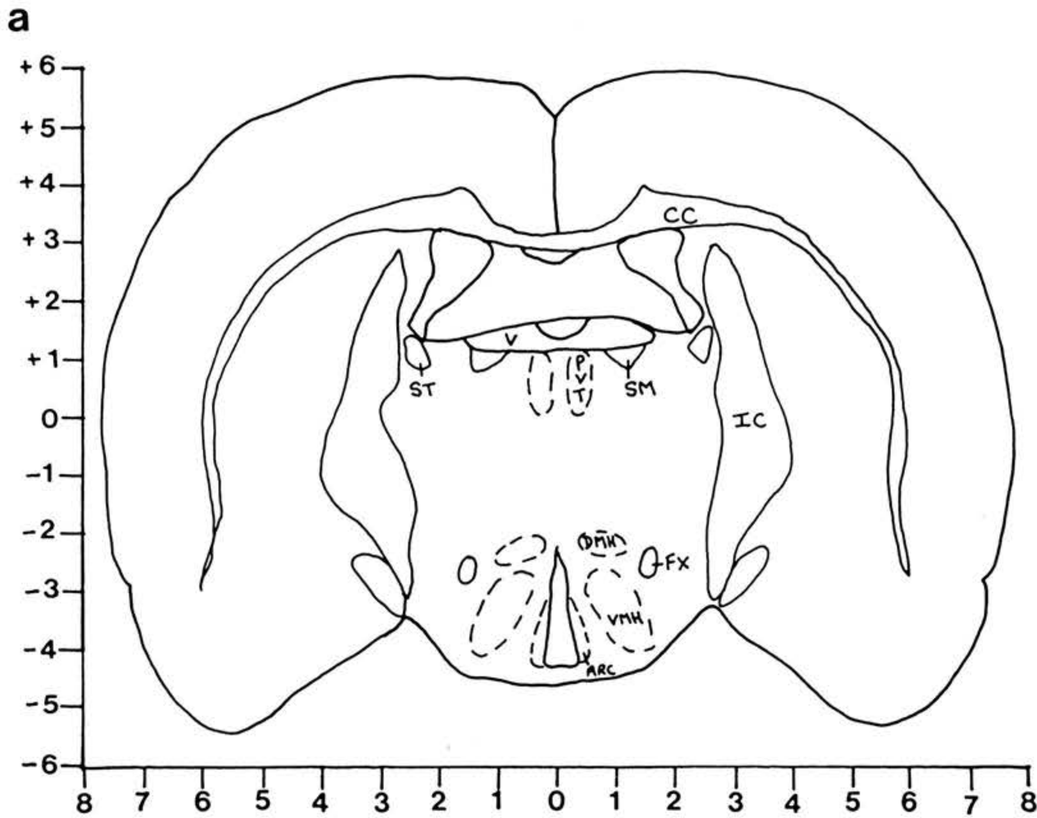
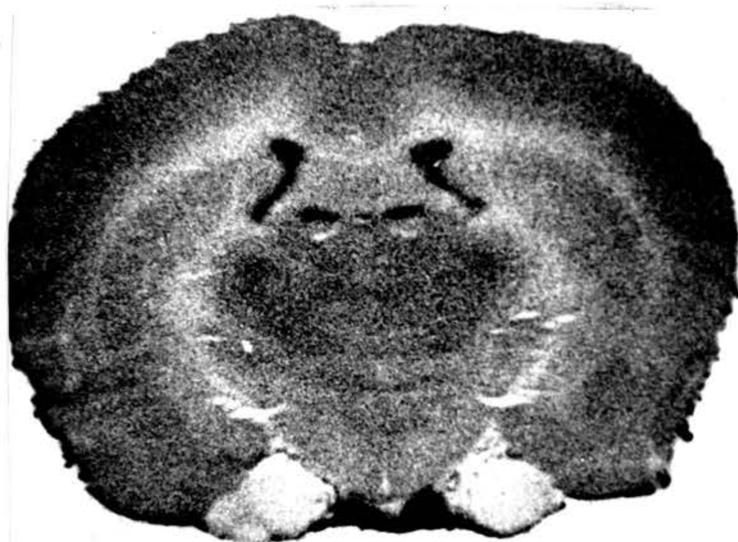


Fig. 6-C: (a) schematic diagram of a coronal section of the brain at A 5.8 (Pellegrino et al., 1967); autoradiographs of coronal sections of the brain at the same level in rats injected with [^{14}C]2-deoxyglucose: control, unstimulated (b) and preoptic area-stimulated (c) animals. Abbreviations: ARC, arcuate nucleus; CC, corpus callosum, DMH, dorsomedial hypothalamic nucleus; FX, fornix; IC, internal capsule; PVT, paraventricular thalamic area; SM, stria medullaris; ST, stria terminalis; V, ventricle; VMH, ventromedial hypothalamic nucleus.

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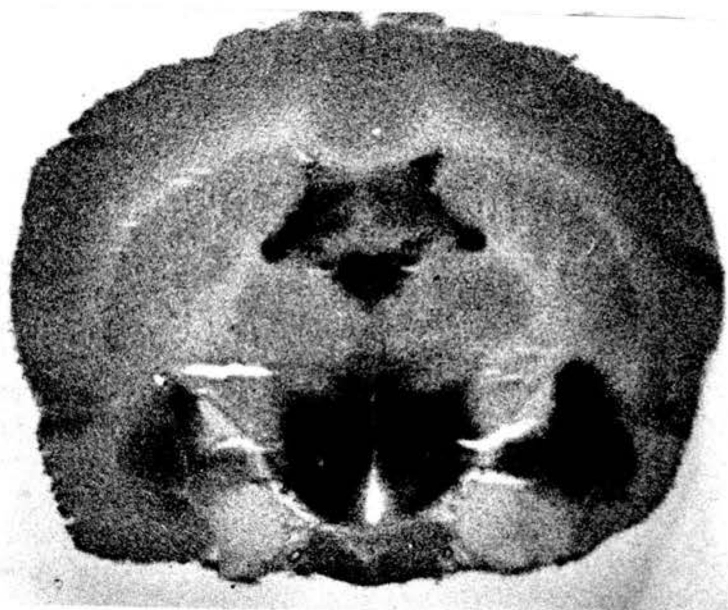

c

increase in the plasma concentration of LH in all but one animal (#2 in OVX+oil-POA stimulated). In animals treated with OB the increases in plasma LH were more consistent, so that at 15 and 45 min the increases were significant ($P < 0.02$). The plasma LH concentrations after 15 min of electrical stimulation in OVX+OB-POA stimulated were significantly greater than values at the same time in POA stimulated ($P < 0.05$) and OVX+oil-POA stimulated animals ($P < 0.02$). The plasma LH concentrations after 45 min of electrical stimulation in OVX+OB-POA stimulated animals were also significantly greater than the values at the same time in OVX+oil-POA stimulated animals ($P < 0.02$).

6.3.1.8 Comparison of uterine weight and appearance in short-term ovariectomised rats treated with oestradiol benzoate or oil

In short-term ovariectomised rats treated with OB the uteri were filled with fluid. In these animals the uterine wet weight (539 ± 41mg, mean ± S.E.M., n = 8) was significantly ($P < 0.005$ unpaired t-test) greater than in short-term ovariectomised rats treated with oil (360 ± 28 mg, n=8). The uterine 'dry' weight was not significantly different in short-term ovariectomised rats treated with OB (411 ± 32mg, mean ± S.E.M., n = 8) compared with that in short-term ovariectomised rats treated with oil (331 ± 27mg, n = 8).

6.3.2 Electrical stimulation of the suprachiasmatic nucleus, the anterior hypothalamic area and the paraventricular nucleus

6.3.2.1 Stimulation of the suprachiasmatic nucleus

A representative photomicrograph showing the position of the tips of the electrode in the SCN is shown in Fig. 6-D (a). The

anterior-posterior and vertical position of the electrode tips in all the animals in this study are shown in Fig. 6-D (b).

The rma of the following areas of brain and pituitary gland increased in SCN stimulated animals compared with combined, control unstimulated animals: S-m, S-l, DBB, POA-m, POA-l, SCN, SON, AHA, FX, PVN, MFB, ST, ARC, VMH, DMH, SM, AMYG CO+M, AMYG CE+L, PN, HAB-l, CG, RF, PVT and LGEN (Table 6-3). The increased rma of some of the brain areas listed above can easily be seen in the autoradiograph shown in Fig. 6-E.

The plasma LH concentrations increased significantly in the SCN stimulated animals from a value below the lower limit of detection of the assay (< 2.5 ng/ml, n=4) to 4.5 ± 0.2 ng/ml (mean ± S.E.M., P < 0.005, paired t-test) after 15 min of electrical stimulation, and to 5.0 ± 0.7 ng/ml (mean ± S.E.M., P < 0.005, paired t-test) after 45 min of electrical stimulation. There was no significant change in plasma LH concentration in the combined control unstimulated animals which remained undetectable throughout the experiment.

6.3.2.2 <u>Stimulation of the anterior hypothalamic area</u>

A representative photomicrograph showing the position of the electrode tips in the AHA is shown in Fig. 6-F (a), and the anterior- posterior and vertical positions of the electrode tips in all the animals are shown in Fig. 6-F (b).

Table 6-3 shows that the same areas of brain and pituitary gland increased in rma in AHA stimulated animals compared with combined control unstimulated animals, as in SCN stimulated animals. In addition, the rma of MM, IP, DHIPPM, VHIPPM, VHIPPG,

HAB-m, PYRCX and AVT also increased in AHA stimulated animals compared with unstimulated control animals (Table 6-3). Some of the areas showing increased rma can be seen in Figs. 6-G and 6-H.

The plasma LH concentrations remained below the lower limit of detection of the assay (< 2.5 ng/ml, n=4) in all AHA stimulated animals.

6.3.2.3 Stimulation of the paraventricular nucleus

Figure 6-I (a) shows a representative photomicrograph showing the position of the electrode tips which straddled the PVN. The anterior-posterior and vertical positions of the electrode tips in all the animals are shown in Fig. 6-I (b).

In the PVN stimulated animals the rma of the same brain areas increased above unstimulated control values as in AHA stimulated animals, apart from VHIPPG, HAB-m and LGEN (Table 6-3). The rma of the PD, DHIPPG and VT also increased in PVN stimulated animals compared with unstimulated control animals (Table 6-3).

The plasma LH concentrations remained below the lower limit of detection of the assay (< 2.5 ng/ml, n=4) in all PVN stimulated animals.

6.3.3 Electrical stimulation of the median eminence

Table 6-4 shows that the rma of the POA-m, SON, PVN, ST, ARC, VMH and PN increased in ME stimulated animals compared with ME unstimulated animals. The increased rma of some of these areas are shown in Fig. 6-J.

Plasma LH concentrations remained below the lower limit of detection of the assay (< 2.5 ng/ml) in all ME unstimulated animals (n=4) and in ME stimulated animals (n=4), apart from 1 animal in

Table 6-3. The relative metabolic activity of selected brain areas and the pituitary gland in anaesthetised rats after electrical stimulation of the suprachiasmatic nucleus (SCN), anterior hypothalamic area (AHA), and the paraventricular nucleus (PVN).

Values are expressed as ratio (mean ± S.E.M.) of [^{14}C] concentration of selected region to [^{14}C] concentration of the corpus callosum.

	Type and number of animal			
	Combined controls, unstimulated (n=6)	SCN stimulated (n=4)	AHA stimulated (n=4)	PVN stimulated (n=5)
S-m	1.31±0.04	3.70±0.64*****	3.74±0.15******	2.80±0.22******
S-1	1.22±0.02	3.61±0.31******	5.24±0.15******	5.24±0.38******
DBB	1.33±0.04	3.90±0.62******	3.26±0.30******	2.23±0.17******
POA-m	1.23±0.02	4.56±0.58******	4.85±0.39******	4.75±0.54******
POA-1	1.20±0.03	4.70±0.60******	5.64±0.29******	5.83±0.46******
SCN	1.89±0.08	5.17±0.47******	4.75±0.53******	3.19±0.26******
SON	1.05±0.04	2.66±0.34******	1.91±0.20******	1.70±0.03******
AHA	1.28±0.04	4.03±0.28******	5.50±0.16******	5.75±0.49******
FX	1.08±0.03	1.73±0.08******	2.43±0.21******	2.38±0.20******
PVN	1.29±0.02	4.16±0.32******	3.58±0.15******	3.87±0.49******

Significantly different from control group; * $P < 0.05$; ** $P < 0.025$; *** $P < 0.02$; **** $P < 0.01$; ***** $P < 0.005$; ****** $P < 0.001$.

S-m, medial septum; S-1, lateral septum; DBB, diagonal band of Broca; POA-m, medial preoptic area; POA-1, lateral preoptic area; SCN, suprachiasmatic nucleus; SON, supraoptic nuclei; AHA, anterior hypothalamic area; FX, fornix; PVN, paraventricular nucleus

Table 6-3 (cont). The relative metabolic activity of selected brain areas and the pituitary gland in anaesthetised rats after electrical stimulation of the suprachiasmatic nucleus (SCN), anterior hypothalamic area (AHA), and the paraventricular nucleus (PVN).

Values are expressed as ratio (mean ± S.E.M.) of [^{14}C] concentration of selected region to [^{14}C] concentration of the corpus callosum.

	Type and number of animal			
	Combined controls, unstimulated (n=6)	SCN stimulated (n=4)	AHA stimulated (n=4)	PVN stimulated (n=5)
MFB	1.25±0.02	3.76±0.46******	3.23±0.22******	3.27±0.18******
ST	1.07±0.02	2.00±0.08******	3.85±0.24******	5.22±0.39******
ARC	1.27±0.02	2.55±0.10******	3.03±0.41******	3.32±0.38******
VMH	1.19±0.03	2.58±0.34******	4.85±0.15******	5.67±0.52******
DMH	1.27±0.02	4.32±0.53******	4.63±0.33******	3.89±0.46******
SM	1.27±0.02	1.83±0.26*	2.12±0.14******	2.26±0.12******
AMYG CO+M	1.15±0.02	2.11±0.15******	4.01±0.52******	4.53±0.35******
AMYG CE+L	1.17±0.02	1.74±0.18*****	1.76±0.15*****	1.71±0.10******
IC	0.95±0.02	0.91±0.04	0.99±0.02	0.95±0.03
MM	1.44±0.03	1.77±0.21	4.67±0.25******	5.04±0.45******

Significantly different from control group; * $P < 0.05$; ** $P < 0.025$; *** $P < 0.02$; **** $P < 0.01$; ***** $P < 0.005$; ****** $P < 0.001$.

MFB, medial forebrain bundle; ST, stria terminalis; ARC, arcuate nucleus; VMH, ventromedial hypothalamic nucleus; DMH, dorsomedial hypothalamic nucleus; SM, stria medullaris; AMYG CO+M, cortical and medial amygdaloid nuclei; AMYG CE+L, central and lateral amygdaloid nuclei; IC, internal capsule; MM, mamillary body

Table 6-3 (cont). The relative metabolic activity of selected brain areas and the pituitary gland in anaesthetised rats after electrical stimulation of the suprachiasmatic nucleus (SCN), anterior hypothalamic area (AHA), and the paraventricular nucleus (PVN).

Values are expressed as ratio (mean ± S.E.M.) of [^{14}C] concentration of selected region to [^{14}C] concentration of the corpus callosum.

	Type and number of animal			
	Combined controls, unstimulated (n=6)	SCN stimulated (n=4)	AHA stimulated (n=4)	PVN stimulated (n=5)
IP	1.99±0.05	2.04±0.13	2.51±0.06******	2.63±0.25**
PN	1.26±0.05	1.81±0.17****	2.54±0.18******	2.46±0.22******
PD	1.22±0.05	1.50±0.15	1.44±0.12	1.88±0.26*
DHIPPM	1.37±0.02	1.35±0.12	1.29±0.03*	1.46±0.03*
DHIPPG	1.15±0.02	1.21±0.11	1.20±0.01	1.28±0.02*****
VHIPPM	1.39±0.02	1.43±0.10	1.46±0.01**	1.71±0.06******
VHIPPG	1.19±0.01	1.28±0.11	1.24±0.01*	1.32±0.06
HAB-m	1.85±0.06	2.04±0.40	2.12±0.09*	2.15±0.20
HAB-1	1.59±0.04	2.61±0.38***	3.50±0.13******	3.61±0.27******
PYRCX	1.13±0.02	1.53±0.34	2.79±0.24******	4.00±0.36******

Significantly different from control group; * $P < 0.05$; ** $P < 0.025$; *** $P < 0.02$; **** $P < 0.01$; ***** $P < 0.005$; ****** $P < 0.001$.

IP, interpeduncular nucleus; PN, pars nervosa; PD, pars distalis; DHIPPM, dorsal hippocampus (molecular layer); DHIPPG, dorsal hippocampus (granular layer);
VHIPPM, ventral hippocampus (molecular layer); VHIPPG, ventral hippocampus (granular layer); HAB-m, medial habenular nucleus; HAB-1, lateral habenular nucleus; PRYCX, pyriform cortex

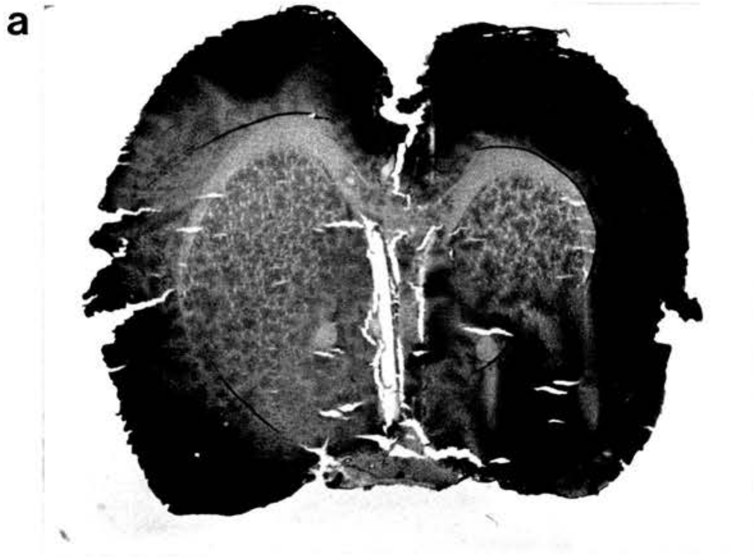
Table 6-3 (cont). The relative metabolic activity of selected brain areas and the pituitary gland in anaesthetised rats after electrical stimulation of the suprachiasmatic nucleus (SCN), anterior hypothalamic area (AHA), and the paraventricular nucleus (PVN).

Values are expressed as ratio (mean ± S.E.M.) of [^{14}C] concentration of selected region to [^{14}C] concentration of the corpus callosum.

	Type and number of animal			
	Combined controls, unstimulated (n=6)	SCN stimulated (n=4)	AHA stimulated (n=4)	PVN stimulated (n=5)
CG	1.33±0.02	2.67±0.19******	4.68±0.29******	3.27±0.35******
RF	1.38±0.02	1.51±0.06*	1.85±0.10******	1.59±0.05*****
PVT	1.31±0.02	3.40±0.25******	5.12±0.15******	3.95±0.42******
VT	1.60±0.01	1.60±0.12	1.63±0.04	1.98±0.19*
AVT	1.43±0.02		2.15±0.10*****	2.47±0.36*
LGEN	1.37±0.02	2.70±0.32******	2.35±0.20******	
MGEN	1.49±0.02	1.50±0.06	1.41±0.04	
CS	1.50±0.03	1.53±0.06	1.64±0.06	

Significantly different from control group; * $P < 0.05$; ** $P < 0.025$; *** $P < 0.02$; **** $P < 0.01$; ***** $P < 0.005$; ****** $P < 0.001$.

CG, central grey; RF, reticular formation; PVT, paraventricular thalamic area; VT, ventral thalamic area; AVT, anteroventral thalamic area; LGEN, lateral geniculate bodies; MGEN, medial geniculate bodies; CS, superior colliculus



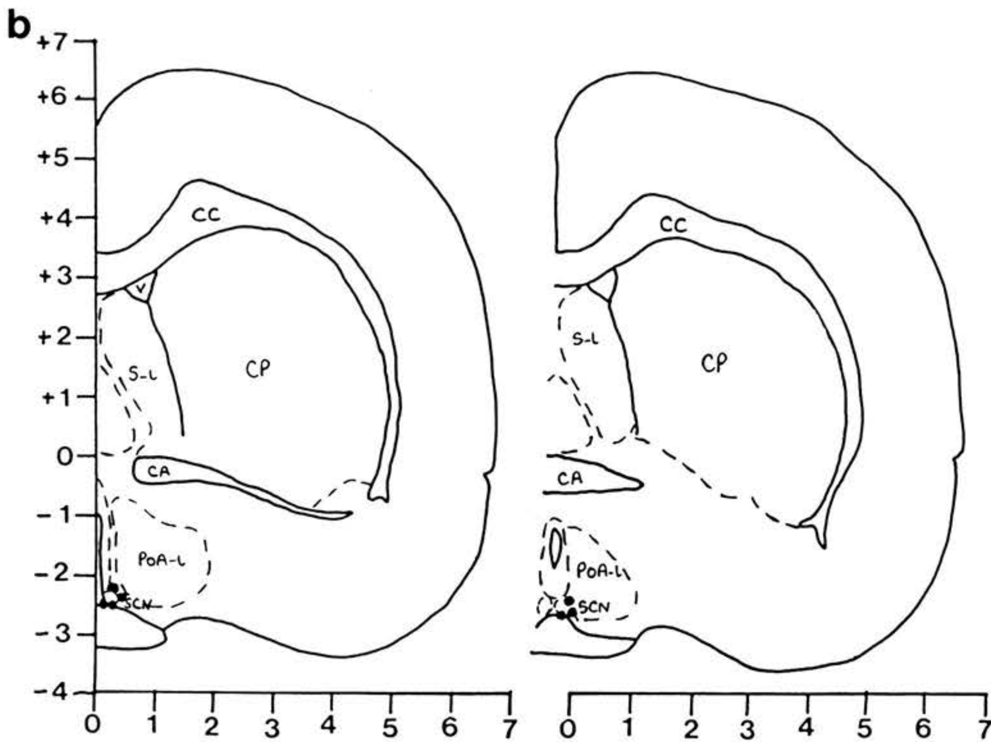
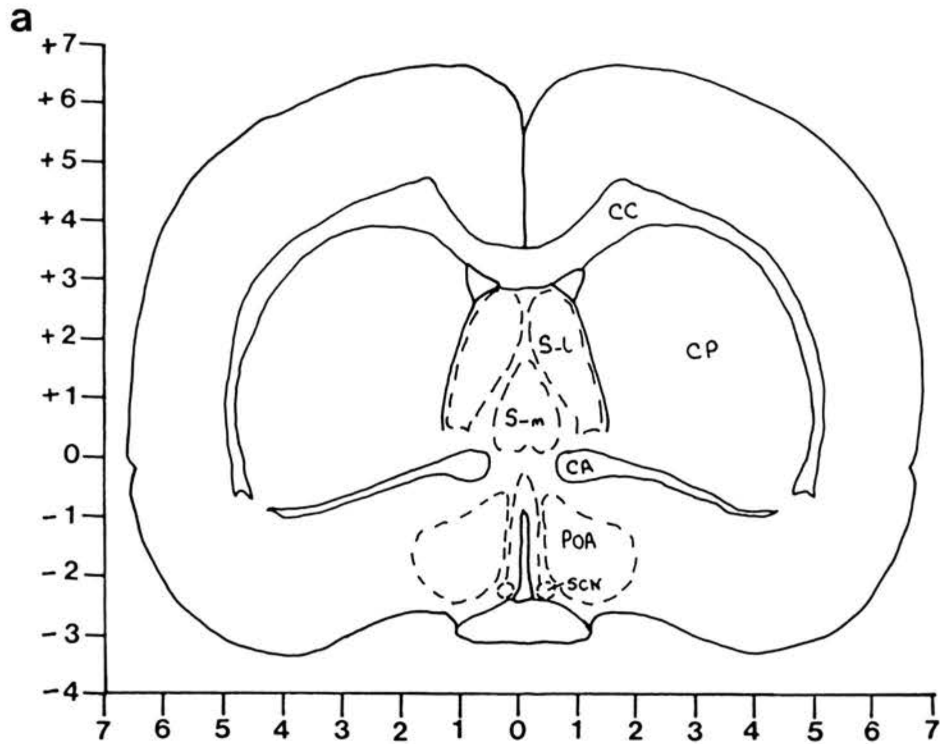


Fig. 6-D: (a) representative low power photomicrograph of a coronal section of the brain showing the position of the electrode tips in the suprachiasmatic nucleus; (b) schematic diagram of coronal sections of the brain at A 7.8 and 7.6 (Pellegrino et al., 1967) showing the anterior-posterior and vertical positions of all the electrodes. Abbreviations: CA, anterior commissure; CC, corpus callosum; CP, caudate putamen; POA-l, lateral preoptic area; SCN, suprachiasmatic nucleus; S-l, lateral septum; V, ventricle.



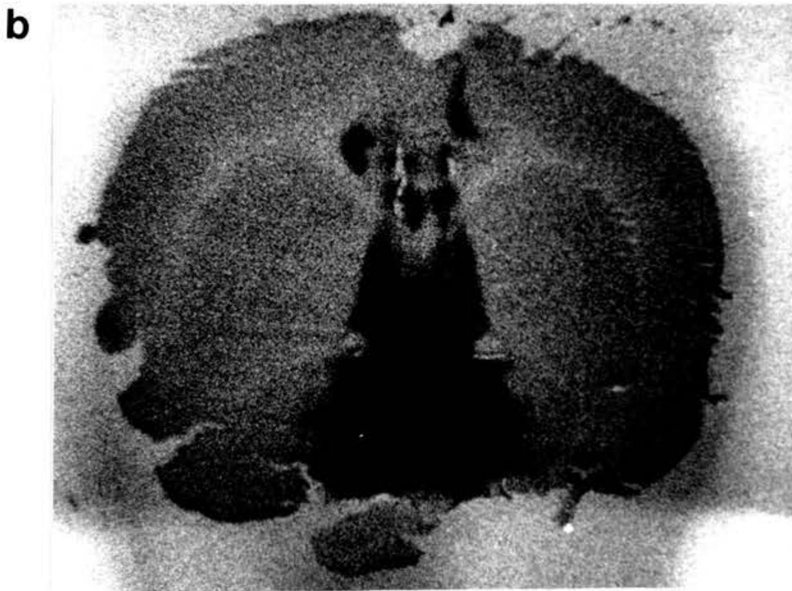
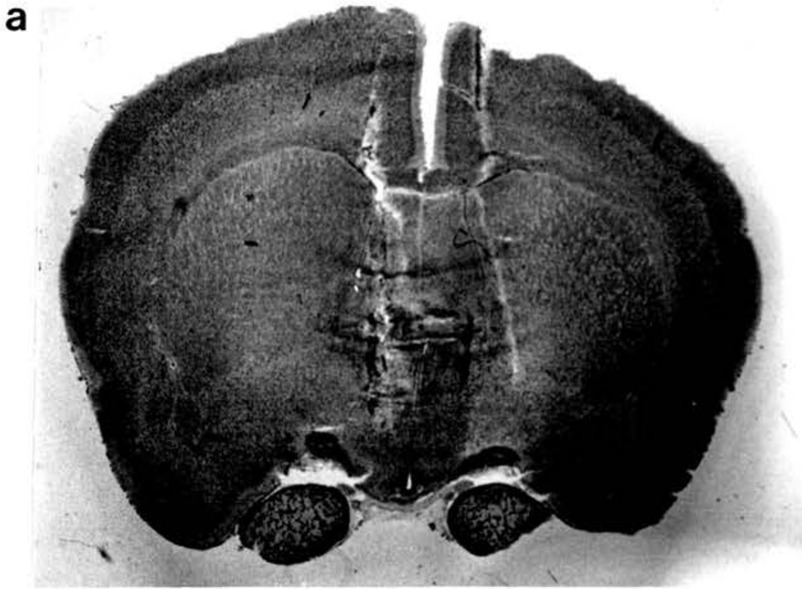


Fig. 6-E: (a) schematic diagram of a coronal section of the brain at A 7.8 (Pellegrino et al., 1967); (b) autoradiograph of a coronal section of the brain at the same level in a rat injected with [^{14}C]2-deoxyglucose and the suprachiasmatic nuclei stimulated. Abbreviations: CA, anterior commissure; CC, corpus callosum; CP, caudate putamen; POA, preoptic area; SCN, suprachiasmatic nucleus; S-l, lateral septum; S-m, medial septum.



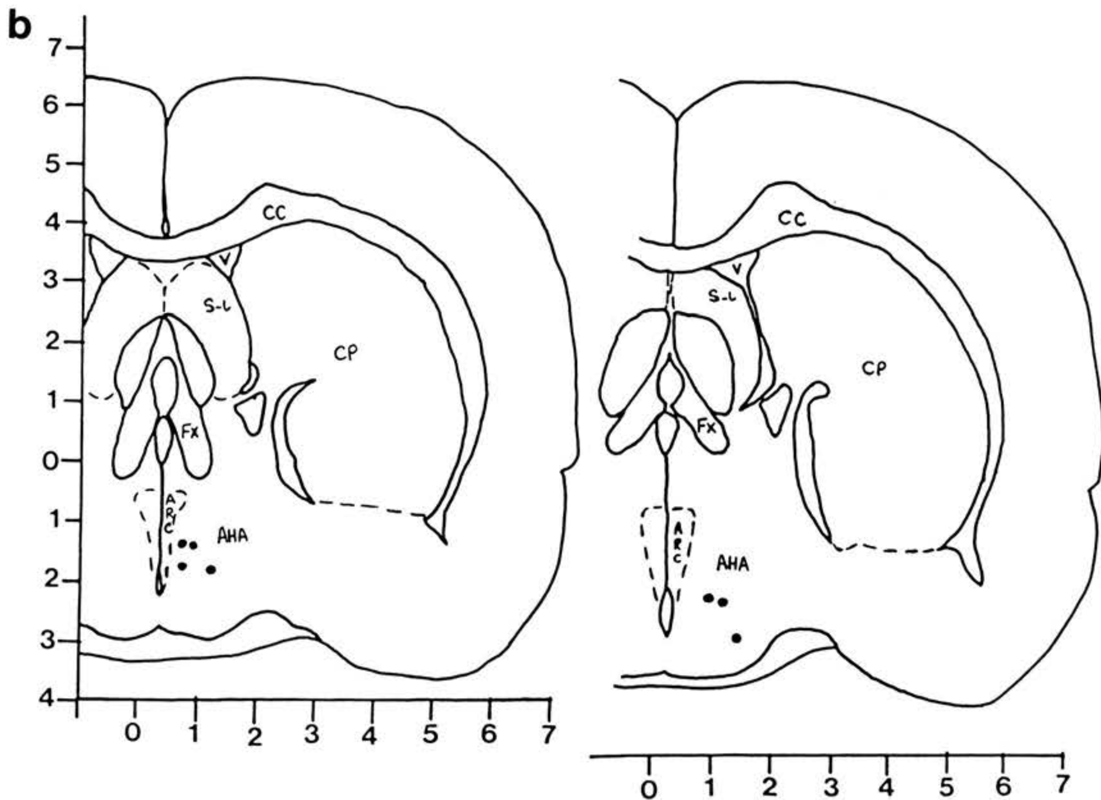
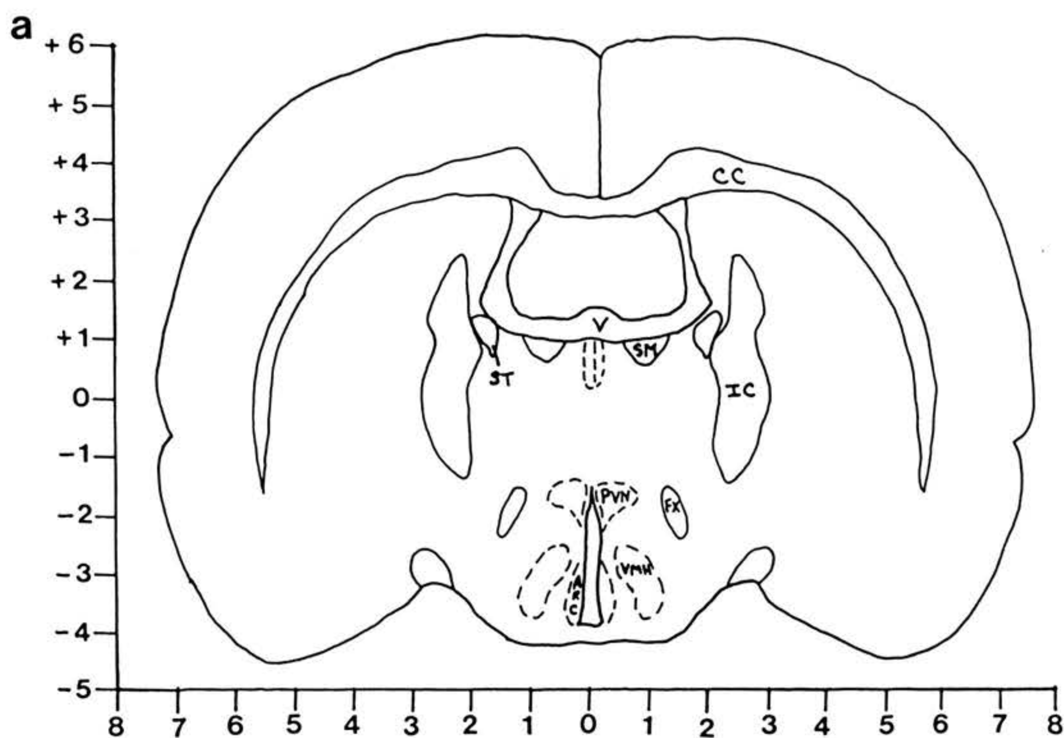


Fig. 6-F: (a) representative low power photomicrograph of a coronal section of the brain showing the position of the electrode tips in the anterior hypothalamic area; (b) schematic diagram of coronal sections of the brain at A 7.0 and 6.8 (Pellegrino et al., 1967) showing the anterior-posterior and vertical positions of the electrodes. Abbreviations: AHA, anterior hypothalamic area; ARC, arcuate nucleus, CC, corpus callosum; CP, caudate putamen; FX, fornix; S-1, lateral septum; V, ventricle.



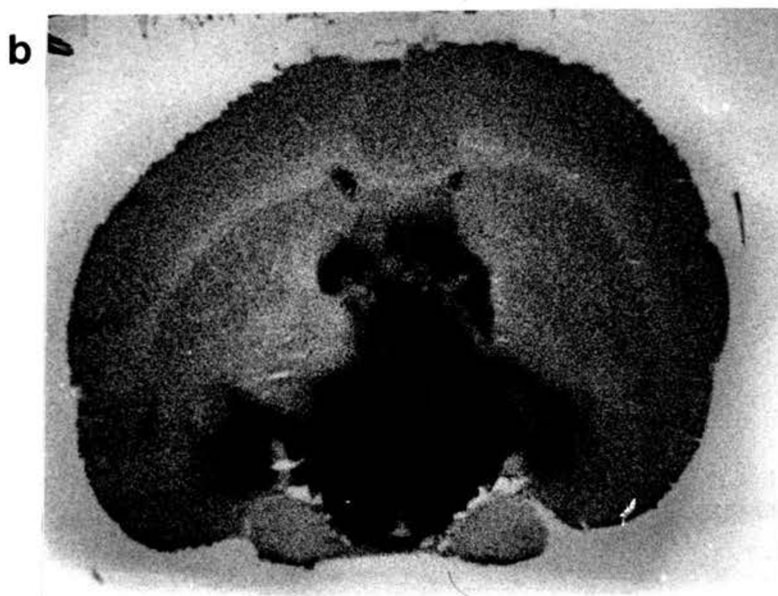
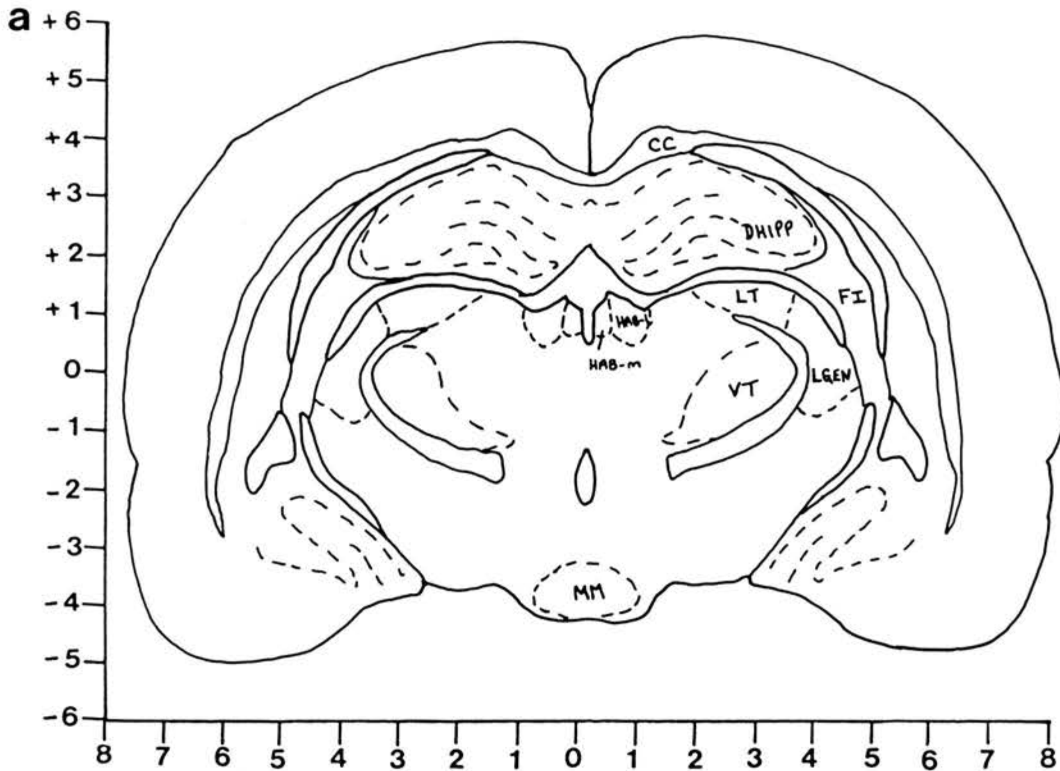


Fig. 6-G: (a) schematic diagram of a coronal section of the brain at A 6.2 (Pellegrino et al., 1967); (b) autoradiograph of a coronal section of the brain at the same level in a rat injected with [^{14}C]2-deoxyglucose and the anterior hypothalamic area stimulated. Abbreviations: ARC, arcuate nucleus; CC, corpus callosum; FX, fornix; IC, internal capsule; PVN, paraventricular nucleus; SM, stria medullaris; ST, stria terminalis; V, ventricle; VMH, ventromedial hypothalamic nucleus.



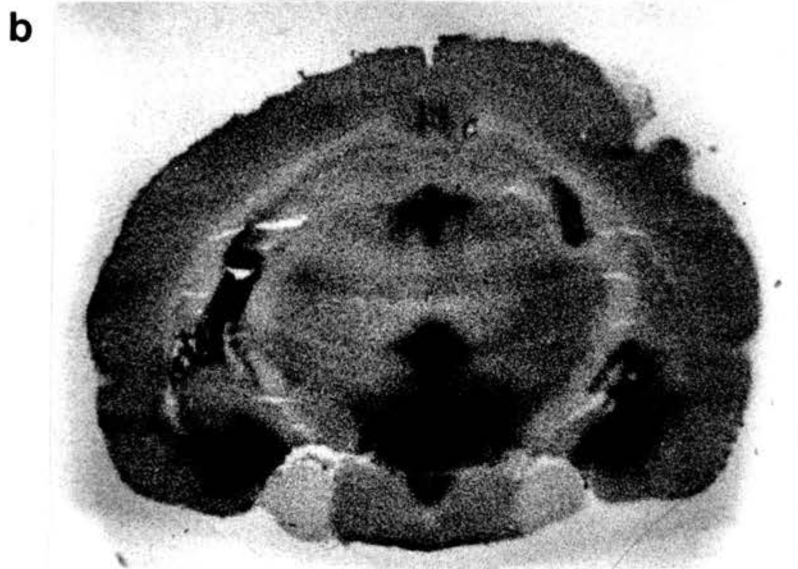
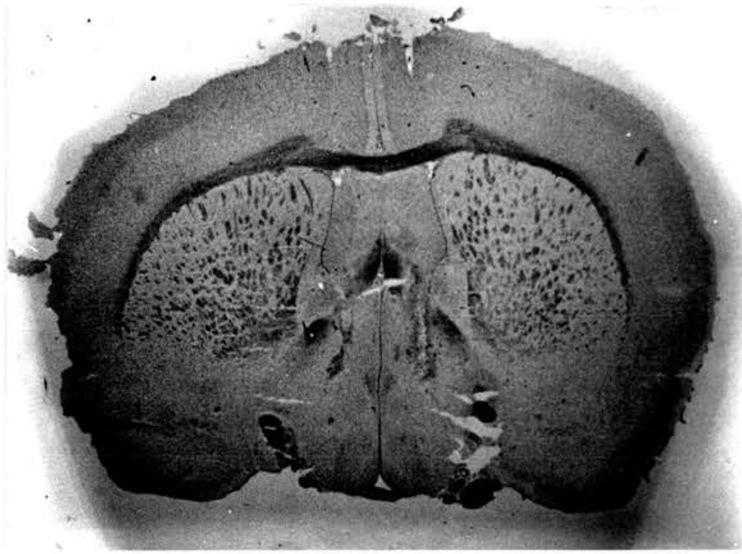


Fig. 6-H: (a) schematic diagram of a coronal section of the brain at A 3.6 (Pellegrino et al., 1967); (b) autoradiograph of a coronal section of the brain at the same level in a rat injected with [^{14}C]2-deoxyglucose and the anterior hypothalamic area stimulated. Abbreviations: CC, corpus callosum; DHIPP, dorsal hippocampus; FI, fimbria; HAB-l, lateral habenular nucleus; HAB-m, medial habenular nucleus; LGEN, lateral geniculate bodies; LT, lateral thalamic area; MM, mamillary body; VT, ventral thalamic area.



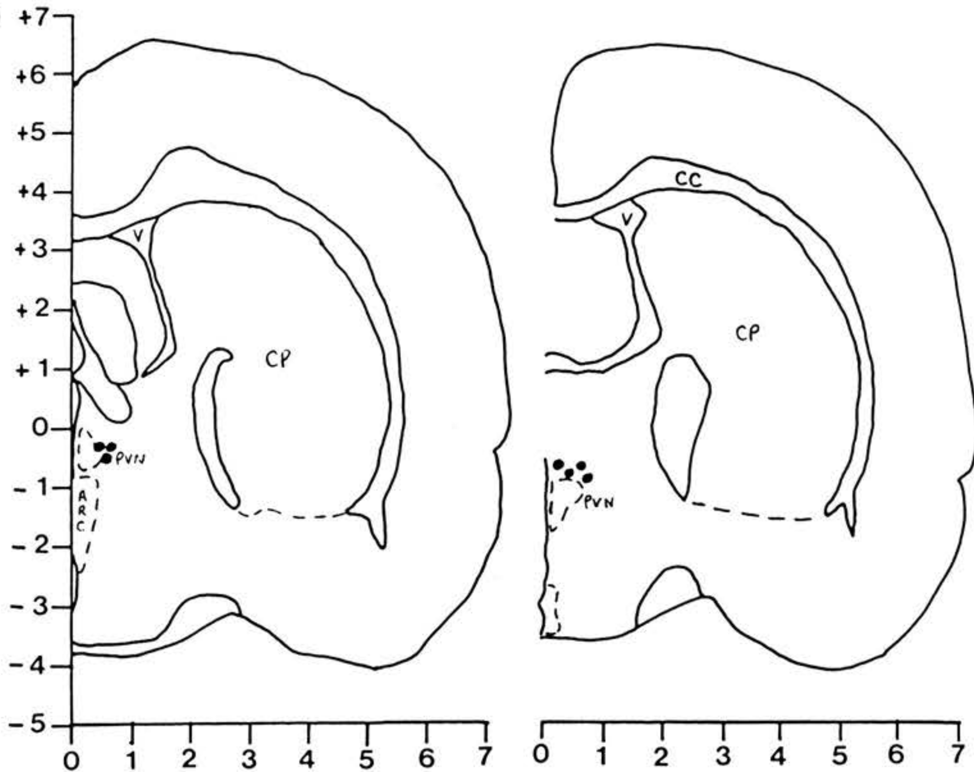


Fig. 6-I: (a) representative low power photomicrograph of a coronal section of the brain showing the position of the electrode tips which straddled the paraventricular nucleus; (b) schematic diagram of coronal sections of the brain at A 6.8 and 6.6 (Pellegrino et al., 1967) showing the anterior- posterior and vertical positions of all the electrodes. Abbreviations: ARC, arcuate nucleus; CC, corpus callosum; CP, caudate putamen; PVN, paraventricular nucleus; V, ventricle.

Table 6-4. The relative metabolic activity of selected brain areas and the pituitary gland in anaesthetised rats after electrical stimulation of the median eminence (ME).

Values are expressed as a ratio (mean ± S.E.M.) of [^{14}C] concentration of selected region to [^{14}C] concentration of the corpus callosum.

	Type and number of animal	
	ME control, unstimulated (n=4)	ME stimulated (n=6)
S-m	1.35 ± 0.07	1.39 ± 0.06
S-l	1.23 ± 0.07	1.31 ± 0.07
DBB	1.41 ± 0.04	1.45 ± 0.05
POA-m	1.36 ± 0.08	1.84 ± 0.15*
POA-l	1.31 ± 0.05	1.71 ± 0.20
SCN	1.82 ± 0.15	1.79 ± 0.16
SON	1.34 ± 0.05	1.61 ± 0.07**
AHA	1.30 ± 0.06	2.21 ± 0.36
FX	1.16 ± 0.02	1.13 ± 0.07
PVN	1.54 ± 0.06	1.95 ± 0.13*
MFB	1.40 ± 0.04	1.45 ± 0.09
ST	1.18 ± 0.01	1.88 ± 0.23*
ARC	1.41 ± 0.04	2.22 ± 0.16*****
VMH	1.33 ± 0.04	2.64 ± 0.28****
DMH	1.41 ± 0.05	1.76 ± 0.17
SM	1.29 ± 0.02	1.39 ± 0.06

Significantly different from control group: * P < 0.05; ** P < 0.025; *** P < 0.02; **** P < 0.01; ***** P < 0.005; ****** P < 0.001.

S-m, medial septum; S-l, lateral septum; DBB, diagonal band of Broca; POA-m, medial preoptic area; POA-l, lateral preoptic area; SCN, suprachiasmatic nucleus; SON, supraoptic nuclei; AHA, anterior hypothalamic area; FX, fornix; PVN, paraventricular nucleus; MFB, medial forebrain bundle; ST, stria terminalis; ARC, arcuate nucleus; VMH, ventromedial hypothalamic area; DMH, dorsomedial hypothalamic nucleus; SM, stria medullaris

Table 6-4 (cont). The relative metabolic activity of selected brain areas and the pituitary gland in anaesthetised rats after electrical stimulation of the median eminence(ME).

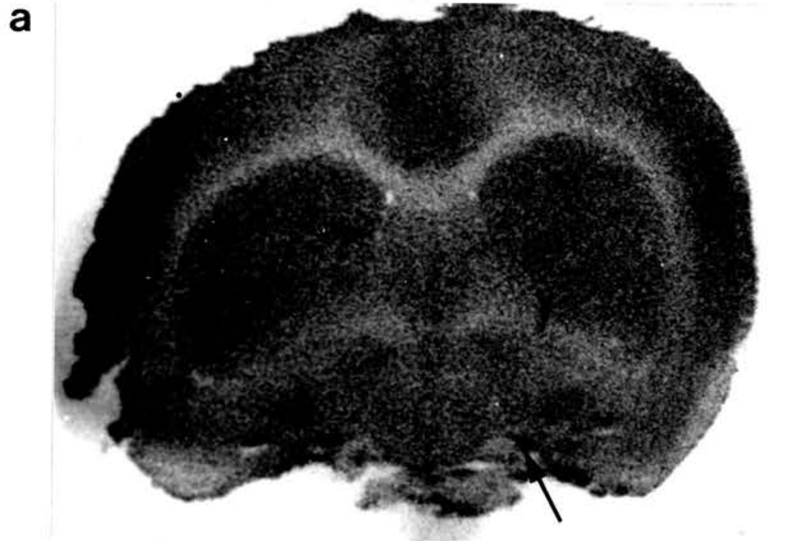
Values are expressed as a ratio (mean ± S.E.M.) of [^{14}C] concentration of selected region to [^{14}C] concentration of the corpus callosum.

	Type and number of animal	
	ME control, unstimulated (n=4)	ME stimulated (n=6)
AMYG CO+M	1.26 ± 0.03	1.54 ± 0.19
AMYG CE+L	1.26 ± 0.01	1.32 ± 0.10
IC	1.00 ± 0.02	0.98 ± 0.02
MM	1.81 ± 0.06	1.78 ± 0.11
IP	2.21 ± 0.12	2.26 ± 0.16
PN	1.36 ± 0.06	2.23 ± 0.29*
PD	1.36 ± 0.06	1.52 ± 0.12
DHIPPM	1.43 ± 0.04	1.49 ± 0.07
DHIPPG	1.24 ± 0.03	1.27 ± 0.05
VHIPPM	1.53 ± 0.03	1.51 ± 0.07
VHIPPG	1.28 ± 0.02	1.28 ± 0.05
HAB-m	1.92 ± 0.06	1.91 ± 0.09
HAB-l	2.11 ± 0.12	2.12 ± 0.13
PYRCX	1.28 ± 0.02	1.70 ± 0.24
CG	1.47 ± 0.06	1.61 ± 0.08
RF	1.47 ± 0.05	1.51 ± 0.07

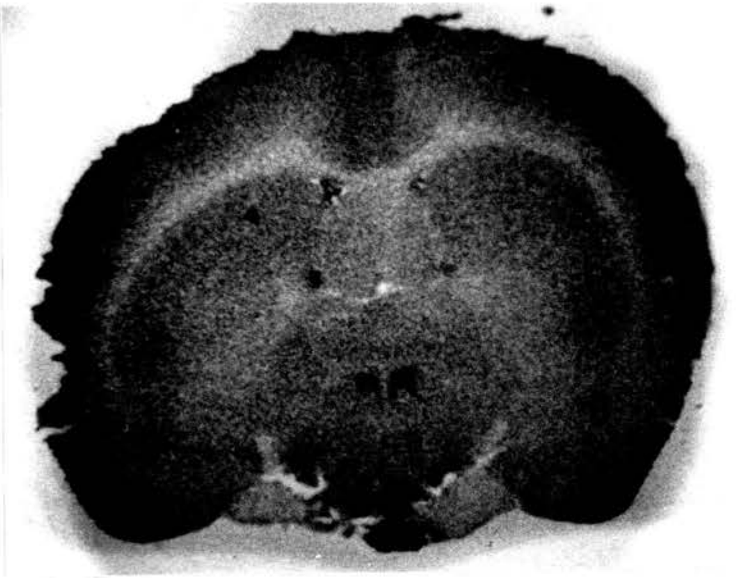
Significantly different from control group: * $P < 0.05$; ** $P < 0.025$; *** $P < 0.02$; **** $P < 0.01$; ***** $P < 0.005$; ****** $P < 0.001$

AMYG CO+M, cortical and medial amygdaloid nuclei; AMYG CE+L, central and lateral amygdaloid nuclei; IC, internal capusle; MM, mamillary body; IP, interpeduncular nucleus; PN, pars nervosa; PD, pars distalis; DHIPPM, dorsal hippocampus (molecular layer); DHIPPG, dorsal hippocampus (granular layer); VHIPPM, ventral hippocampus (molecular layer); VHIPPG, ventral hippocampus (granular layer); HAB-m, medial habenular nucleus; HAB-l, lateral habenular nucleus; PYRCX, pyriform cortex; CG, central grey; RF, reticular formation

Fig. 6-J: autoradiographs of coronal sections of the brain in rats injected with [^{14}C]2-deoxyglucose and the median eminence stimulated; (a) brain section at the level of the supraoptic nucleus (arrow); (b) brain section at the level of the paraventricular nucleus (arrow); (c) schematic diagram of a coronal section of the brain at A 6.4 (Pellegrino et al., 1967) corresponding to the brain level of the autoradiograph (b). Abbreviations: ARC, arcuate nucleus; CC, corpus callosum; FX, fornix; IC, internal capsule; PVN, paraventricular nucleus; ST, stria terminalis; V, ventricle; VMH, ventromedial hypothalamic nucleus.



b



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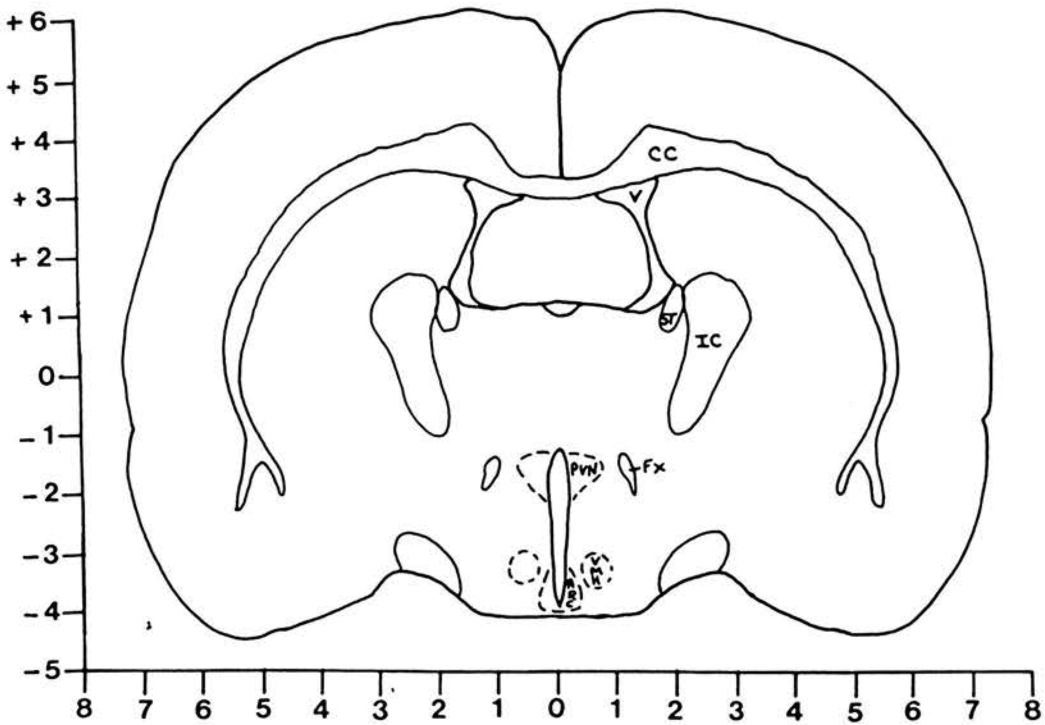


which the plasma LH concentration increased from < 2.5 ng/ml to 6.0 ng/ml after 45 min of electrical stimulation.

6.3.4 Electrical stimulation of the amygdala, ventral hippocampus and dorsal hippocampus

6.3.4.1 Stimulation of the amygdala

Fig. 6-K (a) shows a representative photomicrograph of the position of the electrode tips in the AMYG. The anterior-posterior and vertical positions of the electrode tips in all the animals are shown in Fig. 6-K (b).

6.3.4.1.1 Differences between areas on the side of brain ipsilateral and contralateral to the implanted electrode in unstimulated animals

Although there was increased 2DG uptake along the electrode track, there were no significant differences in the rma of any brain area measured between i and c sides of the brain in the combined control unstimulated animals (Table 6-5).

6.3.4.1.2 Effect of electrical stimulation on areas on the side of brain ipsilateral to the implanted electrode

The rma of the following i brain areas increased significantly in AMYG stimulated animals compared with control unstimulated animals: S-1, DBB, POA-1, MFB, ST, SM, AMYG CO+M, AMYG CE+L, VHIPPM, VHIPPG, PYRCX, CA, FRCX and PVT (Table 6-5).

6.3.4.1.3 Effect of electrical stimulation on areas on the side of brain contralateral to the implanted electrode

Table 6-5 shows that the rma of the AMYG CE+L, PYRCX and CA increased significantly on the c side in AMYG stimulated animals compared with control unstimulated animals. The increased 2DG

uptake in these areas and some i areas are shown in Figs. 6-L - 6-N.

6.3.4.1.4 Comparison of the effects of electrical stimulation on areas on the side of brain ipsilateral and contralateral to the implanted electrode

The rma of the i side was significantly greater than of the c side in AMYG stimulated animals in the following brain areas: S-l, POA-l, MFB, ST, AMYG CO+M, AMYG CE+L, VHIPPM, VHIPPG, PYRCX, FRCX and PVT (Table 6-5). There was no significant difference in rma between the two sides of brain in the CA (Table 6-5).

6.3.4.2 Stimulation of the ventral hippocampus

A representative photomicrograph showing the position of the electrode tips in the VHIPP is shown in Fig. 6-O (a). The anterior-posterior and vertical positions of the electrode tips in all the animals in this study are shown in Fig. 6-O (b).

6.3.4.2.1 Effect of electrical stimulation on areas on the side of brain ipsilateral to the implanted electrode

Table 6-5 shows that the rma of the following i brain areas increased significantly in VHIPP stimulated animals compared with control unstimulated animals: S-l, DBB, POA-m, POA-l, AHA, FX, MFB, ST, ARC, VMH, DMH, SM, AMYG CO+M, AMYG CE+L, VHIPPM, VHIPPG and PVT. In addition, the rma of the IP was also increased in VHIPP stimulated animals compared with control unstimulated animals. The increase in rma of most of the areas listed above can be seen in Figs. 6-P and 6-Q.

6.3.4.2.2 Effect of electrical stimulation on areas on the side of brain contralateral to the implanted electrode

The rma of the S-l, ST, VHIPPM, VHIPPG and PVT also increased

Table 6-5. The relative metabolic activity of selected brain areas and the pituitary gland in anaesthetised rats after electrical stimulation of the amygdala (AMYG) and the ventral hippocampus (VHIPP).

Values are expressed as a ratio (mean ± S.E.M.) of [^{14}C] concentration of selected region to [^{14}C] concentration of the corpus callosum.

	Type and number of animal					
	Combined controls, unstimulated (n=6)		AMYG stimulated (n=5)		VHIPP stimulated (n=4)	
	c	i	c	i	c	i
S-m	1.28±0.04		1.35±0.03		1.37±0.04	
S-l	1.20±0.03	1.16±0.03	1.16±0.05	1.49±0.18*	2.08±0.24******	5.01±0.12******
DBB	1.29±0.04	1.26±0.04	1.35±0.03	1.56±0.10*	1.40±0.02	1.54±0.05****
POA-m	1.23±0.01	1.21±0.02	1.26±0.02	1.52±0.17	1.25±0.04	1.45±0.04******
POA-l	1.16±0.02	1.16±0.01	1.17±0.03	1.58±0.15****	1.16±0.02	2.06±0.21******
SCN	2.04±0.06	1.92±0.06	2.14±0.10	2.05±0.09	2.09±0.08	2.00±0.13
SON	1.09±0.03	1.06±0.03	0.95±0.06	1.15±0.09	1.10±0.07	1.15±0.02
AHA	1.22±0.02	1.19±0.02	1.24±0.02	1.57±0.26	1.29±0.04	2.76±0.25******
FX	1.05±0.03	1.08±0.02	0.99±0.03	1.05±0.05	1.06±0.05	1.42±0.07******

Significantly different from corresponding side of control brain: * $P<0.05$; ** $P<0.025$; *** $P<0.02$; **** $P<0.01$; ***** $P<0.005$; ****** $P<0.001$

c, structure on the side of brain contralateral to the implanted electrode;
i, structure on the side of brain ipsilateral to the implanted electrode;

S-m, medial septum; S-l, lateral septum; DBB, diagonal band of Broca; POA-m, medial preoptic area; POA-l, lateral preoptic area; SCN, suprachiasmatic nucleus; SON, supraoptic nucleus; AHA, anterior hypothalamic area; FX, fornix

Table 6-5 (cont). The relative metabolic activity of selected brain areas and the pituitary gland in anaesthetised rats after electrical stimulation of the amygdala (AMYG) and the ventral hippocampus (VHIPP).

Values are expressed as a ratio (mean ± S.E.M.) of [^{14}C] concentration of selected region to [^{14}C] concentration of the corpus callosum.

	Type and number of animal					
	Combined controls, unstimulated (n=6)		AMYG stimulated (n=5)		VHIPP stimulated (n=4)	
	c	i	c	i	c	i
PVN	1.33±0.03	1.31±0.04	1.35±0.04	1.45±0.09	1.30±0.05	1.34±0.02
MFB	1.22±0.02	1.22±0.02	1.35±0.04	2.45±0.38****	1.30±0.03	1.41±0.06*
ST	1.10±0.02	1.10±0.02	1.36±0.02	4.46±0.33******	1.69±0.22*	5.14±0.27******
ARC	1.20±0.02	1.17±0.03	1.24±0.03	1.32±0.10	1.21±0.03	1.38±0.04*****
VMH	1.15±0.02	1.12±0.02	1.23±0.02	2.65±0.82	1.22±0.02	3.70±0.23******
DMH	1.27±0.01	1.25±0.02	1.27±0.02	1.39±0.09	1.33±0.04	1.42±0.04******
SM	1.20±0.02	1.22±0.02	1.27±0.02	1.35±0.02****	1.29±0.01	1.44±0.04******
AMYG CO+M	1.14±0.02	1.11±0.03	1.34±0.09	4.28±0.08******	1.26±0.05	5.19±0.35******
AMYG CE+L	1.17±0.02	1.17±0.04	2.76±0.33******	4.78±0.26******	1.23±0.04	4.73±0.21******

Significantly different from corresponding side of control brain: * P<0.05; ** P<0.025; *** P<0.02; **** P<0.01; ***** P<0.005; ****** P<0.001

c, structure on the side of brain contralateral to the implanted electrode;
i, structure on the side of brain ipsilateral to the implanted electrode;

PVN, paraventricular nucleus; MFB, medial forebrain bundle; ST, stria terminalis; ARC, arcuate nucleus; VMH, ventromedial hypothalamic nucleus; DMH, dorsomedial hypothalamic nucleus; SM, stria medullaris; AMYG CO+M, cortical and medial amygdaloid nuclei; AMYG CE+L, central and lateral amygdaloid nuclei

Table 6-5 (cont). The relative metabolic activity of selected brain areas and the pituitary gland in anaesthetised rats after electrical stimulation of the amygdala (AMYG) and the ventral hippocampus (VHIPP).

Values are expressed as a ratio (mean ± S.E.M.) of [^{14}C] concentration of selected region to [^{14}C] concentration of the corpus callosum.

	Type and number of animal					
	Combined controls, unstimulated (n=6)		AMYG stimulated (n=5)		VHIPP stimulated (n=4)	
	c	i	c	i	c	i
IC	0.97±0.03		0.97±0.05		1.00±0.04	
MM	1.47±0.03		1.51±0.03		1.53±0.03	
IP	2.04±0.09		2.07±0.09		3.17±0.08*****	
PN	1.28±0.03		1.23±0.05		1.21±0.06	
PD	1.34±0.07		1.15±0.09		1.54±0.03	
DHIPPM	1.38±0.05	1.35±0.05	1.34±0.03	1.33±0.04	1.41±0.05	1.59±0.15
DHIPPG	1.20±0.04	1.19±0.04	1.20±0.01	1.11±0.03	1.22±0.03	1.27±0.16
VHIPPM	1.40±0.06	1.37±0.05	1.43±0.06	2.41±0.19******	2.41±0.27****	4.49±0.35******
VHIPPG	1.23±0.04	1.24±0.03	1.27±0.03	1.67±0.09******	4.38±1.03******	5.59±0.35******

Significantly different from corresponding side of control brain: * P<0.05; ** P<0.025; *** P<0.02; **** P<0.01; ***** P<0.005; ****** P<0.001

c, structure on the side of brain contralateral to the implanted electrode;
i, structure on the side of brain ipsilateral to the implanted electrode;

IC, internal capsule; MM, mamillary body; IP, interpeduncular nucleus; PN, pars nervosa; PD, pars distalis; DHIPPM, dorsal hippocampus (molecular layer); DHIPPG, dorsal hippocampus (granular layer); VHIPPM, ventral hippocampus (molecular layer); VHIPPG, ventral hippocampus (granular layer)

Table 6-5 (cont). The relative metabolic activity of selected brain areas and the pituitary gland in anaesthetised rats after electrical stimulation of the amygdala (AMYG) and the ventral hippocampus (VHIPP).

Values are expressed as a ratio (mean ± S.E.M.) of [^{14}C] concentration of selected region to [^{14}C] concentration of the corpus callosum.

	Type and number of animal					
	Combined controls, unstimulated (n=6)		AMYG stimulated (n=5)		VHIPP stimulated (n=4)	
	c	i	c	i	c	i
HAB-1	1.76±0.09	1.76±0.07	1.92±0.13	1.99±0.12	2.05±0.10	2.16±0.15
PYRCX	1.24±0.02	1.17±0.02	1.72±0.03*	5.06±0.26******	1.31±0.04	1.20±0.04
CG	1.34±0.03	1.30±0.03	1.41±0.03	1.44±0.07	1.38±0.06	1.32±0.08
RF	1.30±0.05	1.31±0.05	1.35±0.04	1.38±0.03	1.33±0.03	1.37±0.06
CA	1.06±0.02	1.03±0.02	4.03±0.37******	4.71±0.37******	1.14±0.02	1.10±0.03
FRCX	1.42±0.03	1.33±0.02	1.44±0.02	1.91±0.16*****	1.42±0.02	1.46±0.10
PVT	1.31±0.02	1.28±0.03	1.47±0.02	1.84±0.07******	1.69±0.11*****	2.08±0.10******

Significantly different from corresponding side of control brain: * $P<0.05$; ** $P<0.025$; *** $P<0.02$; **** $P<0.01$; ***** $P<0.005$; ****** $P<0.001$

c, structure on the side of brain contralateral to the implanted electrode;
i, structure on the side of brain ipsilateral to the implanted electrode;

HAB-1, lateral habenular nucleus; PYRCX, pyriform cortex; CG, central grey; RF, reticular formation; CA, anterior commissure; FRCX, frontal cortex; PVT, paraventricular thalamic area

Table 6-5 (cont). Significance of differences in the relative metabolic activity of brain areas and the pituitary gland in Table 6-5 (Duncan's multiple range test):

$P < 0.05$

S-1	i-AMYG stimulated v c-AMYG stimulated, i-control unstimulated, c-control unstimulated
DBB	i-AMYG stimulated v i-control unstimulated, c-control unstimulated
PYRCX	c-AMYG stimulated v i-control unstimulated, c-control unstimulated
PVT	c-AMYG stimulated v i-control unstimulated

$P < 0.01$

POA-1	i-AMYG stimulated v c-AMYG stimulated, i-control unstimulated, c-control unstimulated
MFB	i-AMYG stimulated v c-AMYG stimulated, i-control unstimulated, c-control unstimulated
SM	i-AMYG stimulated v i-control unstimulated

$P < 0.005$

SM	i-AMYG stimulated v c-control unstimulated
FRCX	i-AMYG stimulated v c-AMYG stimulated, i-control unstimulated, c-control unstimulated

$P < 0.001$

ST	i-AMYG stimulated v c-AMYG stimulated, i-control unstimulated, c-control unstimulated
AMYG CO+M	i-AMYG stimulated v c-AMYG stimulated, i-control unstimulated, c-control unstimulated
AMYG CE+L	i-AMYG stimulated v c-AMYG stimulated, i-control unstimulated, c-control unstimulated
	c-AMYG stimulated v i-AMYG stimulated, c-control unstimulated, c-control unstimulated

Table 6-5 (cont). Significance of differences in the relative metabolic activity of brain areas and the pituitary gland in Table 6-5 (Duncan's multiple range test):

VHIPPM	i-AMYG stimulated v c-AMYG stimulated, i-control unstimulated, c-control unstimulated
VHIPPG	i-AMYG stimulated v c-AMYG stimulated, i-control unstimulated, c-control unstimulated
PYRCX	i-AMYG stimulated v c-AMYG stimulated, i-control unstimulated, c-control unstimulated
CA	i-AMYG stimulated v i-control unstimulated, c-control unstimulated c-AMYG stimulated v i-control unstimulated, c-control unstimulated
PVT	i-AMYG stimulated v c-AMYG stimulated, i-control unstimulated, c-control unstimulated

Table 6-5 (cont). Significance of differences in the relative metabolic activity of brain areas and the pituitary gland in Table 6-5 (Duncan's multiple range test:

$P < 0.05$

MFB	i-VHIPP stimulated v i-control unstimulated, c-control unstimulated
ST	c-VHIPP stimulated v i-control unstimulated, c-control unstimulated
DMH	i-VHIPP stimulated v c-VHIPP stimulated c-VHIPP stimulated v i-VHIPP stimulated, i-control unstimulated

$P < 0.01$

DBB	i-VHIPP stimulated v i-control unstimulated, c-control unstimulated
ARC	i-VHIPP stimulated v c-VHIPP stimulated, c-control unstimulated
VHIPPM	c-VHIPP stimulated v i-control unstimulated, c-control unstimulated

$P < 0.005$

POA-m	i-VHIPP stimulated v c-VHIPP stimulated, c-control unstimulated
ARC	i-VHIPP stimulated v i-control unstimulated
DMH	i-VHIPP stimulated v c-control unstimulated
SM	i-VHIPP stimulated v c-VHIPP stimulated
IP	VHIPP stimulated v control unstimulated
PVT	i-VHIPP stimulated v c-VHIPP stimulated c-VHIPP stimulated v i-VHIPP stimulated, i-control unstimulated c-control unstimulated

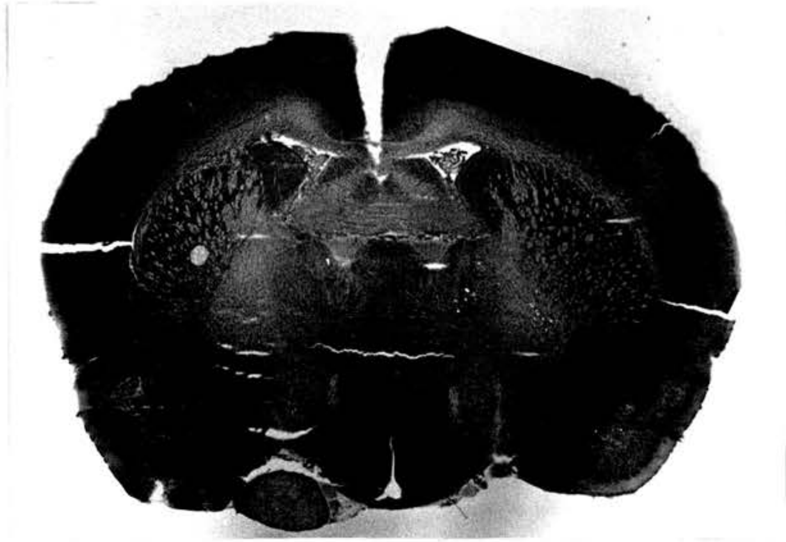
$P < 0.001$

S-1	i-VHIPP stimulated v c-VHIPP stimulated, i-control unstimulated, c-control unstimulated c-VHIPP stimulated v i-VHIPP stimulated, i-control unstimulated c-control unstimulated

Table 6-5 (cont). Significance of differences in the relative metabolic activity of brain areas and the pituitary gland in Table 6-5 (Duncan's multiple range test:

POA-m	i-VHIPP stimulated v i-control unstimulated
POA-l	i-VHIPP stimulated v c-VHIPP stimulated, i-control unstimulated c-control unstimulated
AHA	i-VHIPP stimulated v c-VHIPP stimulated, i-control unstimulated, c-control unstimulated
FX	i-VHIPP stimulated v c-VHIPP stimulated, i-control unstimulated, c-control unstimulated
ST	i-VHIPP stimulated v c-VHIPP stimulated, i-control unstimulated, c-control unstimulated
VMH	i-VHIPP stimulated v c-VHIPP stimulated, i-control unstimulated, c-control unstimulated
DMH	i-VHIPP stimulated v i-control unstimulated
SM	i-VHIPP stimulated v i-control unstimulated, c-control unstimulated
AMYG CO+M	i-VHIPP stimulated v c-VHIPP stimulated, i-control unstimulated, c-control unstimulated
AMYG CE+L	i-VHIPP stimulated v c-VHIPP stimulated, i-control unstimulated, c-control unstimulated
VHIPPM	i-VHIPP stimulated v c-VHIPP stimulated, i-control unstimulated, c-control unstimulated
VHIPPG	i-VHIPP stimulated v i-control unstimulated, c-control unstimulated, c-VHIPP stimulated v i-control unstimulated, c-control unstimulated
PVT	i-VHIPP stimulated v i-control unstimulated, c-control unstimulated

a



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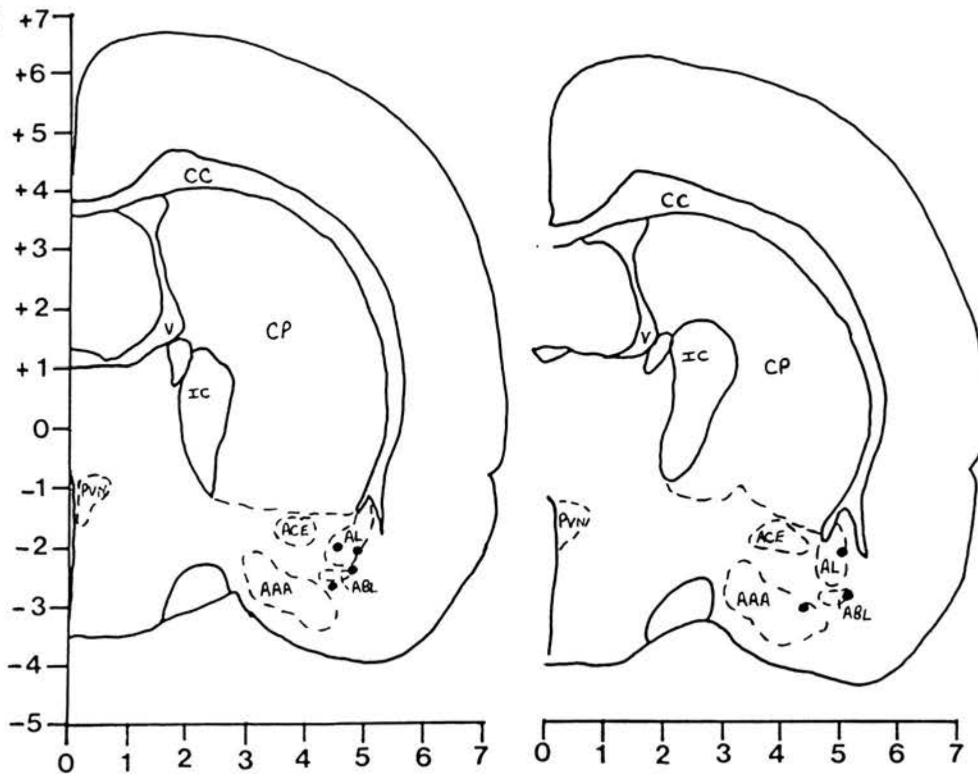
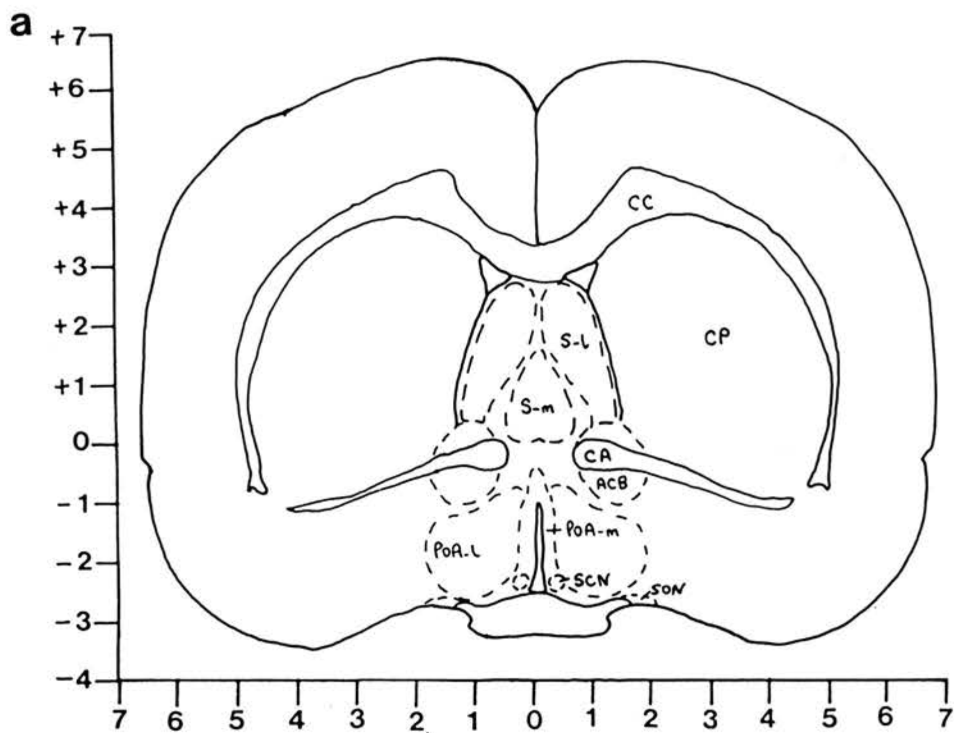


Fig. 6-K: (a) representative low power photomicrograph of a coronal section of the brain showing the position of the electrode tips in the amygdala; (b) schematic diagram of coronal sections of the brain at A 6.6 and 6.4 (Pellegrino et al., 1967) showing the anterior-posterior and vertical positions of all the electrodes. Abbreviations: AAA, anterior amygdaloid nucleus; ABL, basolateral amygdaloid nucleus; ACE, central amygdaloid nucleus; AL, lateral amygdaloid nucleus; CC, corpus callosum; CP, caudate putamen; IC, internal capsule; PVN, paraventricular nucleus; V, ventricle.



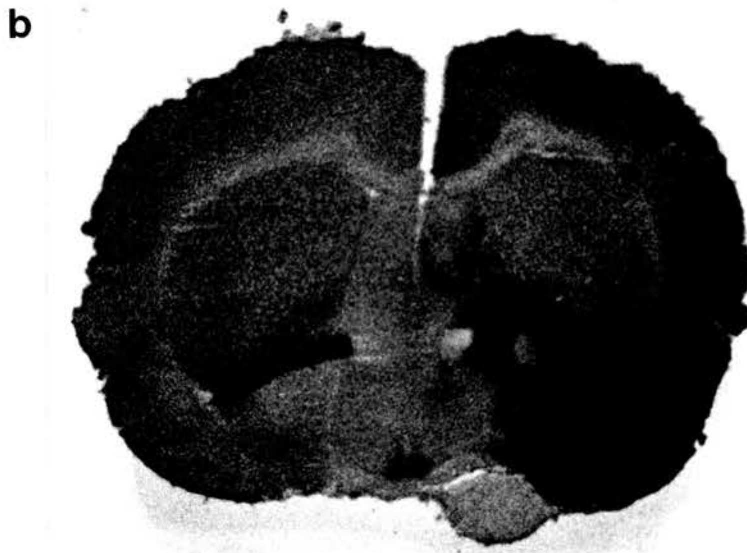
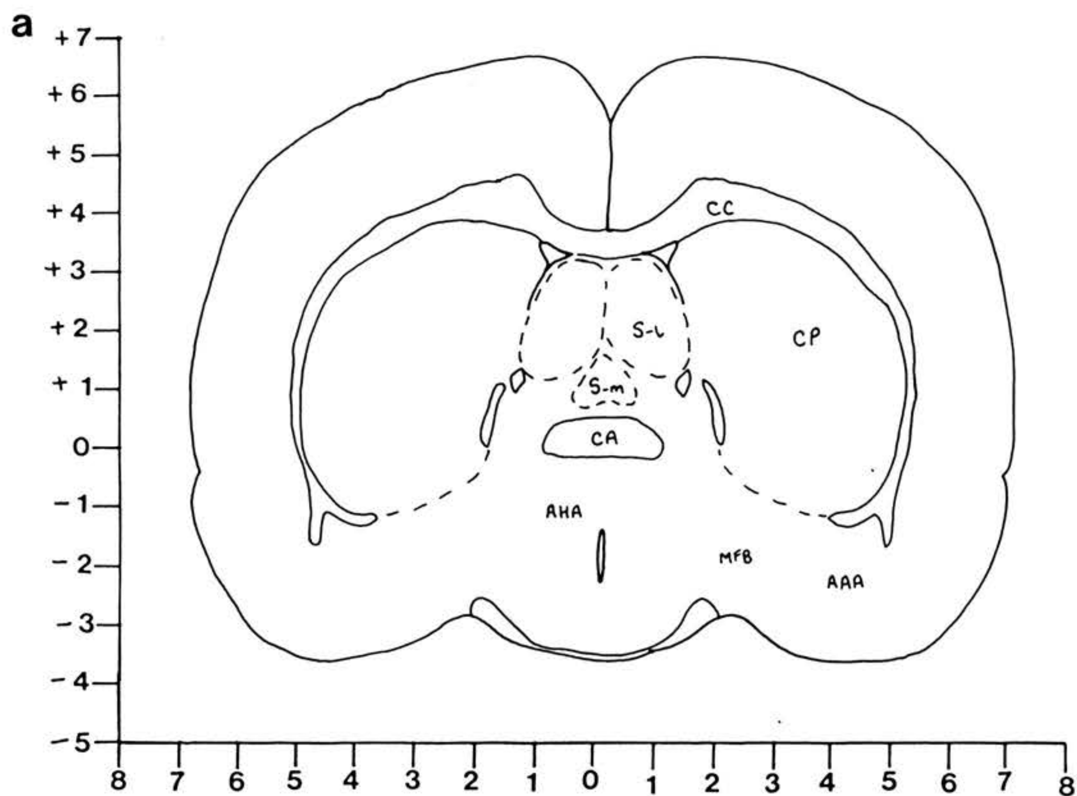


Fig. 6-L: (a) schematic diagram of a coronal section of the brain at A 7.8 (Pellegrino et al., 1967); (b) autoradiograph of a coronal section of the brain at the same level in a rat injected with [^{14}C]2-deoxyglucose and the amygdala stimulated. Abbreviations: ACB, nucleus accumbens; CA, anterior commissure; CC, corpus callosum; CP, caudate putamen; POA-l, lateral preoptic area; POA-m, medial preoptic area; SCN, suprachiasmatic nucleus; S-l, lateral septum; S-m, medial septum; SON, supraoptic nucleus.



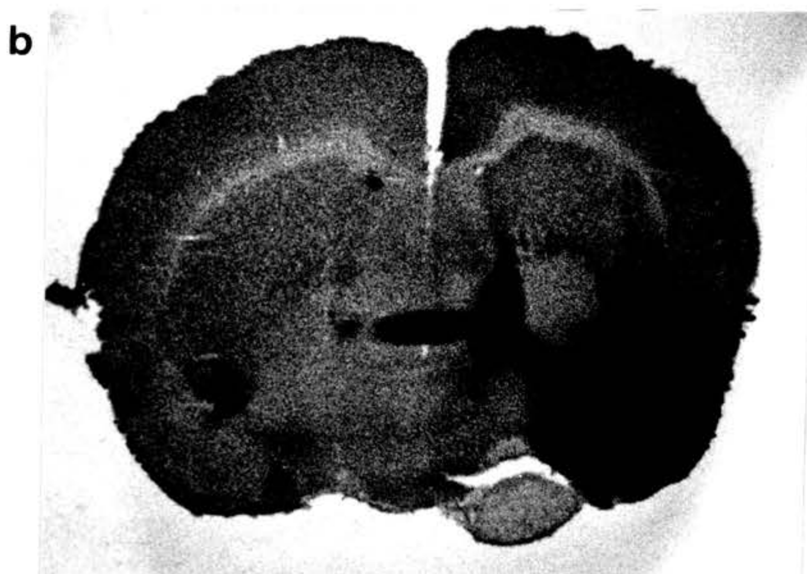
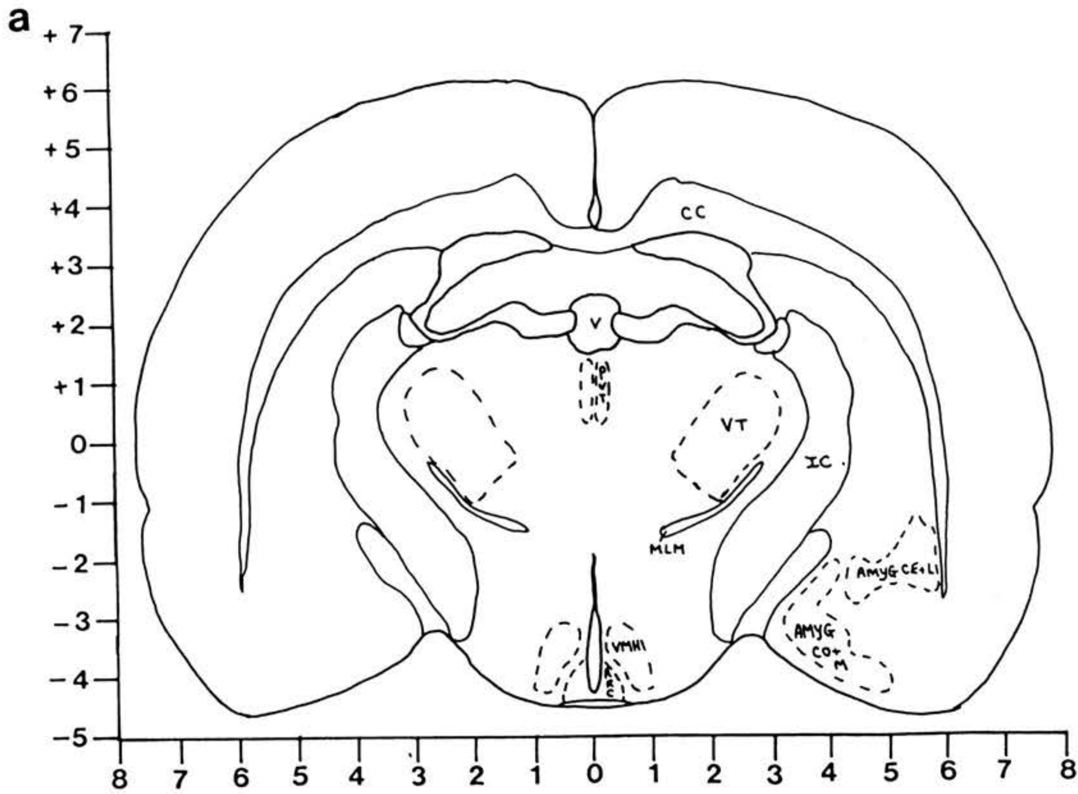


Fig. 6-M: (a) schematic diagram of a coronal section of the brain at A 7.1 (Pellegrino et al., 1967); (b) autoradiograph of a coronal section of the brain at the same level in a rat injected with [^{14}C]2-deoxyglucose and the amygdala stimulated. Abbreviations: AAA, anterior amygdaloid nucleus; AHA, anterior hypothalamic area; CA, anterior commissure; CC, corpus callosum; CP, caudate putamen; MFB, medial forebrain bundle; S-l, lateral septum; S-m, medial septum.



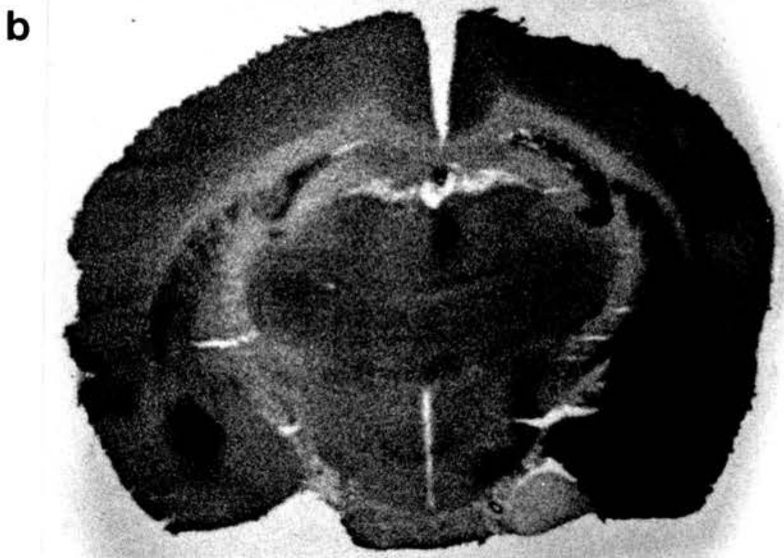
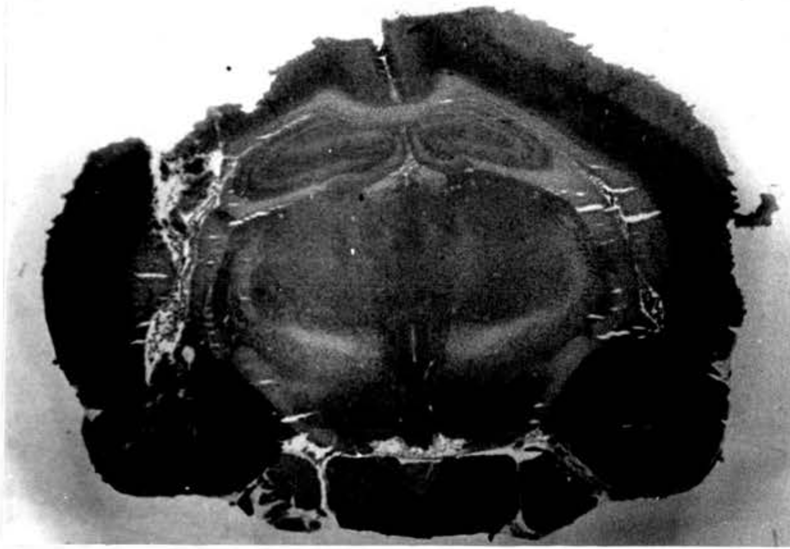


Fig. 6-N: (a) schematic diagram of a coronal section of the brain at A 5.2 (Pellegrino et al., 1967); (b) autoradiograph of a coronal section of the brain at the same level in a rat injected with [^{14}C]2-deoxyglucose and the amygdala stimulated. Abbreviations: ARC, arcuate nucleus; AMYG CE+L, central and lateral amygdaloid nuclei; AMYG CO+M, cortical and medial amygdaloid nuclei; CC, corpus callosum; IC, internal capsule; MLM, medial lemniscus; PVT, paraventricular thalamic area; V, ventricle; VMH, ventromedial hypothalamic nucleus; VT, ventral thalamic area.



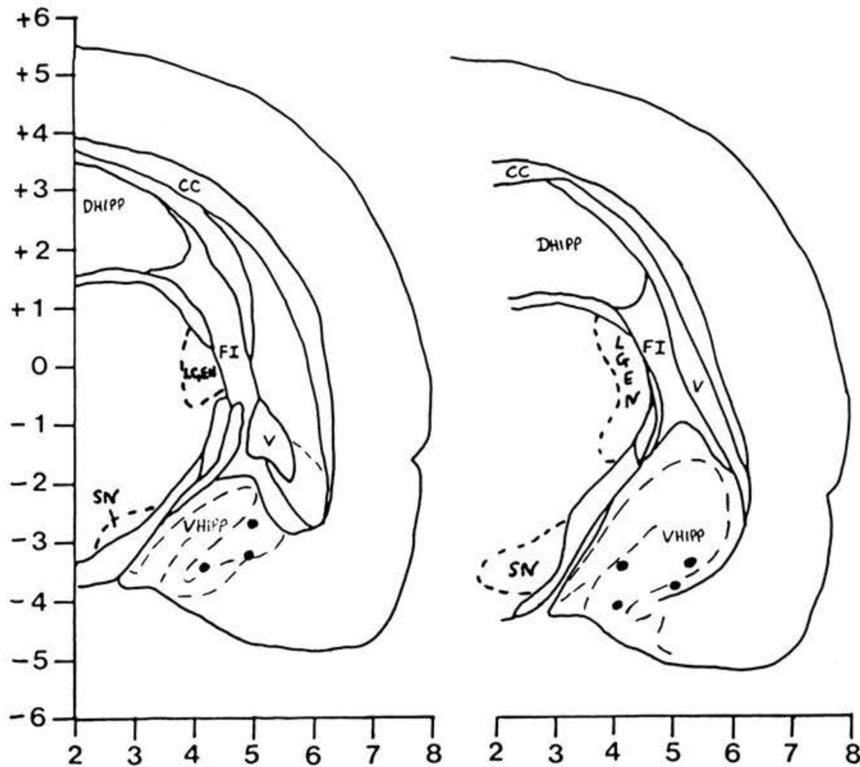
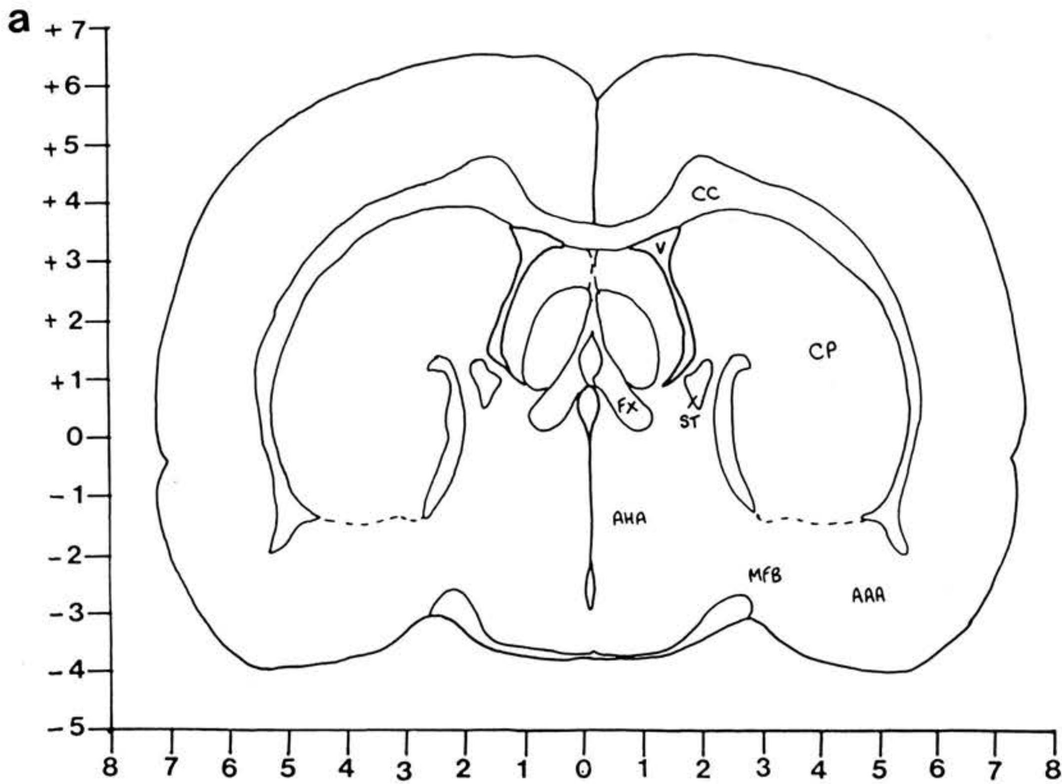


Fig. 6-0: (a) representative low power photomicrograph of a coronal section of the brain showing the position of the electrode tips in the ventral hippocampus; (b) schematic diagram of coronal sections of the brain at A 3.6 and 3.4 (Pellegrino et al., 1967) showing the anterior-posterior and vertical positions of all the electrodes. Abbreviations: CC, corpus callosum; DHIPP, dorsal hippocampus; FI, fimbria; LGEN, lateral geniculate bodies; SN, substantia nigra; V, ventricle; VHIPP, ventral hippocampus.



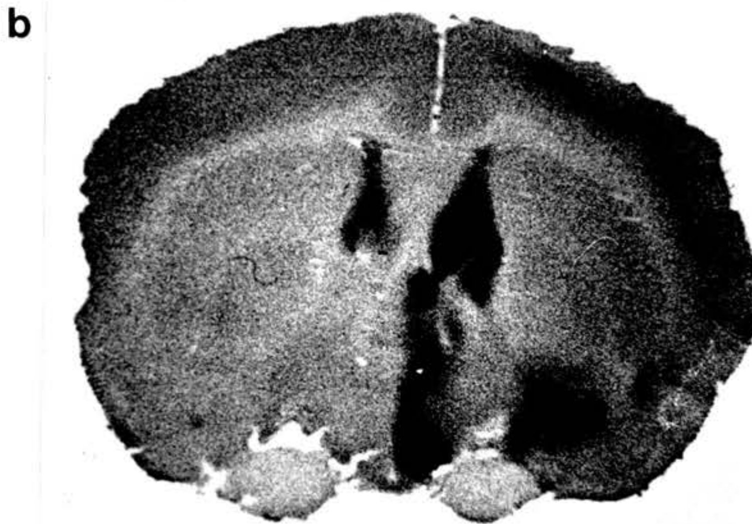
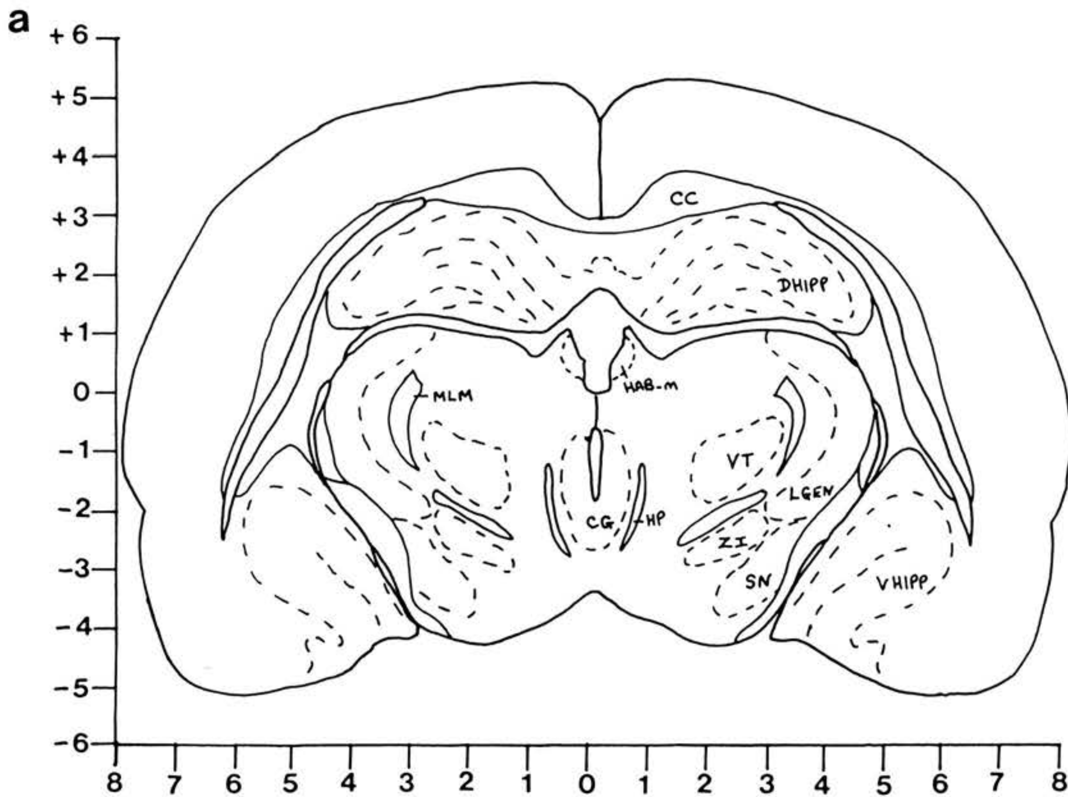


Fig. 6-P: (a) schematic diagram of a coronal section of the brain at A 6.8 (Pellegrino et al., 1967); (b) autoradiograph of a coronal section of the brain at the same level in a rat injected with [^{14}C]2-deoxyglucose and the ventral hippocampus stimulated.

Fig. 6-Q: (a) schematic diagram of a coronal section of the brain at A 3.2 (Pellegrino et al., 1967); autoradiographs of coronal sections of the brain at the same level in rats injected with [^{14}C]2-deoxyglucose in control unstimulated (b) and ventral hippocampus-stimulated (c) animals. Abbreviations: CC, corpus callosum; CG, central grey; DHIPP, dorsal hippocampus; HAB-m, medial habenular nucleus; HP, habenular-interpeduncular tract; LGEN, lateral geneiculate bodies; MLM, medial lemniscus; SN, substantia nigra; VHIPP, ventral hippocampus; VT, ventral thalamic area; ZI, zona incerta.



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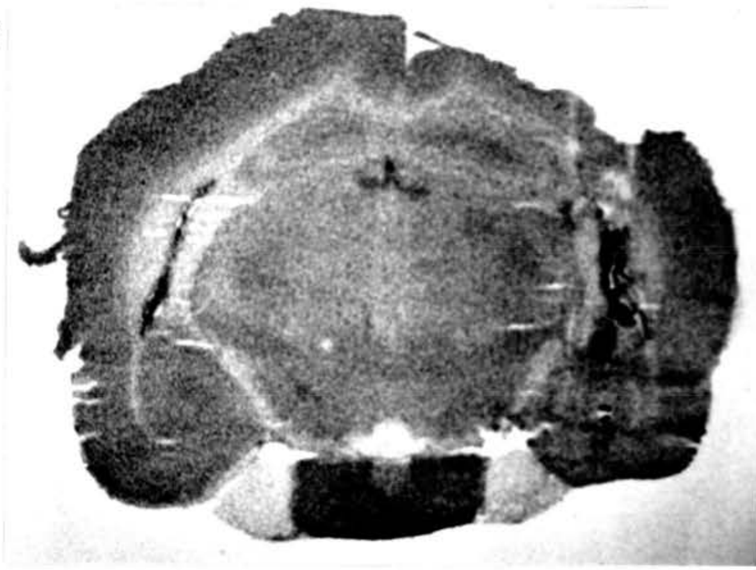
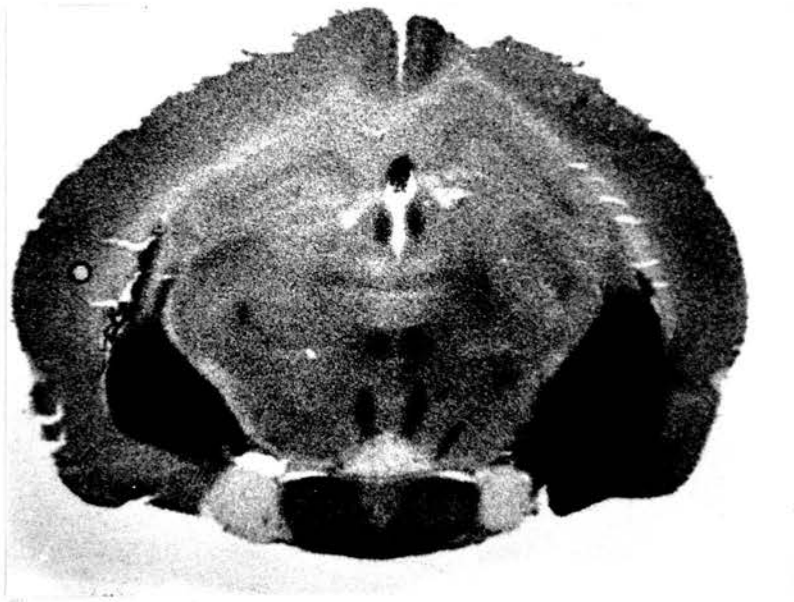


c



on the c side in VHIPP stimulated animals compared with control unstimulated animals (Table 6-5 and Figs. 6-P and 6-Q).

6.3.4.2.3 Comparison of the effects of electrical stimulation on areas on the side of the brain ipsilateral and contralateral to the implanted electrode

The rma of the i side was significantly greater than that of the c side in VHIPP stimulated animals in the following brain areas: S-1, POA-m, POA-l, AHA, FX, ST, ARC, VMH, DMH, SM, AMYG CO+M, AMYG CE+L, VHIPPM, and PVT (Table 6-5). There was no significant difference in rma between the two sides of brain in the VHIPPG (Table 6-5).

6.3.4.3 Stimulation of the dorsal hippocampus

Figure 6-R (a) shows a representative photomicrograph of the position of the electrode tips in the DHIPP. The anterior-posterior and vertical positions of the electrode tips in all the animals are shown in Fig. 6-R (b).

6.3.4.3.1 Differences between areas on the side of brain ipsilateral and contralateral to the implanted electrode in unstimulated animals

As in section 6.3.4.1, although there was increased 2DG uptake along the electrode track, there were no significant differences in rma between the 2 sides of brain in any area measured in the DHIPP unstimulated animals (Table 6-6).

6.3.4.3.2 Effect of electrical stimulation on areas on the side of brain ipsilateral to the implanted electrode

In DHIPP stimulated animals the rma of the following i brain areas increased compared with DHIPP unstimulated animals: S-1, DBB,

FX, DHIPPM, DHIPPG, VHIPPM, VHIPPG, AVT, LT and CX (Table 6-6).

6.3.4.3.3 Effect of electrical stimulation on areas on the side of brain contralateral to the implanted electrode

Table 6-6 shows that the rma of the S-1, DHIPPM, DHIPPG and VHIPPG increased on the c side in DHIPP stimulated animals compared with DHIPP unstimulated animals. The increased rma of these areas and some i areas are shown in Figs. 6-S and 6-T.

6.3.4.3.4 Comparison of the effects of electrical stimulation on areas on the side of brain ipsilateral and contralateral to the implanted electrode

The rma of the i side was significantly greater than that of the c side in the DHIPP stimulated animals in the following areas: DBB, FX, DHIPPM, AVT, LT and CX (Table 6-6). There was no significant difference in the rma of i and c sides in the S-1, DHIPPG and VHIPPG in DHIPP stimulated animals (Table 6-6).

6.3.4.3.5 Effects of electrical stimulation of the amygdala, ventral hippocampus and dorsal hippocampus on plasma concentration of luteinising hormone

There were no significant changes in plasma LH concentrations after 15 min and 45 min of electrical stimulation in AMYG stimulated, VHIPP stimulated and DHIPP stimulated animals, or in the combined control unstimulated animals and DHIPP unstimulated animals, and the concentrations of LH remained undetectable throughout the experiment.

6.3.5 Electrical stimulation and electrothermal lesioning of the ventrolateral geniculate body and the effect of a midbrain knife cut rostral to the raphe nuclei

Table 6-6. The relative metabolic activity of selected brain areas and the pituitary gland in anaesthetised rats after electrical stimulation of the dorsal hippocampus (DHIPP).

Values are expressed as a ratio (mean ± S.E.M.) of [^{14}C] concentration of selected region to [^{14}C] concentration of the corpus callosum.

	Type and number of animal			
	DHIPP control unstimulated (n=4)		DHIPP stimulated (n=4)	
	c	i	c	i
S-m	1.33±0.05		1.41±0.15	
S-1	1.20±0.04	1.20±0.04	5.73±0.24******	5.46±0.24******
DBB	1.34±0.08	1.34±0.08	1.53±0.07	1.87±0.07******
POA-m	1.23±0.03		1.17±0.12	
POA-1	1.21±0.03	1.20±0.03	1.14±0.13	1.21±0.18
SCN	1.98±0.06	1.98±0.05	1.69±0.22	1.95±0.18
SON	1.07±0.03	1.08±0.01	1.03±0.10	1.42±0.33
AHA	1.29±0.03	1.27±0.03	1.14±0.11	1.29±0.21
FX	1.11±0.02	1.10±0.02	1.25±0.10	3.00±0.81*
PVN	1.37±0.04	1.38±0.04	1.15±0.09	1.14±0.12
MFB	1.29±0.05	1.28±0.04	1.22±0.12	1.31±0.17
ST	1.05±0.01	1.14±0.03	1.12±0.07	1.51±0.30
ARC	1.25±0.01	1.22±0.01	1.10±0.08	1.08±0.13
VMH	1.19±0.01	1.22±0.01	1.08±0.07	1.20±0.16
DMH	1.29±0.03	1.30±0.03	1.15±0.10	1.17±0.14
SM	1.28±0.04	1.27±0.04	1.32±0.03	1.43±0.07

Significantly different from the corresponding side of control brain: * $P<0.05$; ** $P<0.025$; *** $P<0.02$; **** $P<0.01$; ***** $P<0.005$; ****** $P<0.001$

c, structure on the side of brain contralateral to the implanted electrode;
i, structure on the side of brain ipsilateral to the implanted electrode;

S-m, medial septum; S-1, lateral septum; DBB, diagonal band of Broca; POA-m, medial preoptic area; POA-1, lateral preoptic area; SCN, suprachiasmatic nucleus; SON, supraoptic nucleus, AHA, anterior hypothalamic area; FX, fornix; PVN, paraventricular nucleus; MFB, medial forebrain bundle; ST, stria terminalis; ARC, arcuate nucleus; VMH, ventromedial hypothalamic nucleus; DMH, dorsomedial hypothalamic nucleus; SM, stria medullaris

Table 6-6 (cont). The relative metabolic activity of selected brain areas and the pituitary gland in anaesthetised rats after electrical stimulation of the dorsal hippocampus (DHIPP).

Values are expressed as a ratio (mean ± S.E.M.) of [^{14}C] concentration of selected region to [^{14}C] concentration of the corpus callosum.

	Type and number of animal			
	DHIPP control unstimulated (n=4)		DHIPP stimulated (n=4)	
	c	i	c	i
AMYG CO+M	1.21±0.02	1.21±0.04	1.04±0.10	1.13±0.10
AMYG CE+L	1.20±0.03	1.19±0.02	1.10±0.10	1.20±0.11
IC	0.98±0.03		0.89±0.06	
MM	1.60±0.06		3.15±0.49	
IP	2.22±0.12		2.79±0.44	
PN	1.44±0.07		1.14±0.13	
PD	1.40±0.22		1.05±0.10	
DHIPPM	1.39±0.02	1.43±0.02	3.04±0.17*****	4.77±0.45******
DHIPPG	1.18±0.03	1.19±0.03	5.24±0.25******	5.23±0.42******
VHIPPM	1.51±0.08	1.49±0.05	2.04±0.19	3.00±0.55*
VHIPPG	1.24±0.05	1.26±0.03	4.47±0.56******	4.66±0.38******
HAB-m	2.01±0.06	1.98±0.06	1.97±0.24	1.98±0.26
HAB-l	1.90±0.11	1.89±0.12	1.74±0.15	1.87±0.25
PYRCX	1.14±0.03	1.16±0.02	1.14±0.07	1.14±0.04
CG	1.37±0.04	1.37±0.05	1.17±0.12	1.24±0.17
RF	1.41±0.04	1.42±0.05	1.17±0.12	1.25±0.13

Significantly different from the corresponding side of control brain: * $P<0.05$; ** $P<0.025$; *** $P<0.02$; **** $P<0.01$; ***** $P<0.005$; ****** $P<0.001$

c, structure on the side of brain contralateral to the implanted electrode;
i, structure on the side of brain ipsilateral to the implanted electrode;

AMYG CO+M, cortical and medial amygdaloid nuclei; AMYG CE+L, central and lateral amygdaloid nuclei; IC, internal capsule; MM, mamillary body; IP, interpeduncular nucleus; PN, pars nervosa, PD, pars distalis; DHIPPM, dorsal hippocampus (molecular layer); DHIPPG, dorsal hippocampus (granular layer); VHIPPM, ventral hippocampus (molecular layer); VHIPPG, ventral hippocampus (granular layer); HAB-m, medial habenular nucleus; HAB-l, lateral habenular nucleus; PYRCX, pyriform cortex; CG, central grey; RF, reticular formation

Table 6-6 (cont). The relative metabolic activity of selected brain areas and the pituitary gland in anaesthetised rats after electrical stimulation of the dorsal hippocampus (DHIPP).

Values are expressed as a ratio (mean ± S.E.M.) of [^{14}C] concentration of selected region to [^{14}C] concentration of the corpus callosum.

	Type and number of animal			
	DHIPP control unstimulated (n=4)		DHIPP stimulated (n=4)	
	c	i	c	i
CP	1.43±0.03	1.45±0.02	1.43±0.03	1.51±0.11
FRCX	1.46±0.04	1.44±0.03	1.28±0.08	1.46±0.08
AVT	1.42±0.02	1.44±0.04	1.65±0.10	4.20±0.48******
LT	1.42±0.05	1.47±0.06	1.65±0.23	4.77±0.69******
VT	1.73±0.10	1.74±0.09	1.44±0.15	2.08±0.33
MLM	1.50±0.09	1.53±0.09	1.28±0.07	1.71±0.23
LGEN	1.35±0.04	1.43±0.07	1.27±0.15	2.70±1.23
MGEN	1.60±0.06	1.59±0.06	1.34±0.11	2.33±0.91
CX	1.43±0.03	1.39±0.04	2.08±0.52	3.90±0.37******
CS	1.55±0.06	1.55±0.04	1.32±0.12	2.04±0.56

Significantly different from the corresponding side of control brain: * $P<0.05$; ** $P<0.025$; *** $P<0.02$; **** $P<0.01$; ***** $P<0.005$; ****** $P<0.001$

c, structure on the side of brain contralateral to the implanted electrode;
i, structure on the side of brain ipsilateral to the implanted electrode;

CP, caudate putamen, FRCX, frontal cortex; AVT, anteroventral thalamic area; LT, lateral thalamic area; VT, ventral thalamic area; MLM, medial lemniscus; LGEN, lateral geniculate bodies; MGEN, medial geniculate bodies; CX, visual cortex; CS, superior colliculus

Table 6-6 (cont). Significance of differences in the relative metabolic activity of brain areas and the pituitary gland in Table 6-6 (Duncan's multiple range test):

$P < 0.05$

DBB — i-DHIPP stimulated v c-DHIPP stimulated

FX — i-DHIPP stimulated v c-DHIPP stimulated, i-DHIPP unstimulated, c-DHIPP unstimulated

VHIPPM — i-DHIPP stimulated v i-DHIPP unstimulated, c-DHIPP unstimulated

$P < 0.005$

DHIPPM — c-DHIPP stimulated v i-DHIPP unstimulated, c-DHIPP unstimulated

CX — i-DHIPP stimulated v c-DHIPP stimulated

$P < 0.001$

S-1 — i-DHIPP stimulated v i-DHIPP unstimulated, c-DHIPP unstimulated
c-DHIPP stimulated v i-DHIPP unstimulated, c-DHIPP unstimulated

DBB — i-DHIPP stimulated v i-DHIPP unstimulated, c-DHIPP unstimulated

DHIPPM — i-DHIPP stimulated v c-DHIPP stimulated, i-DHIPP unstimulated, c-DHIPP unstimulated

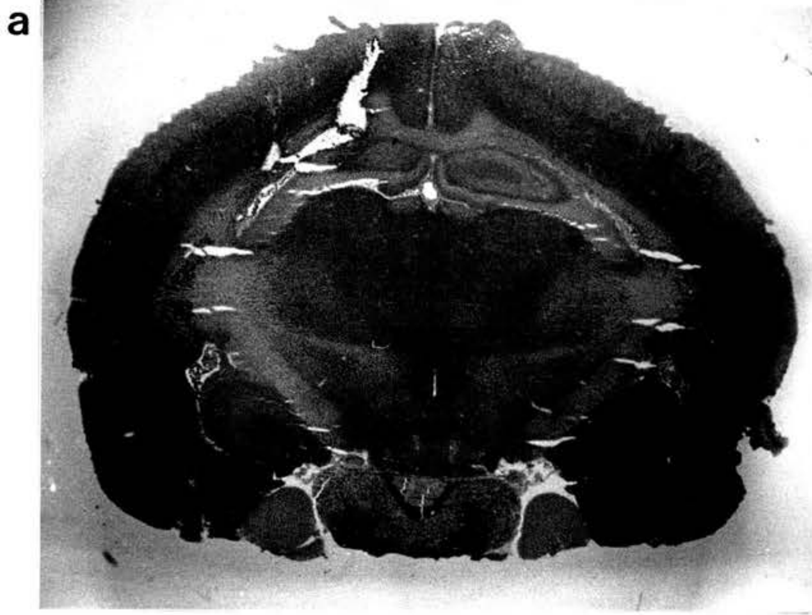
DHIPPG — i-DHIPP stimulated v i-DHIPP unstimulated, c-DHIPP unstimulated
c-DHIPP stimulated v i-DHIPP unstimulated, c-DHIPP unstimulated

VHIPPG — i-DHIPP stimulated v i-DHIPP unstimulated, c-DHIPP unstimulated
c-DHIPP stimulated v i-DHIPP unstimulated, c-DHIPP unstimulated

AVT — i-DHIPP stimulated v c-DHIPP stimulated, i-DHIPP unstimulated, c-DHIPP unstimulated

LT — i-DHIPP stimulated v c-DHIPP stimulated, i-DHIPP unstimulated, c-DHIPP unstimulated

CX — i-DHIPP stimulated v i-DHIPP unstimulated, c-DHIPP unstimulated



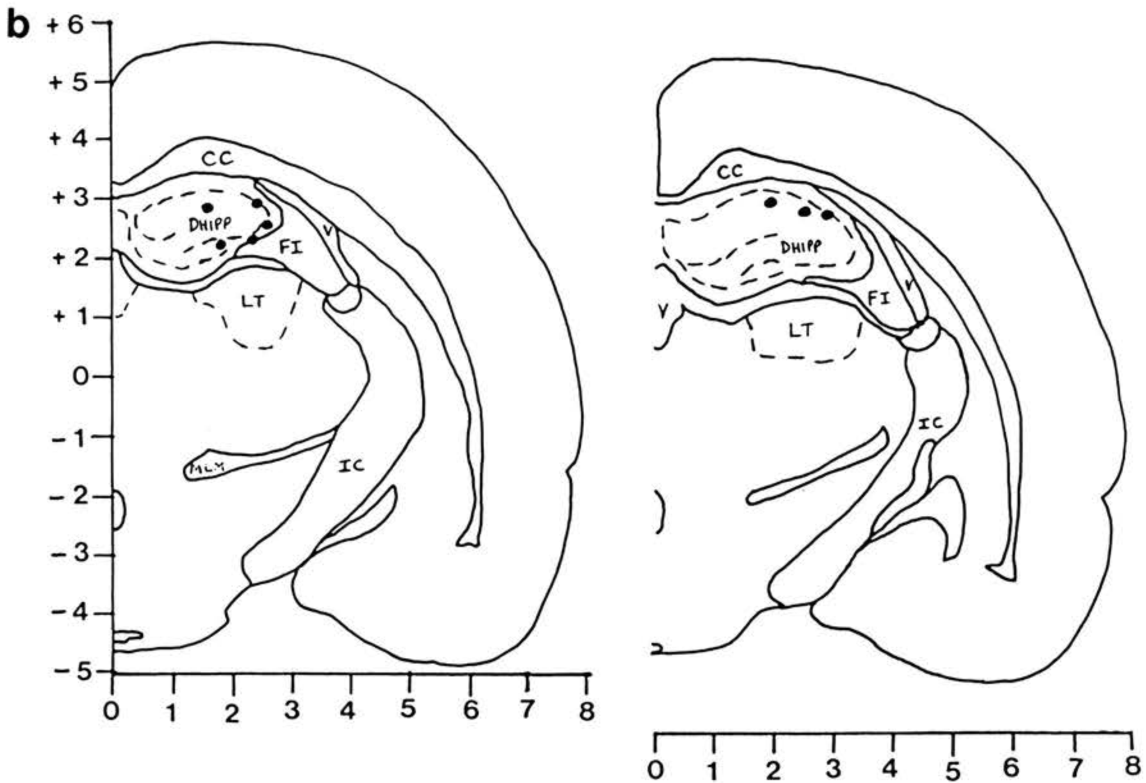
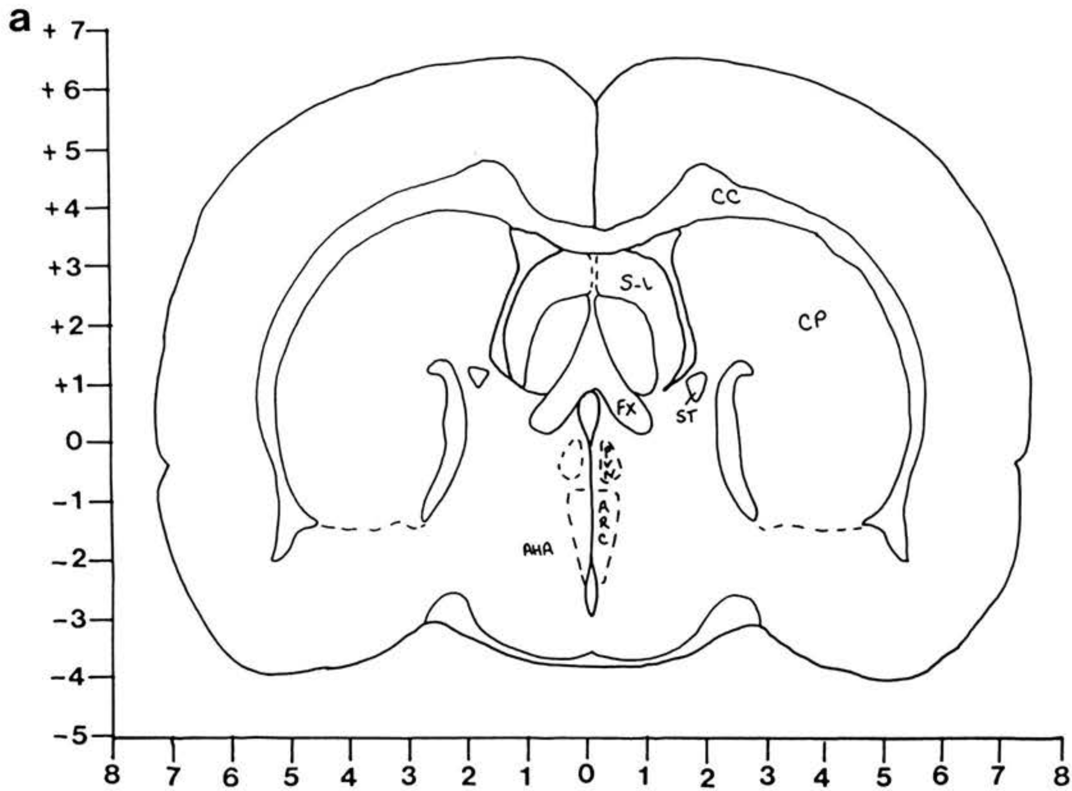


Fig. 6-R: (a) representative low power photomicrograph of a coronal section of the brain showing the position of the electrode tips in the dorsal hippocampus; (b) schematic diagram of coronal sections of the brain at A 4.4 and 4.0 (Pellegrino et al., 1967) showing the anterior-posterior and vertical positions of all the electrodes: Abbreviations: CC, corpus callosum; DHIPP, dorsal hippocampus; FI, fimbria; IC, internal capsule; LT, lateral thalamic area; MLM, medial lemniscus; V, ventricle.

a



b

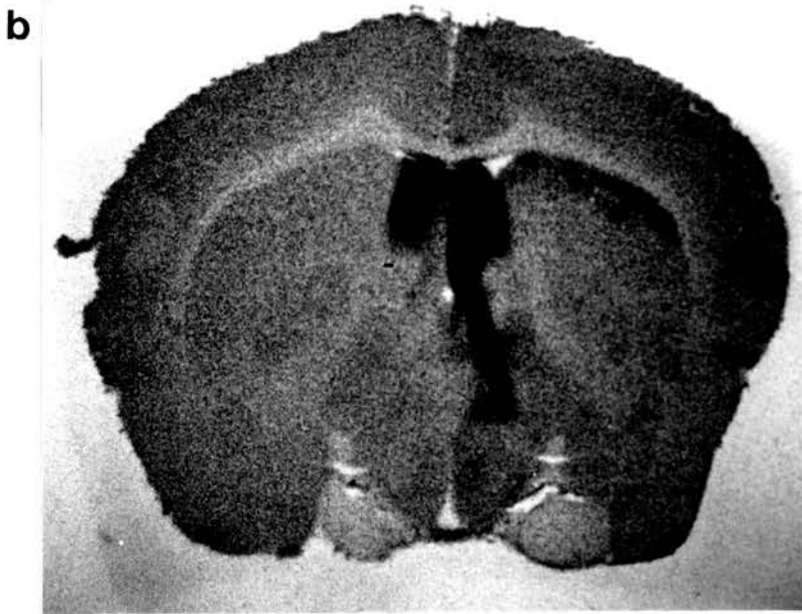
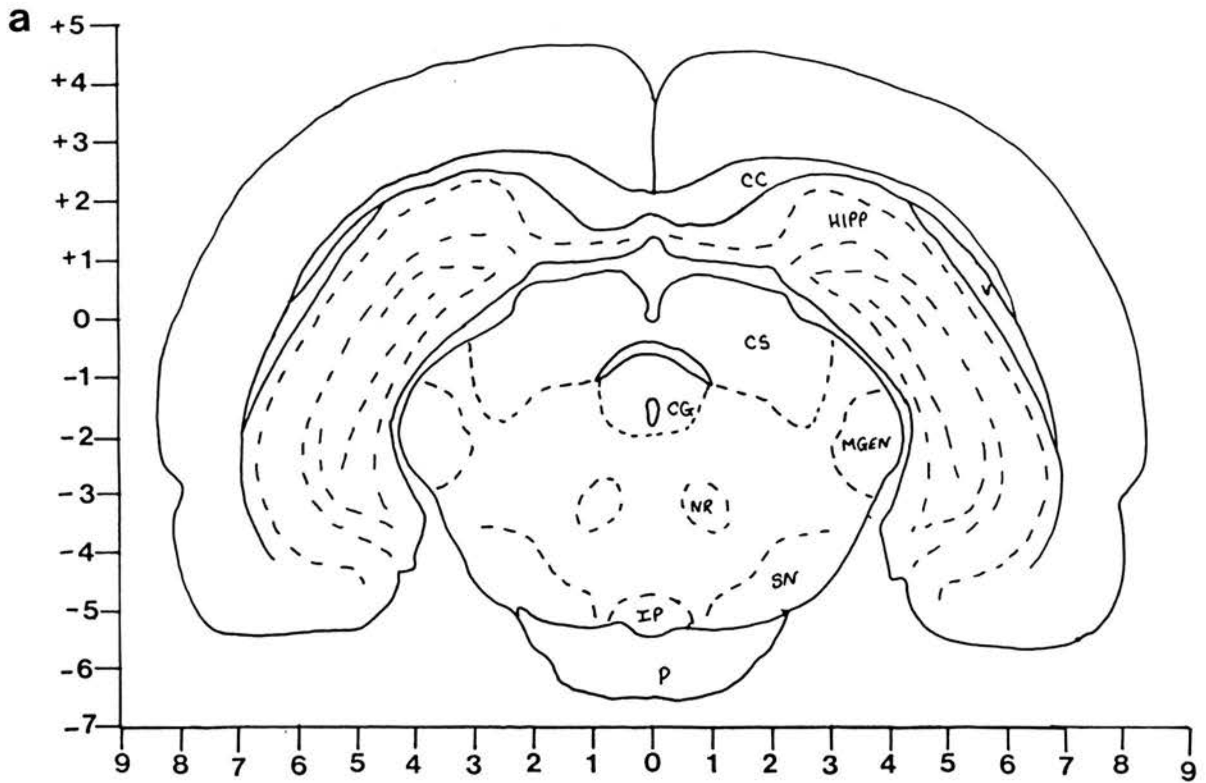


Fig. 6-S: (a) schematic diagram of a coronal section of the brain at A 6.8 (Pellegrino et al., 1967); (b) autoradiograph of a coronal section of the brain at the same level in a rat injected with [^{14}C]2-deoxyglucose and the dorsal hippocampus stimulated. Abbreviations: AHA, anterior hypothalamic area; ARC, arcuate nucleus; CC, corpus callosum; CP, caudate putamen; FX, fornix; PVN, paraventricular nucleus; S-l, lateral septum; ST, stria terminalis.



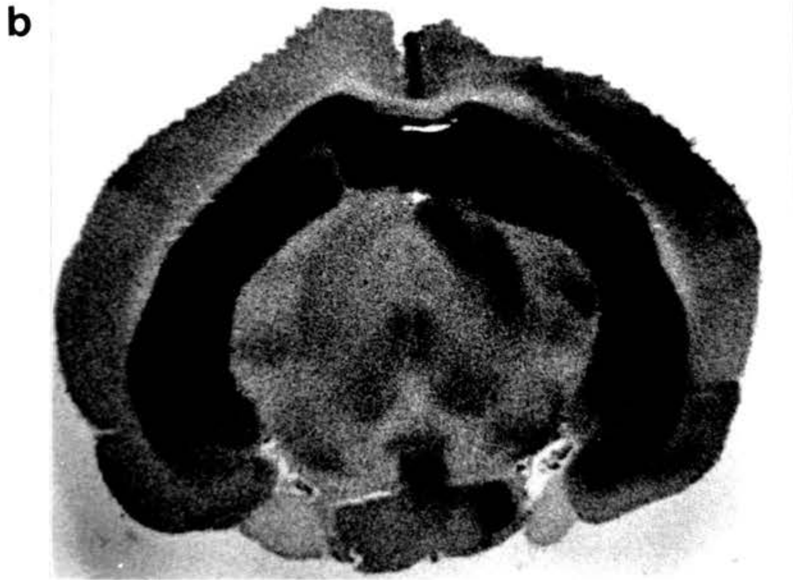


Fig. 6-T: (a) schematic diagram of a coronal section of the brain at A 1.8 (Pellegrino et al., 1967); (b) autoradiograph of a coronal section of the brain at the same level in a rat injected with [^{14}C]2-deoxyglucose and the dorsal hippocampus stimulated. Abbreviations: CC, corpus callosum; CG, central grey; CS, superior colliculus; HIPP, hipoocampus; IP, interpeduncular nucleus; MGEN, medial geniculate bodies; NR, red nucleus; P, pons; SN, substantia nigra; V, ventricle.

6.3.5.1 Stimulation of the ventrolateral geniculate body

Fig. 6-U (a) shows a representative photomicrograph of the position of the electrode tips in the VLGEN. The anterior-posterior and vertical positions of the electrode tips from all the animals included in this study are shown in Fig. 6-U (b).

6.3.5.1.1 Differences between areas on the side of brain ipsilateral and contralateral to the implanted electrode in unstimulated animals

The rma of the DHIPPG and VHIPPG were significantly ($P < 0.01$) increased in the i side compared with the c side in VLGEN unstimulated animals (Table 6-7).

6.3.5.1.2 Effects of electrical stimulation on areas on the side of brain ipsilateral to the implanted electrode

The rma of the following i brain areas increased in VLGEN stimulated animals compared with VLGEN unstimulated animals: DBB, POA-1, SCN, AHA, MFB, VMH, DMH, IC, CG, RF, CP, ZI, VMT, LT, LGEN, MGEN, CX, CS and CI (Table 6-7). In addition, the rma of the RE also increased in VLGEN stimulated animals compared with VLGEN unstimulated animals (Table 6-7). The increases in rma of some of the areas listed above are shown in Fig. 6-V.

6.3.5.1.3 Effect of electrical stimulation on areas on the side of brain contralateral to the implanted electrode

The rma of only 2 areas, the DBB and SCN, increased on the c side in VLGEN stimulated animals compared with VLGEN unstimulated animals. The increase in rma of the c and i SCN can be seen in Fig. 6-W.

6.3.5.1.4 Comparison of the effects of electrical stimulation on areas on the side of brain ipsilateral and contralateral to the implanted electrode

The rma of the i brain areas that increased after electrical stimulation of the VLGEN were all significantly higher than those of the c side (Table 6-7).

6.3.5.1.5 Effect of electrical stimulation of the ventrolateral geniculate body on plasma luteinising hormone concentration

The concentration of LH in plasma remained below the lower limit of detection of the assay (2.5 ng/ml) in VLGEN stimulated and VLGEN unstimulated animals.

6.3.5.2 Lesioning of the ventrolateral geniculate body

Fig. 6-X (a) shows a representative photomicrograph of the position and size of the lesion in the VLGEN. The anterior-posterior and vertical positions and sizes of the lesions in all the animals are shown in Fig. 6-X (b).

6.3.5.2.1 Effect of lesioning on areas on the side of brain ipsilateral to the lesion

Table 6-7 shows that the rma of the i FX, AMYG CE+L, DHIPPG and VHIPPG were significantly higher in VLGEN lesioned animals compared with VLGEN unstimulated animals. The increase in rma along the electrode track and the site of the lesion are shown in Fig. 6-X (c).

6.3.5.2.2 Effect of lesioning on areas on the side of brain contralateral to the lesion

The rma of the c DBB, FX, DHIPPG and VHIPPG were significantly higher, and the rma of the c CI was significantly lower in VLGEN lesioned animals compared with VLGEN unstimulated animals

(Table 6-7).

6.3.5.2.3 Differences between areas on the side of brain ipsilateral and contralateral to the lesion

The rma of the AMYG CE+L, IC, DHIPPM, VHIPPM and VHIPPG were significantly higher on the i side to the lesion than on the c side in VLGEN lesioned animals (Table 6-7).

6.3.5.3 Midbrain knife cut rostral to the raphe nuclei

Fig. 6-Y (a) shows a representative photomicrograph of the position and size of the midbrain knife cut. The anterior-posterior and vertical positions of the cuts from all the animals in this study are shown in Fig. 6-Y (b).

6.3.5.3.1 Effect of the knife cut on areas on the side of brain ipsilateral to the cut

The rma of the i FX and VHIPPG were significantly increased in raphe cut animals compared with VLGEN unstimulated animals (Table 6-7). In addition, the rma of the PD was significantly decreased in raphe cut animals compared with VLGEN unstimulated animals.

6.3.5.3.2 Effect of the knife cut on areas on the side of brain contralateral to the cut

The rma of the c SCN and DHIPPG were significantly increased in raphe cut animals compared with VLGEN unstimulated animals (Table 6-7).

6.3.5.3.3 Differences between areas on the 2 sides of brain

The rma of the DHIPPG and VHIPPG were significantly greater on the i side than on the c side in raphe cut animals (Table 6-7).

Table 6-7. The relative metabolic activity of selected brain areas and the pituitary gland in anaesthetised rats after electrical stimulation or electrothermal lesioning of the ventrolateral geniculate body (VLGEN), and a midbrain knife cut immediately rostral to the raphe nuclei (Raphe cut).

Values are expressed as a ratio (mean ± S.E.M.) of [^{14}C] concentration of the selected region to [^{14}C] concentration of the corpus callosum.

	Type and number of animal							
	VLGEN control unstimulated (n=4)		VLGEN stimulated (n=6)		VLGEN lesioned (n=5)		Raphe cut (n=5)	
	c	i	c	i	c	i	c	i
S-m	1.35±0.02		1.38±0.02		1.43±0.03		1.42±0.04	
S-1	1.19±0.02	1.22±0.02	1.19±0.03	1.23±0.04	1.26±0.02	1.27±0.03	1.22±0.01	1.25±0.02
DBB	1.30±0.03	1.36±0.07	1.43±0.05*	1.71±0.07******	1.42±0.04	1.43±0.04	1.41±0.03*	1.43±0.03
POA-m	1.25±0.01		1.28±0.05		1.32±0.03		1.30±0.03	
POA-1	1.23±0.02	1.22±0.02	1.28±0.03	1.46±0.07******	1.27±0.03	1.28±0.04	1.26±0.02	1.25±0.03
SCN	1.80±0.12	1.90±0.07	2.18±0.14*	2.52±0.21*****	2.06±0.07	2.09±0.08	2.16±0.09*	2.17±0.10
SON	1.07±0.02	1.10±0.02	1.09±0.17	1.12±0.15	1.08±0.06	1.11±0.05	1.07±0.07	1.07±0.08
AHA	1.28±0.02	1.28±0.01	1.30±0.03	1.62±0.11******	1.33±0.03	1.35±0.04	1.29±0.02	1.29±0.02

Significantly different from the corresponding side of the control brain: * P<0.05; ** P<0.025; *** P<0.02
**** P<0.01; ***** P<0.005; ****** P<0.01

c, structure on the side of brain contralateral to the implanted electrode or lesion;
i, structure on the side of brain ipsilateral to the implanted electrode or lesion;

S-m, medial septum; S-1, lateral septum; DBB, diagonal band of Broca; POA-m, medial preoptic area;
POA-1, lateral preoptic area; SCN, suprachiasmatic nucleus; SON, supraoptic nucleus; AHA, anterior hypothalamic area

Table 6-7 (cont). The relative metabolic activity of selected brain areas and the pituitary gland in anaesthetised rats after electrical stimulation or electrothermal lesioning of the ventrolateral geniculate body (VLGEN), and a midbrain knife cut immediately rostral to the raphe nuclei (Raphe cut).

Values are expressed as a ratio (mean ± S.E.M.) of [^{14}C] concentration of the selected region to [^{14}C] concentration of the corpus callosum.

Type and number of animal

	VLGEN control unstimulated (n=4)		VLGEN stimulated (n=6)		VLGEN lesioned (n=5)		Raphe cut (n=5)	
	c	i	c	i	c	i	c	i
FX	1.10±0.03	1.09±0.01	1.05±0.02	1.05±0.02	1.17±0.02*	1.17±0.02****	1.14±0.01	1.17±0.02****
PVN	1.35±0.04	1.34±0.04	1.32±0.04	1.44±0.07	1.39±0.04	1.39±0.04	1.39±0.01	1.42±0.01
MFB	1.24±0.03	1.27±0.02	1.28±0.04	1.63±0.14******	1.31±0.02	1.38±0.02	1.31±0.02	1.34±0.03
ST	1.05±0.02	1.12±0.02	1.06±0.02	1.18±0.05	1.09±0.03	1.21±0.02	1.08±0.04	1.15±0.03
ARC	1.30±0.05	1.31±0.06	1.32±0.03	1.41±0.04	1.31±0.01	1.28±0.01	1.30±0.06	1.29±0.06
VMH	1.22±0.04	1.25±0.04	1.32±0.04	1.96±0.11******	1.23±0.02	1.30±0.03	1.26±0.04	1.27±0.04
DMH	1.27±0.02	1.29±0.03	1.38±0.03	1.94±0.10******	1.35±0.07	1.39±0.08	1.37±0.03	1.38±0.03
SM	1.20±0.02	1.24±0.03	1.29±0.04	1.33±0.03	1.28±0.02	1.28±0.02	1.34±0.03	1.31±0.02

Significantly different from the corresponding side of the control brain: * P<0.05; ** P<0.025; *** P<0.02 **** P<0.01; ***** P<0.005; ****** P<0.01

c, structure on the side of brain contralateral to the implanted electrode or lesion;
i, structure on the side of brain ipsilateral to the implanted electrode or lesion;

FX, fornix; PVN, paraventricular nucleus; MFB, medial forebrain bundle; ST, stria terminalis; ARC, arcuate nucleus; VMH, ventromedial hypothalamic nucleus, DMH, dorsomedial hypothalamic nucleus; SM, stria medullaris

Table 6-7 (cont). The relative metabolic activity of selected brain areas and the pituitary gland in anaesthetised rats after electrical stimulation or electrothermal lesioning of the ventrolateral geniculate body (VLGEN), and a midbrain knife cut immediately rostral to the raphe nuclei (Raphe cut).

Values are expressed as a ratio (mean ± S.E.M.) of [^{14}C] concentration of the selected region to [^{14}C] concentration of the corpus callosum.

Type and number of animal

	VLGEN control unstimulated (n=4)		VLGEN stimulated (n=6)		VLGEN lesioned (n=5)		Raphe cut (n=5)	
	c	i	c	i	c	i	c	i
AMYG CO+M	1.15±0.02	1.25±0.02	1.15±0.03	1.31±0.08	1.20±0.03	1.34±0.03	1.26±0.05	1.25±0.03
AMYG CE+L	1.19±0.01	1.26±0.03	1.16±0.03	1.31±0.07	1.22±0.02	1.38±0.02*	1.24±0.04	1.25±0.03
IC	0.94±0.03	0.99±0.01	0.95±0.02	1.22±0.05******	0.96±0.02	1.05±0.02	0.96±0.02	1.03±0.01
MM	1.60±0.04		1.61±0.04		1.67±0.05		1.68±0.03	
IP	2.20±0.09		2.19±0.10		2.44±0.23		2.09±0.04	
PN	1.41±0.06		1.68±0.12		1.36±0.04		1.43±0.04	
PD	2.02±0.35		1.34±0.12		1.72±0.16		1.30±0.05*	

Significantly different from the corresponding side of the control brain: * $P<0.05$; ** $P<0.025$; *** $P<0.02$
**** $P<0.01$; ***** $P<0.005$; ****** $P<0.01$

c, structure on the side of brain contralateral to the implanted electrode or lesion;
i, structure on the side of brain ipsilateral to the implanted electrode or lesion;

AMYG CO+M, cortical and medial amygdaloid nuclei; AMYG CE+L, central and lateral amygdaloid nuclei; IC, internal capsule; MM, mamillary body; IP, interpeduncular nucleus; PN, pars nervosa; PD, pars distalis

Table 6-7 (cont). The relative metabolic activity of selected brain areas and the pituitary gland in anaesthetised rats after electrical stimulation or electrothermal lesioning of the ventrolateral geniculate body (VLGEN), and a midbrain knife cut immediately rostral to the raphe nuclei (Raphe cut).

Values are expressed as a ratio (mean ± S.E.M.) of [^{14}C] concentration of the selected region to [^{14}C] concentration of the corpus callosum.

	Type and number of animal							
	VLGEN control unstimulated (n=4)		VLGEN stimulated (n=6)		VLGEN lesioned (n=5)		Raphe cut (n=5)	
	c	i	c	i	c	i	c	i
DHIPPM	1.39±0.01	1.47±0.03	1.37±0.04	1.43±0.04	1.44±0.02	1.53±0.02	1.42±0.05	1.47±0.06
DHIPPG	1.16±0.01	1.26±0.02	1.18±0.04	1.22±0.04	1.23±0.02*	1.35±0.02*****	1.22±0.01	1.30±0.03
VHIPPM	1.40±0.03	1.48±0.03	1.39±0.03	1.40±0.03	1.47±0.03	1.59±0.03	1.47±0.04	1.56±0.05
VHIPPG	1.17±0.02	1.28±0.03	1.19±0.03	1.23±0.04	1.24±0.02*	1.39±0.02******	1.23±0.02	1.34±0.02
HAB-m	1.92±0.04	1.94±0.07	1.98±0.08	1.92±0.08	2.05±0.09	2.05±0.07	1.95±0.11	1.93±0.12
HAB-1	1.72±0.10	1.77±0.10	1.90±0.05	1.88±0.03	1.75±0.08	1.80±0.09	1.68±0.06	1.67±0.05
PYRCX	1.18±0.04	1.21±0.02	1.24±0.05	1.26±0.06	1.21±0.03	1.17±0.04	1.24±0.01	1.20±0.07
CG	1.33±0.02	1.37±0.02	1.55±0.04	2.99±0.25******	1.39±0.05	1.44±0.04	1.49±0.03	1.48±0.03

Significantly different from the corresponding side of the control brain: * P<0.05; ** P<0.025; *** P<0.02
**** P<0.01; ***** P<0.005; ****** P<0.01

c, structure on the side of brain contralateral to the implanted electrode or lesion;
i, structure on the side of brain ipsilateral to the implanted electrode or lesion;

DHIPPM, dorsal hippocampus (molecular layer); DHIPPG, dorsal hippocampus (granular layer);
VHIPPM, ventral hippocampus (molecular layer); VHIPPG, ventral hippocampus (granular layer);
HAB-m, medial habenular nucleus; HAB-1, lateral habenular nucleus; PYRCX, pyriform cortex; CG, central grey

Table 6-7 (cont). The relative metabolic activity of selected brain areas and the pituitary gland in anaesthetised rats after electrical stimulation or electrothermal lesioning of the ventrolateral geniculate body (VLGEN), and a midbrain knife cut immediately rostral to the raphe nuclei (Raphe cut).

Values are expressed as a ratio (mean ± S.E.M.) of $[^{14}C]$ concentration of the selected region to $[^{14}C]$ concentration of the corpus callosum.

	Type and number of animal							
	VLGEN control unstimulated (n=4)		VLGEN stimulated (n=6)		VLGEN lesioned (n=5)		Raphe cut (n=5)	
	c	i	c	i	c	i	c	i
RF	1.34±0.02	1.36±0.03	1.56±0.04	3.29±0.20******	1.41±0.04	1.46±0.05	1.49±0.02	1.53±0.03
CP	1.37±0.02	1.43±0.05	1.37±0.03	2.02±0.17******	1.43±0.02	1.55±0.03	1.45±0.02	1.53±0.03
RE	1.34±0.02		2.12±0.05******		1.44±0.04		1.45±0.04	
ZI	1.30±0.02	1.39±0.02	1.55±0.05	5.18±0.63******	1.50±0.04	1.57±0.05	1.68±0.05	1.74±0.06
VMT	1.60±0.04	1.69±0.06	1.79±0.10	5.16±0.64******	1.77±0.05	1.81±0.12	1.72±0.04	1.85±0.05
LT	1.39±0.02	1.51±0.08	1.43±0.05	2.99±0.24******	1.49±0.04	1.61±0.04	1.59±0.02	1.63±0.03
LGEN	1.35±0.01	1.36±0.02	1.42±0.06	4.95±0.77******	1.39±0.01	--	1.51±0.03	1.60±0.06
MGEN	1.51±0.01	1.59±0.03	1.62±0.06	5.65±0.74******	1.60±0.03	1.83±0.09	1.64±0.04	1.67±0.04

Significantly different from the corresponding side of the control brain: * P<0.05; ** P<0.025; *** P<0.02
**** P<0.01; ***** P<0.005; ****** P<0.01

c, structure on the side of brain contralateral to the implanted electrode or lesion;
i, structure on the side of brain ipsilateral to the implanted electrode or lesion;

RF, reticular formation; CP, caudate putamen; RE, reuniens thalamic nucleus; ZI, zona incerta; VMT, ventromedial thalamic area; LT, lateral thalamic area; LGEN, lateral geniculate body, MGEN, medial geniculate body

Table 6-7 (cont). The relative metabolic activity of selected brain areas and the pituitary gland in anaesthetised rats after electrical stimulation or electrothermal lesioning of the ventrolateral geniculate body (VLGEN), and a midbrain knife cut immediately rostral to the raphe nuclei (Raphe cut).

Values are expressed as a ratio (mean ± S.E.M.) of [^{14}C] concentration of the selected region to [^{14}C] concentration of the corpus callosum.

	Type and number of animal							
	VLGEN control unstimulated (n=4)		VLGEN stimulated (n=6)		VLGEN lesioned (n=5)		Raphe cut (n=5)	
	c	i	c	i	c	i	c	i
CX	1.31±0.02	1.36±0.04	1.27±0.05	3.89±0.36******	1.41±0.02	1.45±0.03	1.46±0.03	1.53±0.02
DR	1.54±0.04		1.80±0.06		1.48±0.03		1.88±0.19	
CS	1.44±0.02	1.50±0.01	1.70±0.05	4.40±0.28******	1.53±0.05	1.60±0.04	1.63±0.02	1.65±0.03
CI	2.38±0.09	2.48±0.09	1.98±0.14	2.87±0.21	1.90±0.09	2.26±0.19	2.00±0.14	2.09±0.17
PIN	1.62±0.05		1.85±0.11		1.67±0.09		1.76±0.10	

Significantly different from the corresponding side of the control brain: * P<0.05; ** P<0.025; *** P<0.02 **** P<0.01; ***** P<0.005; ****** P<0.01

c, structure on the side of brain contralateral to the implanted electrode or lesion;
i, structure on the side of brain ipsilateral to the implanted electrode or lesion;

CX, visual cortex; DR, dorsal raphe nucleus; CS, superior colliculus; CI, inferior colliculus;
PIN, pineal gland

Table 6-7 (cont). Significance of differences in the relative metabolic activity of brain areas and the pituitary gland in Table 6-7 (Duncan's multiple range test):

$P < 0.05$

DBB	c-VLGEN stimulated	v c-VLGEN unstimulated
	c-VLGEN unstimulated	v c-VLGEN stimulated, i-VLGEN lesioned, v i-raphe cut, c-raphe cut
SCN	i-VLGEN stimulated	v c-VLGEN stimulated, i-VLGEN lesioned, c-VLGEN lesioned, i-raphe cut, c-raphe cut
	c-VLGEN stimulated	v i-VLGEN stimulated, c-VLGEN unstimulated
	i-raphe cut	v i-VLGEN stimulated, c-VLGEN unstimulated
	c-raphe cut	v i-VLGEN stimulated, c-VLGEN unstimulated
FX	c-VLGEN unstimulated	v i-VLGEN lesioned, c-VLGEN lesioned, i-raphe cut
ST	i-VLGEN stimulated	v c-VLGEN unstimulated
	i-VLGEN lesioned	v c-VLGEN stimulated, c-raphe cut, c-VLGEN unstimulated
AMYG CE+L	i-VLGEN stimulated	v c-VLGEN stimulated, c-VLGEN unstimulated
	i-VLGEN lesioned	v c-VLGEN lesioned, i-raphe cut, c-raphe cut, i-VLGEN unstimulated
IC	i-VLGEN lesioned	v c-VLGEN stimulated, c-VLGEN lesioned, c-raphe cut
	i-raphe cut	v c-VLGEN unstimulated
PD	raphe cut	v VLGEN unstimulated
DHIPPG	c-VLGEN lesioned	v i-raphe cut, c-VLGEN unstimulated
VHIPPM	i-VLGEN lesioned	v c-VLGEN lesioned, c-raphe cut
	i-raphe cut	v i-VLGEN stimulated, c-VLGEN unstimulated
VHIPPG	c-VLGEN lesioned	v c-VLGEN unstimulated
	i-raphe cut	v i-VLGEN unstimulated
DR	raphe cut	v VLGEN unstimulated

Table 6-7 (cont). <u>Significance of differences in the relative metabolic activity of brain areas and the pituitary gland in Table 6-7 (Duncan's multiple range test)</u>:

$P < 0.01$

FX	i-VLGEN unstimulated v i-VLGEN lesioned, c-VLGEN lesioned i-raphe cut
IC	i-VLGEN lesioned v c-VLGEN unstimulated
DHIPPG	c-VLGEN stimulated v i-VLGEN unstimulated i-raphe cut v i-VLGEN stimulated, c-raphe cut
VHIPPM	c-VLGEN stimulated v i-raphe cut i-VLGEN lesioned v c-VLGEN unstimulated
CI	c-VLGEN lesioned v i-VLGEN unstimulated

$P < 0.005$

SCN	i-VLGEN stimulated v i-VLGEN unstimulated
FX	c-raphe cut v i-VLGEN stimulated, c-VLGEN stimulated
MFB	i-VLGEN stimulated v i-VLGEN lesioned
AMYG CE+L	i-VLGEN lesioned v c-VLGEN stimulated, c-VLGEN unstimulated
DHIPPG	i-VLGEN unstimulated v i-VLGEN lesioned, c-VLGEN unstimulated
VHIPPM	i-VLGEN lesioned v i-VLGEN stimulated, c-VLGEN stimulated
VHIPPG	c-VLGEN lesioned v i-raphe cut i-VLGEN unstimulated v c-VLGEN stimulated, c-VLGEN unstimulated
CI	i-VLGEN stimulated v i-VLGEN lesioned

$P < 0.001$

DBB	i-VLGEN stimulated v all groups

Table 6-7 (cont). Significance of differences in the relative metabolic activity of brain areas and the pituitary gland in Table 6-7 (Duncan's multiple range test):

POA-L — i-VLGEN stimulated v all groups

SCN — i-VLGEN stimulated v c-VLGEN unstimulated

AHA — i-VLGEN stimulated v all groups

FX — i-VLGEN stimulated v i-VLGEN lesioned, c-VLGEN lesioned, i-raphe cut
c-VLGEN stimulated v i-VLGEN lesioned, c-VLGEN lesioned, i-raphe cut

MFB — i-VLGEN stimulated v c-VLGEN stimulated, c-VLGEN lesioned, i-raphe cut, c-raphe cut, i-VLGEN unstimulated c-VLGEN unstimulated

VMH — i-VLGEN stimulated v all groups

DMH — i-VLGEN stimulated v all groups

IC — i-VLGEN stimulated v all groups

DHIPPG — i-VLGEN lesioned v i-VLGEN stimulated, c-VLGEN stimulated, c-VLGEN lesioned, c-raphe cut, c-VLGEN unstimulated
i-raphe cut v c-VLGEN stimulated, c-VLGEN unstimulated

VHIPPG — i-VLGEN lesioned v i-VLGEN stimulated, c-VLGEN stimulated, i-VLGEN lesioned, c-VLGEN lesioned, c-raphe cut, i-VLGEN unstimulated, c-VLGEN unstimulated
i-raphe cut v i-VLGEN stimulated, c-VLGEN stimulated, c-raphe cut, c-VLGEN unstimulated

CG — i-VLGEN stimulated v all groups

RF — i-VLGEN stimulated v all groups

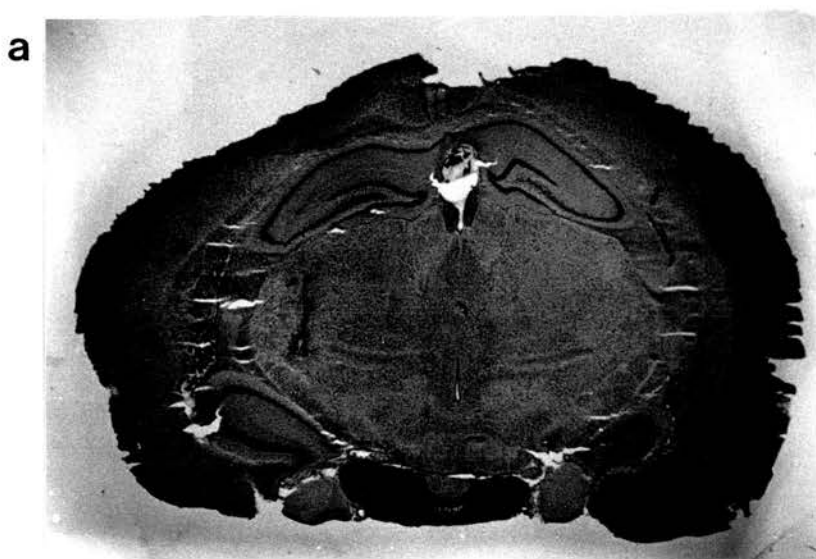
CP — i-VLGEN stimulated v all groups

RE — i-VLGEN stimulated v all groups

Table 6-7 (cont). Significance of differences in the relative metabolic activity of brain areas and the pituitary gland in Table 6-7 (Duncan's multiple range test):

ZI	i-VLGEN stimulated v all groups
VMT	i-VLGEN stimulated v all groups
LT	i-VLGEN stimulated v all groups
LGEN	i-VLGEN stimulated v all groups
MGEN	i-VLGEN stimulated v all groups
CX	i-VLGEN stimulated v all groups
CS	i-VLGEN stimulated v all groups
CI	i-VLGEN stimulated v c-VLGEN stimulated, c-VLGEN lesioned, i-raphe cut, c-raphe cut

a



b

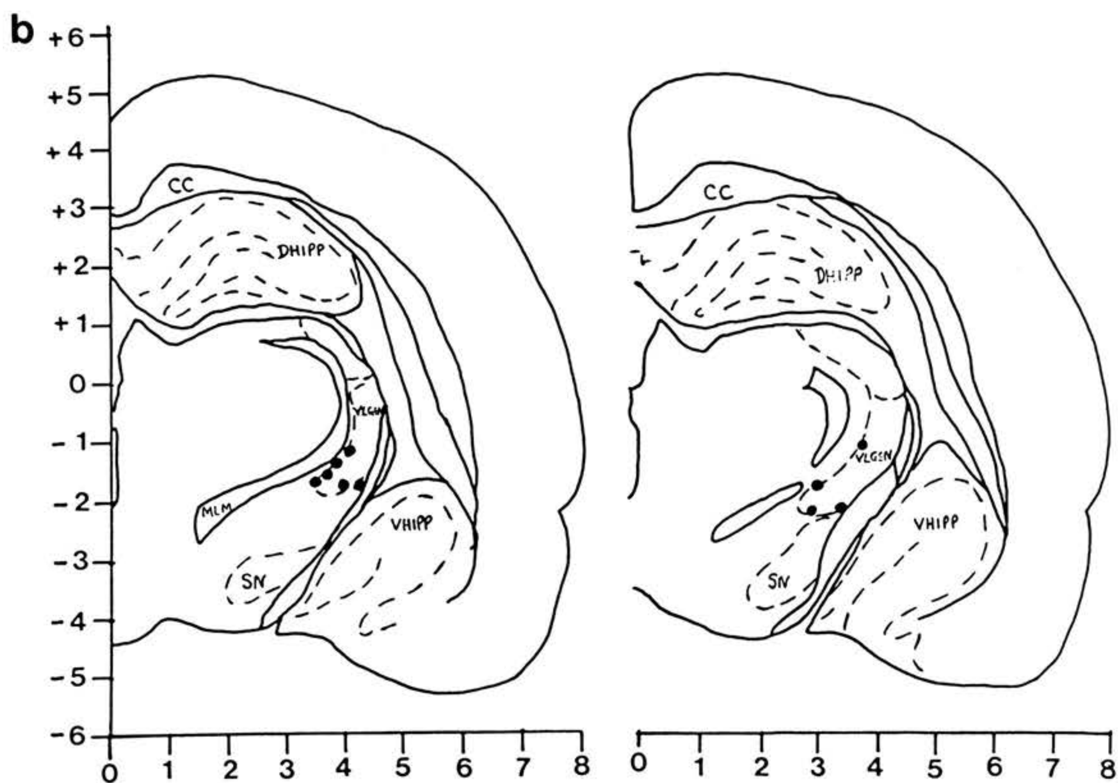


Fig. 6-U: (a) representative low power photomicrograph of a coronal section of the brain showing the position of the electrode tips in the ventrolateral geniculate body; (b) schematic diagram of coronal sections of the brain at A 3.4 and 3.2 (Pellegrino et al., 1967) showing the anterior-posterior and vertical positions of all the electrodes. Abbreviations: CC, corpus callosum; DHIPP, dorsal hippocampus; MLM, medial lemniscus; SN, substantia nigra; VHIPP, ventral hippocampus; VLGEN, ventrolateral geniculate body.

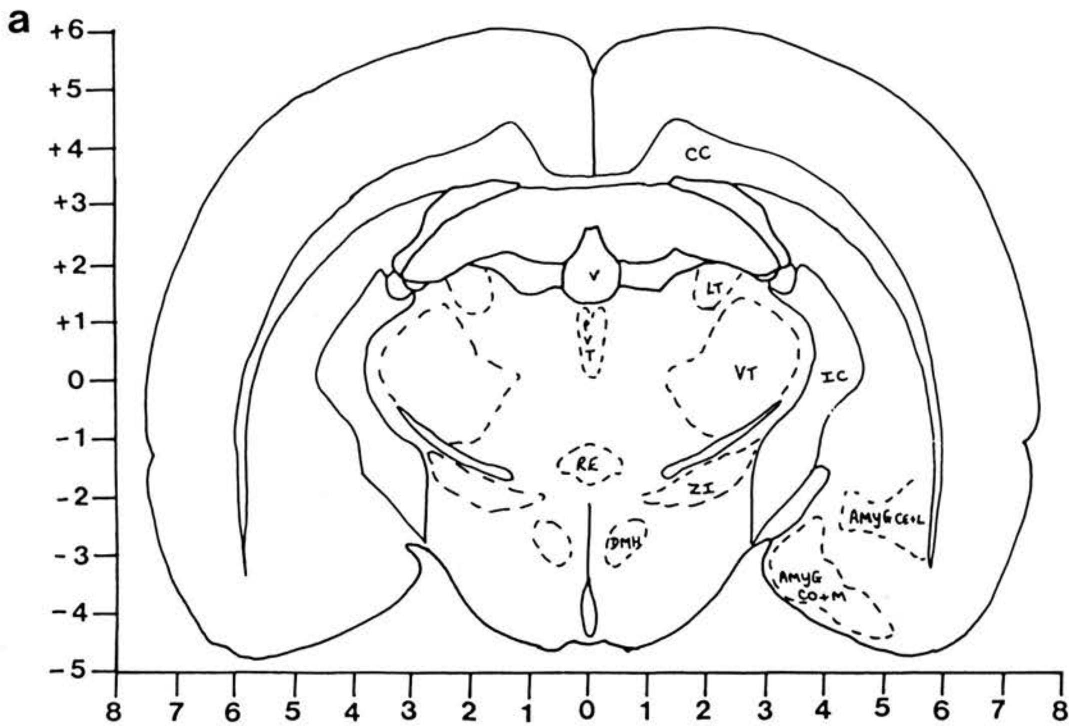
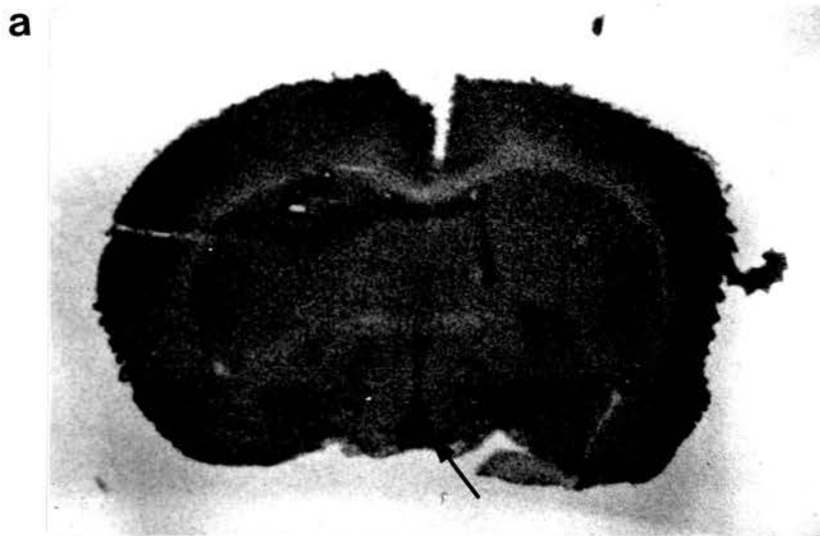


Fig. 6-V: (a) schematic diagram of a coronal section of the brain at A 6.8 (Pellegrino et al., 1967); (b) autoradiograph of a coronal section of the brain at the same level in a rat injected with [^{14}C]2-deoxyglucose an the ventrolateral geniculate body stimulated. Abbreviations: AMYG CE+L, central and lateral amygdaloid nuclei; AMYG CO+M, cortical and medial amygdaloid nuclei; CC, corpus callosum; DMH, dorsomedial hypothalamic nucleus; IC, internal capsule; LT, lateral thalamic area; PVT, paraventricular thalamic area; RE, reuniens thalamic nucleus: V, ventricle; VT, ventral thalamic area; ZI, zona incerta.

a

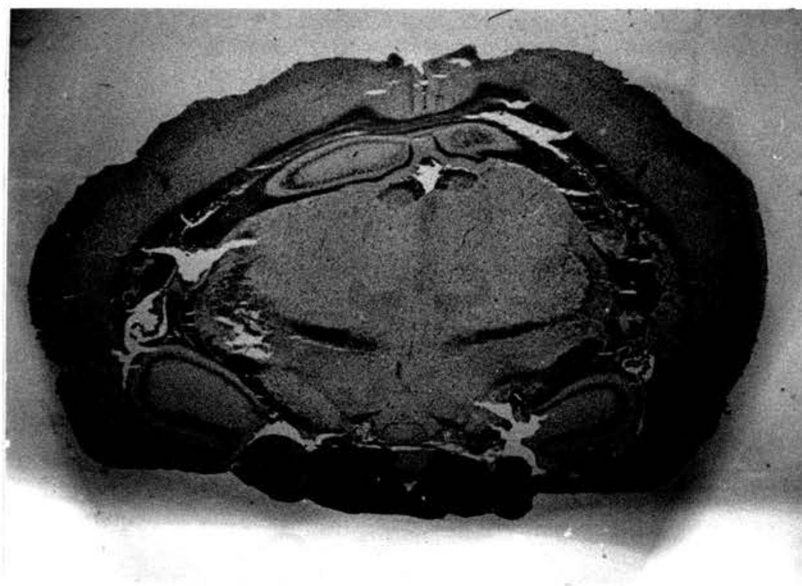


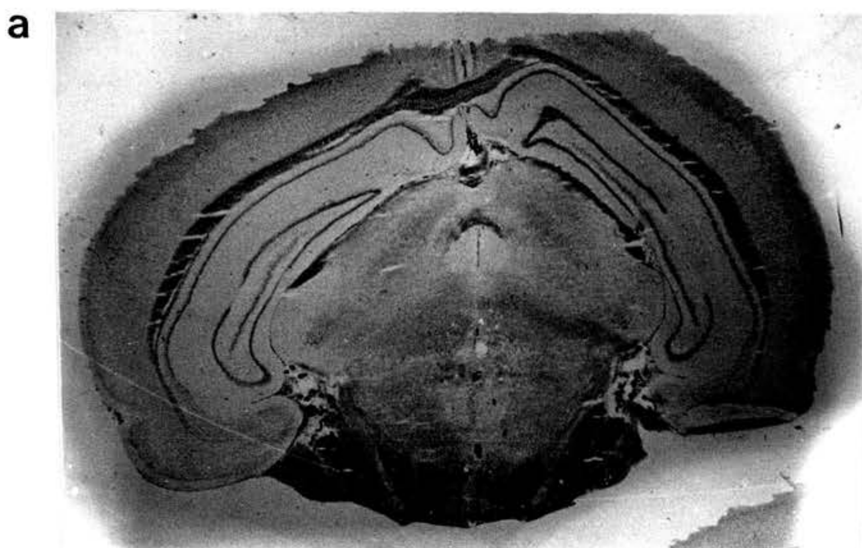
b

Fig.6-W: autoradiographs of coronal sections of the brain at the level of the suprachiasmatic nucleus (arrow) in rats injected with [^{14}C]2-deoxyglucose; control unstimulated (a) and ventrolateral geniculate body-stimulated (b) animals.

Fig. 6-X: (a) representative low power photomicrograph of a coronal section of the brain showing the position of the lesion in the ventrolateral geniculate body; (b) schematic diagram of coronal sections of the brain at A 3.4 and 3.2 (Pellegrino et al., 1967) showing the anterior-posterior and vertical positions and the size of all the lesions; (c) autoradiograph of a coronal section of the brain at the same level in a rat injected with [^{14}C]2-deoxyglucose and a lesion placed in the ventrolateral geniculate body. Abbreviations: CC, corpus callosum; DHIPP, dorsal hippocampus; MLM, medial lemniscus: SN, substantia nigra; VHIPP, ventral hippocampus; VLGEN, ventrolateral geniculate body.

a





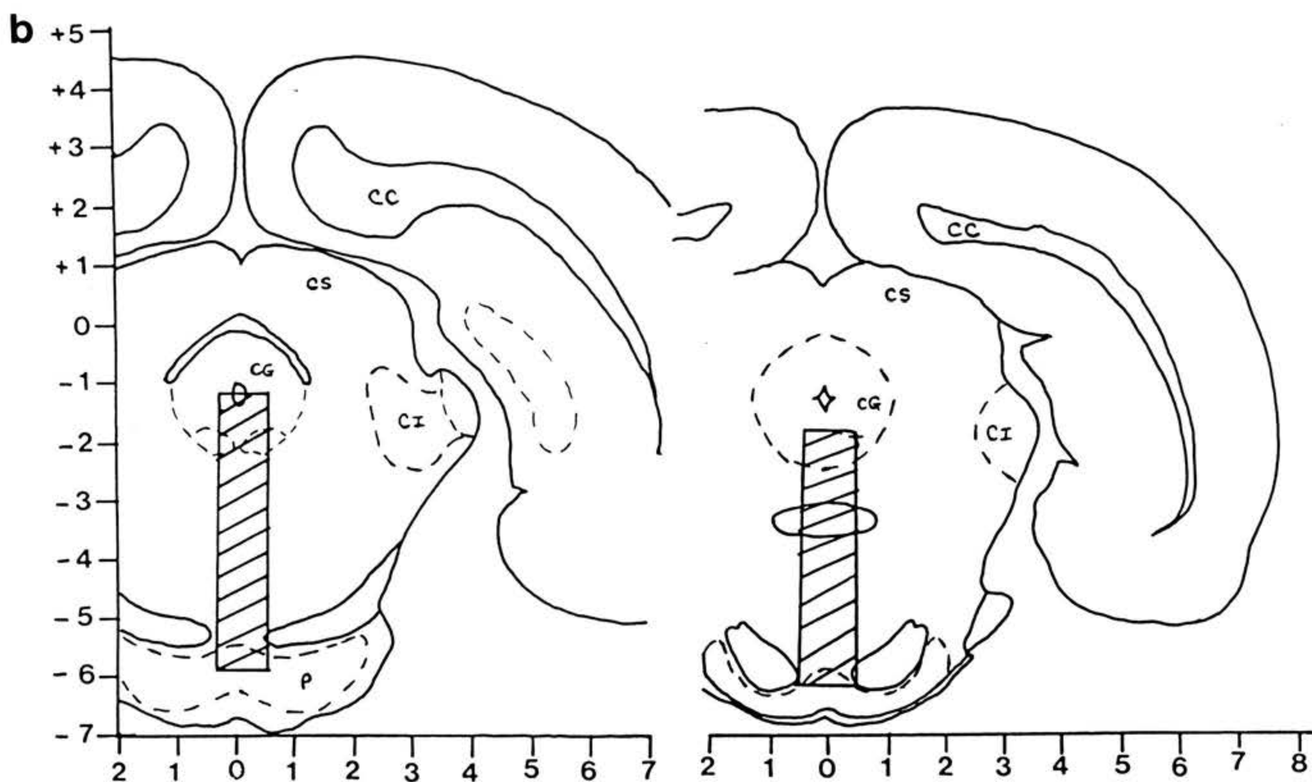
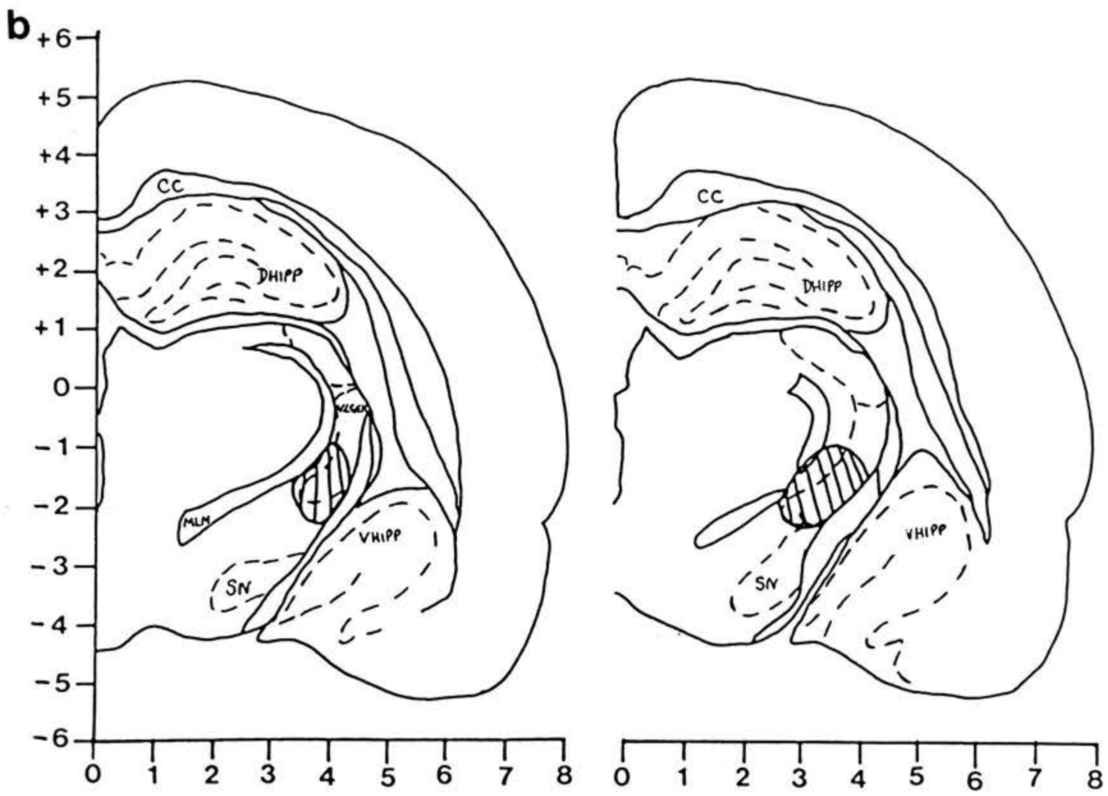


Fig. 6-Y: (a) representative low power photomicrograph of a coronal section of the brain showing the position of the knife cut immediately rostral to the raphe nuclei; (b) schematic diagram of coronal sections of the brain at A 1.0 and 0.6 (Pellegrino et al., 1967) showing the anterior-posterior and vertical positions of all the knife cuts. Abbreviations: CC, corpus callosum; CG, central grey; CI, inferior colliculus; CS, superior colliculus; P, pons.


b
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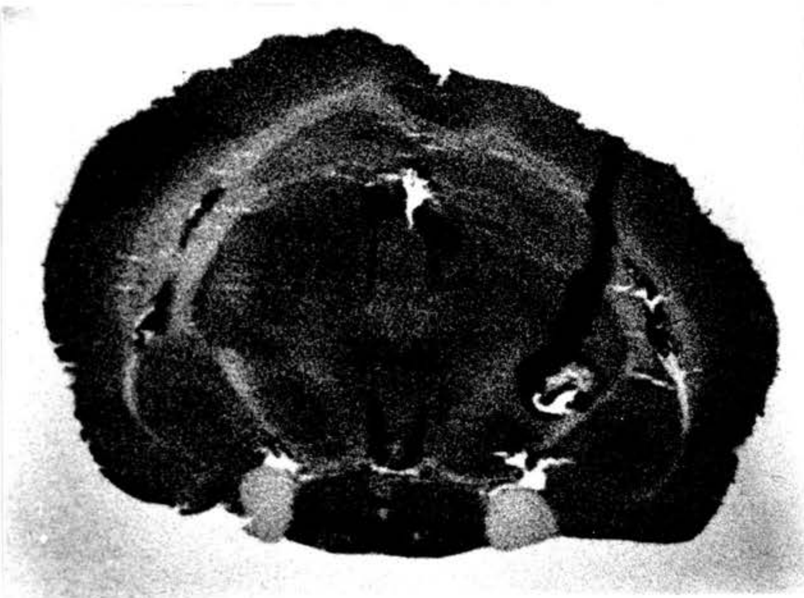

c

6.3.5.3.4 Comparison of the effects of lesioning the ventrolateral geniculate body with the effect of a raphe cut

Table 6-7 shows that the rma of i AMYG CE+L was significantly greater in VLGEN lesioned animals than in raphe cut animals. The rma of the DR was significantly greater in raphe cut animals than in VLGEN lesioned animals (Table 6-7).

6.4 DISCUSSION

The main finding of these studies was that each site of stimulation provided a distinctive pattern of increased metabolic activity throughout the brain, although the rma of certain brain areas, mainly in the hypothalamus, were increased by stimulation at many different sites.

In the interpretation of the increases in rma seen throughout the brain, 3 points should be considered: 1) antidromic as well as orthodromic activation of the neurone could result in increased 2DG uptake; 2) fibres of passage could be affected by the stimulus, and 3) secondary neuronal projections may also show increased rma. In studies using 2DG and electrical stimulation of hypothalamic and limbic structures, Watson and his associates (Watson, Edinger and Siegel, 1983; Watson and Siegel, 1981; Watson, Troiano, Poulakos, Weiner and Siegel, 1982) suggested that 2DG uptake reflected predominantly orthodromic activity of neurones, including fibres of passage, rather than antidromic activation of neurones. Their reasoning was based on the fact that no increase in rma was seen in areas of brain that projected to, but did not receive a reciprocal connection from the site of stimulation, such as the DBB with

hippocampal stimulation (Meibach and Siegel, 1977; Watson et al., 1983). In the present study the effects of antidromic activation on rma were investigated using ME stimulated animals. After stimulation of the ME the rma of the VMH and ARC increased, probably due in part to current spread, but also probably as a result of antidromic invasion of the fibres projecting from these areas to the ME (e.g. Szentágothai, 1964; Björklund et al., 1970; Fuxe and Hökfelt, 1970; Réthelyi and Halász, 1970; Barry et al., 1973a,b; Renaud and Martin, 1975; Wiegand and Price, 1980). Current spread is not a likely explanation for the increased rma of the POA-m in ME stimulated animals, since the POA-m is relatively far removed from the ME. The increased rma of the POA was possibly due to antidromic invasion of the LHRH-containing pathways that run along the dorsal and ventral surfaces of the optic chiasm (Réthelyi et al., 1981). In addition, the rma of the PVN and SON also increased, presumably as a result of antidromic invasion of both the fibres projecting from the PVN to the ME (Rinne, 1960; Dierickx et al., 1976) and the fibres of the hypothalamo-neurohypophysial tract (Cajal, 1894; Rasmussen, 1940; Bodian and Maren, 1951; Vandesande and Dierickx, 1975). The SON does not project to the ME (Wiegand and Price, 1980; Lechan et al., 1980) and so the increase in rma of this nucleus was probably due mainly to activation of the supraoptic-neurohypophysial tract. In the case of some PVN neurones, however, antidromic activation of both pathways may have occurred (Pittman, Blume and Renaud, 1981). The rma of the ST was also increased in ME stimulated animals, presumably as a result of orthodromic and antidromic activation of ST fibres in the hypothalamus (Raisman and

Field, 1971; De Olmos and Ingram, 1972; Swanson and Cowan, 1979; Ottersen, 1980). The absence of evidence for antidromic activity causing increased 2DG uptake in the studies of Watson et al. (1981; 1982; 1983) may have been due to the smaller current used, 200 μA. However, in the present studies areas showing increased rma probably reflected antidromically activated neuronal connections, as well as orthodromic activation of direct connections and fibres of passage. The uptake of 2DG is thought to reflect mainly terminal activity, due to the large surface area: volume ratio of the terminal compared with the perikaryon (Schwartz et al., 1979; Mata et al., 1980). In the case of antidromically activated neural pathways, however, the increased rma of nuclei is more likely to be due to increased uptake of 2DG by perikarya or dendrites.

The POA is important in the control of gonadotrophin release (e.g. Halász and Pupp, 1965; Halász and Gorski, 1967; Tejasen and Everett, 1967) and has direct neuronal connections with the MBH (Millhouse, 1969; Mizuno, Clemente and Sauerland, 1969; Conrad and Pfaff, 1976a; Swanson, 1976). It was not surprising therefore, that the rma of the VMH and ARC increased, as did the concentration of LH in plasma after POA stimulation. Although increases in rma of the DBB, SCN and SON could be attributed to current spread, it is unlikely that current spread caused the increased rma of the VMH and ARC, since these nuclei are further from the POA-m (for approximations of current spread relative to current amplitude see Bagshaw and Evans, 1976; Jamieson and Fink, 1976), and were activated to different extents compared with each other and the nearby AHA. The effects of POA stimulation on the rma of brain

areas was in agreement with the study of Watson et al. (1982) for the majority of areas, although in the present studies a greater number of areas were stimulated, presumably as a result of the greater current amplitude used. The results from POA stimulation demonstrated increased rma of many areas shown by a variety of neuroanatomical tracing techniques to be connected with the POA. Thus, activation of the MFB resulted in increased rma of most hypothalamic nuclei, S and CG (Valverde, 1963; Millhouse, 1969; Mizuno et al., 1969; Conrad and Pfaff, 1976a; Swanson, 1976), stimulation of the ST caused increased rma of the AMYG (Mizuno et al., 1969; De Olmos and Ingram, 1972; Conrad and Pfaff, 1976a) and of the periventricular system caused increased rma of the CG and HAB-1 (Mizuno et al., 1969). The rma of the PYRCX also increased following POA stimulation, which was perhaps as a result of amygdaloid activity (Krettek and Price, 1977). Watson et al. (1981; 1982; 1983) suggested that increased activity in secondary neurones was not reflected in increased 2DG uptake, except in some animals experiencing seizures. Irrespective of the frequency of stimulation, there was no increase in the activity of secondary neurones (Watson et al., 1982; 1983). Watson et al. (1982; 1983) suggested that the absence of a detectable change in the activity of secondary neurones was due to the stimulus from the primary neurone sometimes occurring in the inhibitory phase of the action potential of the secondary neurone, resulting in less frequent stimulation. However, increased 2DG uptake has been demonstrated in known multi-synaptic pathways, such as the visual pathway (Kennedy, Des Rosiers, Sakurada, Shinohara, Reivich, Jehle and Sokoloff, 1976;

Hubel, Wiesel and Stryker, 1978) and the pathway associated with the lordosis reflex (Chapter VII), and in pathways which are probably multisynaptic, such as those associated with the suckling-induced milk ejection reflex (Chapter V) and the pathway conveying osmotic stimuli to the pituitary gland (Sutherland, Martin, McQueen and Fink, 1983; Chapters III and IV). It is likely, therefore, that the present studies showed activation of secondary neurones, and suggests that activation of secondary neurones is dependent on the strength of stimulus and the anatomy of the areas involved.

The rma of the MDT and MM, areas known to receive efferent projections from the POA (Mizuno et al., 1969; Conrad and Pfaff, 1976a; Swanson, 1976) did not increase in the present study or in that of Watson et al. (1982a). Watson et al. (1982a) suggested that the detection of increased activity using the 2DG technique depends on the number of fibres and cell bodies activated by the stimulus, and also the compactness of the activated fibres. This has already been suggested in relation to activity in the SON (Chapters III and IV), and could also explain the presence or absence of increased activity seen in secondary projections.

Short-term ovariectomy and treatment with OB or oil affected the distribution of areas showing increased rma following POA stimulation, and also the magnitude of the increase in some areas. In general, the presence of oestrogen (in POA stimulated animals and OVX + OB-POA stimulated animals) reduced the increase in rma caused by stimulation in the S-m, DBB, SON, MFB, HAB-1 and PVT, but augmented the increase in rma of the AMYG CE+L, PD and AVT. Oestrogen has been shown to increase neuronal activity in the VMH

(Bueno and Pfaff, 1976; Cohen and Pfaff, 1981), although dorsal afferents to the diencephalon appears to be necessary for the facilitation of the LH release mechanism by oestrogen (Van Rees, 1972; Brown-Grant and Raisman, 1972), and oestrogen reduced activity in the POA (Bueno and Pfaff, 1976). Oestrogen has also been shown to affect the hippocampal- and amygdaloid-evoked response in the ARC (Kawakami and Terasawa, 1967). The areas of brain in which the difference in rma following stimulation occurred were not the areas in which large numbers of oestrogen-concentrating cells are located, namely the POA-m, VMH, ARC and ACB (Stumpf, 1970; Pfaff and Keiner, 1973). The changes in rma caused by electrical stimulation in the areas that contain large numbers of oestrogen concentrating cells were so dramatic that any subtle alteration in firing due to the action of oestrogen was probably masked. Whether the differences in rma that were seen in the 3 groups of animals following POA stimulation could be attributed to an altered steroid environment is not known, but seems unlikely since short-term ovariectomy and treatment with OB or oil did not affect the rma of areas of the brain when studied in animals to which no stimulus was applied.

In addition to the POA-m, other brain areas are thought to influence gonadotrophin release. Electrical or electrochemical stimulation of the AHA in either male (Jamieson and Fink, 1976) or female (Clemens, Shaar, Kleber and Tandy, 1971; Kalra et al., 1971) rats produced a significant increase in the plasma concentration of LH. Electrical stimulation of the AHA in female rats also produced a significant increase in the release of LHRH into hypophysial portal plasma, although the amount of LHRH released was only 18% of

that released by stimulation of the POA (Chiappa et al., 1977). If a stimulus was applied to the dorsal region of the AHA, however, ovulation did not occur (Terasawa and Sawyer, 1969). Although the effects of AHA stimulation on gonadotrophin release could have been due to current spread to the POA-m and ME, it seems unlikely that this was the case since stimulation of the POA-l and S-l, which are as close to the POA-m as the AHA, did not produce ovulation or LH release, respectively (Everett, 1965; Jamieson and Fink, 1976). In the present studies stimulation of the AHA caused large increases in rma of the hypothalamus, including the VMH. As with POA stimulation, activation of the major forebrain fibre systems which are known to innervate the AHA, the MFB (Millhouse, 1969; Conrad and Pfaff, 1976c), ST (De Olmos and Ingram, 1972; Conrad and Pfaff, 1976b) and periventricular fibre systems (Swanson and Cowan, 1975; Conrad and Pfaff, 1976b) caused increased rma of most hypothalamic nuclei, the S, some thalamic nuclei (PVT, AVT), AMYG and HAB-l. In spite of the widespread increases in rma of the hypothalamic nuclei, the plasma LH concentration was not increased. Stimulation of the AHA also elicited increases in rma of other brain areas, such as the PYRCX, RF, LGEN, HIPP, HAB-m, IP AND FX, not known to be directly connected with the AHA. The change in rma of these areas, therefore, probably reflected activation of secondary projections. from areas such as the AMYG and HAB-l or of fibres of passage.

There have been conflicting reports as to the effects on gonadotrophin release of hippocampal and amygdaloid stimulation in the rat. Electrochemical stimulation of the VHIPP reduced the plasma LH concentration in long-term ovariectomised rats, but did

not inhibit the ovulatory response to POA stimulation in pentobarbital-blocked pro-oestrous rats (Gallo et al., 1971). Electrochemical stimulation of the HIPP also prevented the ovulatory response to electrochemical stimulation of the POA in rats in persistent oestrus (Velasco and Taleisnik, 1969b), while electrical stimulation of the VHIPP did not significantly affect the LHRH response to POA stimulation in pro-oestrous animals (Chiappa et al., 1977). Similarly, studies on rats in persistent oestrus suggested that the AMYG facilitates gonadotrophin release (Bunn and Everett, 1957; Shealy and Peele, 1957; Hayward, Hilliard and Sawyer, 1964; Velasco and Taleisnik, 1969a; Arai, 1971), while studies involving lesions of the AMYG in ovariectomised rats suggested that the AMYG inhibits gonadotrophin release (Lawton and Sawyer, 1970). Kawakami et al. (1973) found that stimulation of the AMYG in pentobarbital-blocked pro-oestrous rats was accompanied by a small but significant increase in the plasma LH concentration, but no increase was elicited on other days of the cycle. Sawyer (1972) suggested that the AMYG CO + M contains facilitatory and inhibitory neurones with respect to gonadotrophin release. Therefore, activity in the different sets of neurones may explain the apparent discrepancies of the influence of the AMYG on gonadotrophin release. Electrophysiological studies have shown that in cats, stimulation of the AMYG CO + M or ST reduced the activity of neurones in the VMH, and the effect was abolished by lesions of the ST (Dreifuss, Murphy and Gloor, 1968). In rats, activation of the ST resulted in excitatory-inhibitory sequences in the VMH (Renaud, 1976), and stimulation of the basal nucleus of the AMYG caused

increased firing of VMH neurones followed by a period of inhibition and a subsequent period of increased firing (Dreifuss et al., 1968). The AMYG may also influence neuroendocrine regulation through a direct action of amygdaloid neurones on preoptic-tuberoinfundibular neurones (Layton, Lafontaine and Renaud, 1981).

The HIPP and AMYG have lower thresholds for E.E.G. seizure discharges than other areas of the brain (Kaada, 1972; Kawakami et al., 1972) and these thresholds could be altered by gonadal hormones (Terasawa and Timiras, 1968). This may help to explain the differences in effects of stimulation of the HIPP and AMYG applied through short-term and long-term implanted electrodes on LH release and ovulation in pro-oestrus rats (Kawakami et al., 1972; Ellendorff, Colombo, Blake, Whitmoyer and Sawyer, 1973). In studies involving the effects of electrical stimulation of the AMYG and HIPP on 2DG uptake, Watson et al. (1981; 1983) separated the data obtained from animals in which EEG seizure activity was present or absent, and found more extensive increases in 2DG uptake in animals experiencing seizures. In the present studies, 2DG uptake was more extensive in general than in the studies of Watson et al. (1981; 1983). Since different areas of brain were activated by DHIPP, VHIPP and AMYG stimulation, it is unlikely that generalised seizures were responsible for the changes in rma in the present studies.

The uptake of 2DG by the HIPP in anaesthetised animals not subjected to stimulation occurred mainly in the molecular layer. After stimulation, however, although the rma of both the molecular and granular layers increased, the change was most dramatic in the granular layer, as was found in the study of Watson et al. (1983).

Stimulation of the VHIPP, but not DHIPP caused increases in rma of the POA-m, VMH and ARC, probably through activation of the FI and MCHT, through which the information processed in the HIPP reaches the ARC nucleus (Nauta, 1956; Raisman, 1970). Velasco and Taleisnik (1969b) found that the transection of the MCHT prevented the blockade of ovulation produced by VHIPP stimulation, which suggested that this pathway mediates the effects of VHIPP stimulation on gonadotrophin release.

Stimulation of the VHIPP in animals in which seizures were present or absent, also resulted in increased 2DG uptake in a pathway involving the AMYG and ST (Kliot and Poletti, 1979; Watson et al., 1983). The activation occurred in regions of the AMYG distant from the site of stimulation in the VHIPP, and, therefore, could not be accounted for by current spread. Since the ST projects extensively to the hypothalamus (Swanson and Cowan, 1979) it is conceivable that the VHIPP also affects the hypothalamus by this pathway. Electrophysiological studies in the primate support this, as amygdaloid neurones respond to VHIPP stimulation (Morrison and Poletti, 1980) and receive an afferent input from the SCX (Rosene and Van Hoesen, 1977), and VHIPP stimulation has been shown to influence regions of the hypothalamus primarily by pathways other than the FX (Poletti and Sujatanond, 1980). In the present study the i AMYG and i and c ST were also activated by VHIPP stimulation.

Within the HIPP itself, the pattern of metabolic activation depended on the site of stimulation. Unilateral stimulation of the DHIPP caused bilateral increases in the rma of the DHIPP and VHIPP, whereas unilateral stimulation of the VHIPP caused bilaterally

increased rma of the VHIPP alone. The pyramidal cells of the HIPP are known to give rise to commissural projections (Laurberg, 1979; Swanson, Sawchenko and Cowan, 1980; 1981), hence the bilateral activation of the HIPP was not surprising, and can be attributed to orthodromic and antidromic activation of these fibres. In DHIPP stimulated animals the extent of the crossover of activation was such that the i and c sides of the HIPP were not significantly different from each other, except in the VHIPPM, and in VHIPP stimulated animals the rma of the i and c sides of the VHIPPG were also not significantly different. In DHIPP stimulated animals the rma of the c S-1 was also increased, to a value not significantly different from that of the i side. In VHIPP stimulated animals however, the increases in rma of areas other than the HIPP found on the c side (S-1, ST and PVT), were significantly lower than those on the i side, indicating that in VHIPP stimulated animals the increase in activation of the contralateral side of the brain was less than of the i side, apart from the HIPP.

The dorsal-ventral pattern of 2DG uptake is more difficult to explain, since the intrinsic connections of the HIPP are extensive, with complex arrangements of collateral projections (Finch and Babb, 1981; Knowles and Schartzkroin, 1981; Swanson et al., 1980a; 1981b). It is possible that the dorsal and ventral aspects of the hippocampal formation are normally activated as separate functional units, since Watson et al. (1983) only obtained extensive 2DG labelling of the HIPP in animals with after discharge activity. In the present study, only stimulation of the DHIPP caused increased rma throughout the HIPP, which lends further support to the concept

of a functional differentiation between the two regions (Siegel and Flynn, 1968; Siegel and Edinger, 1981).

The AMYG has reciprocal connections with most regions of the hypothalamus, through the ST and ventral amygdalo-fugal pathways (Heimer and Nauta, 1969; De Olmos and Ingram, 1972; Krettek and Price, 1978; Ottersen, 1980), through which the AMYG may influence hypothalamic control of the pituitary gland (Renaud, 1976). However, no change in rma of the POA-m or ARC was found following stimulation of the AMYG in either the present studies or those of Watson et al. (1981), although Watson et al. (1981) observed an increase in 2DG uptake in the VMH; no change in plasma LH concentration was elicited in the present studies by stimulation of the AMYG. Since the ST, MFB and SM were activated in the present studies, the absence of detectable changes in the hypothalamus was surprising, and suggests that the neural connections between the hypothalamus and AMYG were either too diffuse for increased activity to be detected by the 2DG method, or that no activation occurred. The rma of some amygdaloid connections did increase following stimulation of the AMYG, such as the PVT (Ottersen and Ben Ari, 1979), VHIPP and PYRCX (Krettek and Price, 1977), FRCX (Mufson, Mesulam and Pandya, 1981; Porrino, Crane and Goldman-Rakic, 1981) and DBB (Krettek and Price, 1978; Price and Amaral, 1981), while the rma of other known connections such as the RF and CG (Price and Amaral, 1981), MDT (Nauta, 1961; Porrino et al,. 1981) and MGEN (Ottersen and Ben Ari, 1979) did not increase. Why some known pathways were activated and others were not, remains to be explained. The 2DG method, however, is not an anatomical tracing

technique but indicates functionally active pathways. The differential activity of the amygdaloid projections following electrical stimulation could therefore reflect the most important connections of the AMYG, possibly to the cortex, rather than to the hypothalamus, and could also reflect differences in the efferent projections of the various amygdaloid nuclei. The amygdaloid projection that increased in activity most dramatically following stimulation was the CA. Through this route the activity of the AMYG CE + L and PYRCX on the c side of the brain were also increased, although, unlike the HIPP stimulation studies, the cross over of activity was significantly less than the activity of these areas on the i side.

Although the PVN has not been implicated in the control of gonadotrophin release, vasopressin is thought to be important in the control of ACTH release (e.g. Gillies and Lowry, 1980; 1982; Gillies et al., 1982) through a direct input of vasopressinergic neurones to the external lamina of the ME (Rinne, 1960; Dierickx et al., 1976). Since the PVN can, therefore, influence both anterior and posterior pituitary function, and has been studied extensively in this thesis (Chapters III, IV and V), a group of PVN stimulated animals was included in the electrical stimulation studies presented here. Electrical stimulation of the PVN caused increases in rma of areas known to receive projections from the PVN, such as the PN (Cajal, 1894; Rasmussen, 1940; Bodian and Maren, 1951), SON, VMH, ARC, DMH, PVT AND HAB-1 through the periventricular fibre system (Szentágothai, Flerkó, Mess and Halász, 1968; Conrad and Pfaff, 1976b), AMYG through the ST (Conrad and Pfaff, 1976b), S through the

DBB (Conrad and Pfaff, 1976b) and midbrain CG (Conrad and Pfaff, 1976b; Swanson, 1977; Sofroniew and Weindl, 1978). In addition, afferent projections to the PVN from the HIPP (Berk and Finkelstein, 1981a; Silverman et al., 1981; Tribollet and Dreifuss, 1981), ST (Swanson and Cowan, 1979; Sawchenko and Swanson, 1981b) and from most hypothalamic nuclei (Sawchenko and Swanson, 1981b; Swanson et al., 1981a; Sawchenko and Swanson, 1983) may have been driven antidromically to cause increases in the rma of these areas. Several areas, not known to be connected with the PVN, such as thalamic nuclei, PYRCX, MM, IP and SM, also showed increased rma, and may indicate activation of secondary neurones or fibres of passage as a result of the dramatic changes seen throughout the brain.

The effects of electrical stimulation of the PVN were widespread throughout the brain, demonstrating the extensive neuronal connections of the nucleus, which may explain the fact that various higher forebrain and telencephalic structures can influence activity in the PVN. Stress can disrupt vasopressin secretion (Verney, 1947), oxytocin secretion can be conditioned (Cleverley and Folley, 1970) and septal and amygdaloid stimulation usually inhibits magnocellular activity (Koizumi and Yamashita, 1972; Negoro, Visessuwan and Holland, 1973; Pittman et al., 1981). Whether the modulation of activity in the PVN by limbic areas, primarily the S, AMYG and VHIPP, is a result of direct neuronal input from these areas, as suggested by retrograde transport studies (Berk and Finkelstein, 1981a; Silverman et al., 1981; Tribollet and Dreifuss, 1981), or, as suggested by antoradiographic studies, due to an

indirect input from the ST and MCHT (Swanson and Cowan, 1977; Krettek and Price, 1978; Swanson and Cowan, 1979) is not known. In the present studies, activity in the S and AMYG CO + M were increased by 3- to 4-fold following PVN stimulation, while the rma of the AMYG CE + L and VHIPP were increased by only 20 and 50%, respectively. Since Watson et al. (1981; 1982; 1983) suggested that activity in primary projections was preferentially labelled by 2DG, the larger increases in rma of the AMYG CO + M and S may reflect either direct neuronal connections with the PVN, or the fibres in the ST and MCHT connecting these areas with the PVN are more compact.

Light has been known to affect reproductive function for many years, in birds (Rowan, 1926; 1930; Bissonette, 1933) and in mammals, such as the vole (Baker and Ranson, 1932), ferret (Bissonette, 1932; Hill and Parkes, 1933; Marshall and Bowden, 1934), sheep (Hart, 1950) and rats (Browman, 1937; Hemmingsen and Krarup, 1937; Critchlow, 1963). The effects of light were thought to be mediated through the retina and optic nerves (Ceni, 1928; Bissonette, 1938; Clark, Le Gros, McKeown and Zukerman, 1939), and the pituitary gland was also known to be an essential component (Hill and Parkes, 1933; Bissonette, 1935). However, the demonstration of a retino- hypothalamic pathway eluded neuroanatomists for many years. Bilateral retinal projections to the SCN have now been identified in several species (Moore et al., 1971; Hendrickson, Wagoner and Cowan, 1972; Moore, 1973; Mason and Lincoln, 1976; Tigges, Bos and Tigges, 1977), and the retinal neurones have been shown to terminate on dendrites of the SCN cells (Moore and Lenn, 1972; Hendrickson et al., 1972). The SCN also

receives a projection from the VLGEN which may convey visual information to the SCN (Swanson et al., 1974; Ribak and Peters, 1975; Moore, Marchand and Riley, 1979), in a pathway containing avian pancreatic polypeptide-like immunoreactivity (APP) (Card and Moore, 1982). The LCGU in the LGEN has been shown to be lower in albino rats than in pigmented rats (Batipps, Miyaoka, Shinohara, Sokoloff and Kennedy, 1981), and decreases with increasing intensity of light above 7 lux (Miyaoka, Shinohara, Batipps, Pettigrew, Kennedy and Sokoloff, 1979). Stimulation of the optic nerve increased electrical activity in VLGEN and 40% of SCN neurones tested, and inhibited 24% of other SCN neurones tested (Burke and Sefton, 1966). Similarly, SCN neurones respond to photic stimulation of the retina with increased and decreased firing (Nishino, Koizumi and Brooks, 1976; Groos and Mason, 1980). Connections have also been demonstrated between the retina and the limbic system (Itaya, Van Hoesen and Jenq, 1981) and POA-m. In the latter the neurones have dendritic connections in the optic chiasm with retinal axonal boutons (Silver and Brand, 1979).

In the rat, the timing of the LH surge can be altered by changes in the lighting schedule (Colombo, Baldwin and Sawyer, 1974) and can be abolished by constant illumination (Browman, 1937; Everett, 1942; Lawton and Schwartz, 1967). Extensive lesions of the SCN result in failure to ovulate (Brown-Grant and Raisman, 1977) and disruption of other functions normally influenced by the light and dark cycle (Raisman and Brown-Grant, 1977b). Studies in which ovulation and gonadotrophin release were blocked usually involved lesions of the rostral diencephalon including the SCN (Taleisnik and

McCann, 1961; Critchlow, 1963; Kennedy, 1964; Timiras and Sherwood, 1974; Clemens and Smalstig, 1975; Wiegand et al., 1980), or anterior deafferentation of the hypothalamus behind the SCN (Halász and Gorski, 1967; Blake, Weiner, Gorski and Sawyer, 1972; Blake and Sawyer, 1974). Stimulation of the SCN significantly increased the concentration of LHRH in hypophysial portal plasma (Chaippa et al., 1977), and of LH in peripheral plasma in the present studies (6.3.2.1). These results are significant since the SCN is known to contain a small amount of LHRH, and sends projections to the ME (Réthelyi and Halász, 1970; Swanson and Cowan, 1975). However, activation of the LHRH pathways located on the dorsal and ventral surfaces of the optic chiasm (Réthelyi et al., 1981) may have contributed to the increase in plasma LH concentration.

Electrical stimulation of the SCN in the present studies increased the rma of a variety of brain areas. The rma of the POA-m, POA-l, DBB, SON and AHA increased dramatically, possibly due to current spread, but the rma of the VMH, ARC, DMH and PVN also increased, probably as a result of activation of the known efferent projections of the SCN (Krieg, 1932; Swanson and Cowan, 1975; Berk and Finkelstein, 1981b). In addition, the rma of the PVT and the CG were also increased and the SCN has been shown to project as far caudally as these areas (Berk and Finkelstein, 1981b). The bilateral projection to the SCN from the VLGEN was activated antidromically in the SCN stimulated animals, since the rma of the VLGEN increased. Orthodromic activation of the pathway was found in VLGEN stimulated animals, where the rma of the SCN were increased. However, although the rma of the VMH increased in the VLGEN

stimulated animals, no change in the rma of the POA-m or ARC was observed, nor was the concentration of LH in plasma altered.

Although the pathway from the VLGEN to the SCN is bilateral (Swanson et al., 1974), the rma of the c SCN did not increase to the same extent as the i SCN in unilaterally VLGEN stimulated animals. Since increased activity within a neurone results in 2DG becoming trapped (Sokoloff et al., 1977), the difference in rma between the i and c SCN was presumably due to increased activity in a predominantly ipsilateral pathway. This agrees with Card and Moore (1982) who suggested that innervation from the i VLGEN accounts for 75% of the APP-immunoreactivity within a SCN.

The possible route by which the pathway from the VLGEN reaches the SCN has been disputed. Swanson et al. (1974) maintained that fibres from the VLGEN descend in the ZI, while Ribak and Peters (1975) suggested that the fibres traversed the optic tract to the medial aspect of the optic chiasm to innervate each SCN. Card and Moore (1982) found APP-like immunoreactivity present only in the ZI and not in the optic tract, supporting the interpretation of Swanson et al. (1974). In the present study the rma of the ZI increased dramatically in VLGEN stimulated animals. While not necessarily indicating an exclusive pathway to the SCN travelling in the ZI, this finding suggests that the ZI is an important efferent fibre tract from the VLGEN.

The SCN is known to exert an influence on circadian rhythm generation (Moore, 1979; Rusak and Zucker, 1979), and in fact glucose utilisation in the SCN itself varies with a circadian rhythm (Schwartz et al., 1980) and was higher in the afternoon than morning

in ovariectomised rats that had been orbitally enucleated and implanted with oestradiol capsules (Héry, Dusticier and Calas, 1982). In addition, there is evidence for a primary optic projection at the level of the LGEN also affecting entrainment (Rusak, 1977), and the projection from the VLGEN to the SCN would seem to be a likely candidate for this function, as suggested by Card and Moore (1982). However, glucose utilisation of the SCN in anaesthetised animals appears to be independent of the VLGEN, since unilateral lesions of the VLGEN had no effect on the rma of the SCN. The VLGEN lesion did increase the rma of several i brain areas, the IC, DHIPPM, VHIPPM, VHIPPG and AMYG CE+L. The increase in rma of the IC and DHIPPM may have been as a result of damage caused by the passage of the electrode through these areas. The increases in rma of the VHIPPM and VHIPPG and of the AMYG CE+L are more difficult to explain. The increase in the VHIPP may reflect activation caused by damage to the DHIPP, since a VHIPP lesion has been shown to increase glucose utilisation in the DHIPP (Kameyama, Wasterlein, Ackerman, Finch, Lear and Kuhl, 1983). The increase in rma of the AMYG CE+L may therefore have been secondary to the increased activity in the VHIPP through the neural connection between the two areas (Rosene and Van Hoesen, 1977; Morrison and Poletti, 1980). In VLGEN unstimulated animals the rma of the i DHIPPG and VHIPPG were also increased compared with those of the c side, suggesting further that the hippocampal formation is susceptible to damage, resulting in increased 2DG uptake.

To see whether the rma of the SCN could be altered by the transection of other known afferent inputs to the SCN, such as from

the RA (Dahlström and Fuxe, 1964; Ungerstedt, 1971; Björklund et al., 1973a,b; Parent et al., 1983) the animals with raphe cuts were included in the study. Only the rma of the c SCN in the VLGEN unstimulated animals was significantly different from that of the SCN in the animals with raphe cuts, and was in fact increased, indicating that any changes in rma of the SCN caused by activity in the afferent input to the SCN from the RA, if present, were too subtle for detection by the 2DG method. An additional problem in these studies is that the SCN tended to show a variable rma between animals, which may have prevented demonstration of subtle changes in rma. This variability in rma was possibly a result of the effects of light on the rma of the SCN (Schwartz and Gainer, 1977) or the circadian rhythm in glucose utilisation of the SCN itself (Schwartz et al., 1980). However, the experiments were always carried out in the same laboratory at the same time of day, so the variability in rma of the SCN may have been due to other factors, such as the level of anaesthesia in the animal. Since all groups of animals comprised at least one animal in each stage of the oestrous cycle, the variability in the mean rma of the SCN of each group could not be due to animals being in different stages of the oestrous cycle.

CHAPTER VII

Pathways Involved in Reflex Ovulation in the Vole (*Microtus agrestis*)

7.1 INTRODUCTION

Reflex ovulation induced by mating has long been known to occur in several species of mammal such as the rabbit (Barry, 1839; Heape, 1905; Hammond and Marshall, 1917), cat (Courrier and Gros, 1933) and the short-tailed field vole *Microtus agrestis* (Breed, 1967). Ovulation has been shown to be preceded by a surge of LH triggered by mating in the rabbit (Dufy-Barbe, Franchimont and Faure, 1973; Kanematsu, Scaramuzzi, Hilliard and Sawyer, 1974), cat (Concannon, Hodgson and Lein, 1980) and vole (Charlton, Naftolin, Sood and Worth, 1975). This surge of LH in the vole is due, at least in part, to a reflex surge of LHRH, since the hypothalamic content of LHRH decreased markedly after mating (Versi, Chiappa, Fink and Charlton, 1982). The occurrence of a reflex surge of LHRH in the rabbit is suggested by studies with an i.v. injection of cupric acetate, which causes ovulation (Fevold, Hisaw and Greep, 1936; Hiroi, Sugita and Suzuki, 1965) and a pattern of gonadotrophin release similar to that seen after mating (R.L. Goodman and J.D. Neill, unpublished observations; see Goodman and Neill, 1976), and increased the concentration of LHRH in hypophysial portal plasma and the concentration of LH in peripheral plasma (Tsou, Dailey, McLanahan, Parent, Tindall and Neill, 1977).

Rats exposed to constant light (LL) are converted from spontaneous to reflex ovulators; the animals lose their normal ovarian cycles (Browman, 1937; Everett, 1942), become persistently sexually receptive (Hardy, 1970) and ovulate in response to copulation (Dempsey and Searles, 1943). Ovulation in LL rats results from a reflex surge of LH (Brown-Grant, Davidson and Greig,

1973; Davidson, Smith and Bowers, 1973) which is probably due, as in the other species, to a reflexly induced surge of LHRH, since the hypothalamic content of LHRH decreases significantly in LL rats after mating (Smith and Davidson, 1974). Furthermore, progesterone, which stimulates a surge release of LH in LL rats (Brown-Grant et al., 1973), also increased significantly the concentration of LHRH in hypophysial portal plasma collected from LL rats (Sarkar and Fink, 1979). 20α Hydroxy-progesterone was also implicated in the reflex surge of LH in the rabbit by Hilliard, Penardi and Sawyer (1967) but this could not be confirmed by Goodman and Neill (1976). The pathways through which the stimulus of copulation is conveyed to the hypothalamus, resulting in the release of LHRH, have not yet been defined. The aim of the present studies was to investigate the effect of mating on brain neuronal metabolism in the female vole, and so elucidate the brain areas involved in the control of reflex ovulation.

7.2 MATERIALS AND METHODS AND EXPERIMENTAL DESIGN

The animals used in these studies were adult female and male voles (*Microtus agrestis*), 20-30 g body weight, obtained from a breeding colony at the Department of Human Anatomy, University of Oxford, and maintained as described in section 2.1.

Experiments were carried out between 10:00 and 14:00 h. Males were placed in mating boxes, 200 x 200 x 100 mm, made from clear perspex and equipped with ventilation holes in the lids. After 5 min a female was introduced into the box and the pair were observed for several minutes for lordoses and mounts. Sham-mated females had the vagina taped over with masking tape to prevent

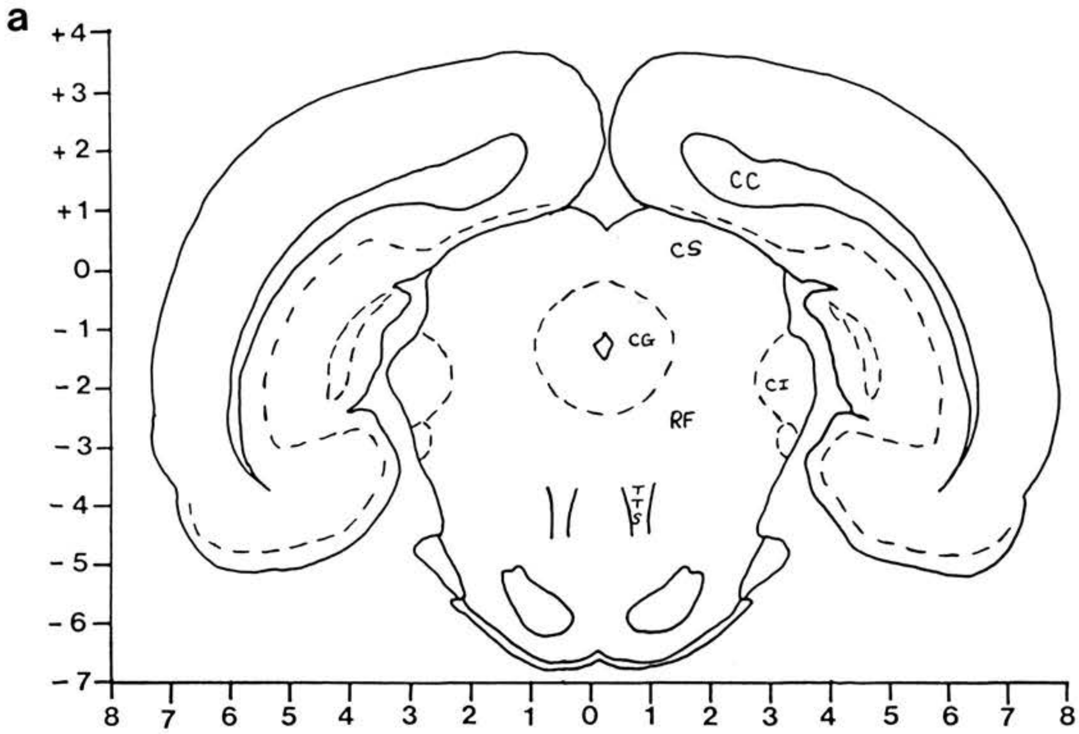
intromission by the males. Control, unmated females were placed in the mating boxes, but males were never introduced into the box. The female was injected with 2DG, i.p. at a dose of 400-500 μCi/kg body weight (2.2.1). The numbers of mounts, lordoses, intromissions and ejaculations shown by the voles were noted during a period of 45 min after the injection of 2DG. The female was then decapitated and the trunk blood was collected into ice-cooled LP2 tubes using a small, heparinised filter funnel. Plasma was prepared from the blood samples (2.2.7) and the concentration of LH was measured in each plasma sample by RIA (2.3.2.3).

The brain and pituitary gland were removed en bloc from each female, and were processed for autoradiography (2.2.1). The rma of the following areas of the brain and pituitary gland were then determined from the autoradiographs (2.3.1.1); S, DBB, POA, MFB, SCN, PVN, AHA, VmArc, DMH, PH, IC, AMYG, MM, IP, LGEN, MGEN, VT, DHIPPM, DHIPPG, VHIPPM, VHIPPG, DG, CS, CI, CG, RF, LLM, LC, PN and PD. Significance of differences in rma was assessed by the unpaired t-test.

7.3 RESULTS

Figure 7-A shows a representative autoradiograph from a vole brain at the midbrain level, and a schematic diagram at the same level of the brain showing the position of the CG and RF. Table 7-1 shows that the rma of the CG and RF were increased significantly in mated animals compared with control, unmated animals. The rma of the CG and RF in sham-mated animals were also greater than in control, unmated animals, but were not significantly different from those in mated animals (Table 7-1). There were no differences in

a



b

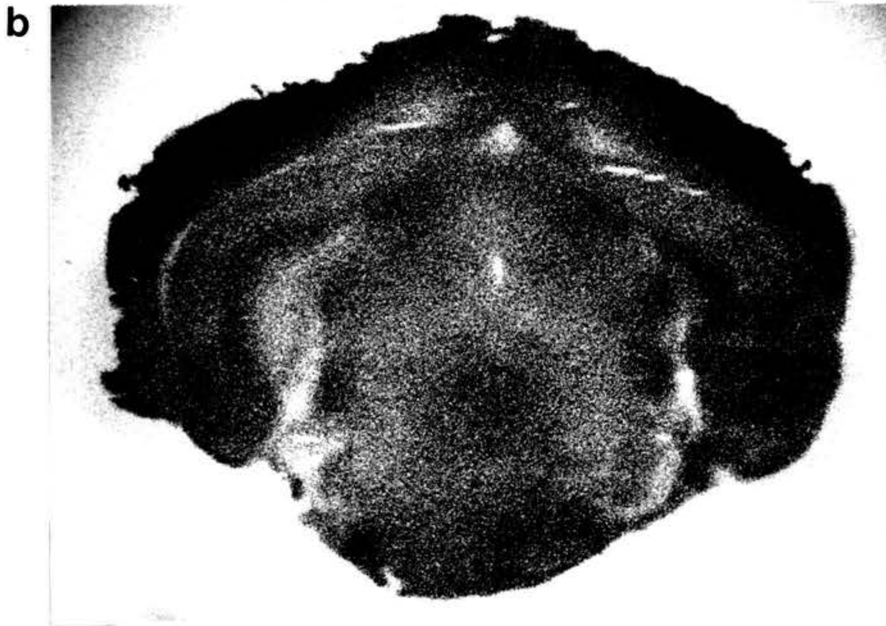


Fig. 7-A: (a) schematic diagram of a coronal section of the brain at A 0.6 (Pellegrino et al., 1967) and (b) autoradiograph of the brain at the same level in a vole injected with $[^{14}C]$2-deoxyglucose. Abbreviations: CC, corpus callosum; CG, central grey; CI, inferior colliculus; CS, superior colliculus; RF, reticular formation; TTS, tectospinal tract.

Table 7-1. Relative metabolic activity of selected brain regions and the pituitary gland in voles

Values are expressed as a ratio (mean ± S.E.M.) of [^{14}C] concentration of selected region to [^{14}C] concentration of the corpus callosum

	Type and number of animal		
	Mated (n=5)	Sham-mated (n=5)	Control unmated (n=6)
CG	1.05±0.03****	1.08±0.06***	0.92±0.02
RF	1.20±0.03***	1.26±0.05****	1.06±0.03
IC	0.91±0.03	0.88±0.02	0.90±0.02
POA	0.97±0.08	0.89±0.05	0.83±0.04
MFB	1.18±0.10	1.14±0.04	1.12±0.04
VmArc	0.89±0.04	0.85±0.10	0.83±0.05
AMYG	0.97±0.04	1.07±0.04	0.99±0.04
AHA	0.92±0.04	1.05±0.07	0.95±0.03
PVN	0.84±0.04	0.95±0.04	0.86±0.04
SCN	1.21±0.07	1.16±0.07	1.01±0.06
S	1.19±0.03	1.38±0.08	1.23±0.02
DBB	1.30±0.07	1.40±0.06	1.38±0.06
DMH	0.96±0.03	1.00±0.06	0.97±0.06
PH	1.03±0.04	1.12±0.07	1.04±0.06
MM	1.82±0.08	1.99±0.21	1.92±0.11

Significantly different from Control, unmated group: *** $P < 0.02$; **** $P < 0.01$

CG, central grey; RF, midbrain reticular formation; IC, internal capsule; POA, preoptic area; MFB, medial forebrain bundle; VmArc, ventromedial-arcuate hypothalamic area; AMYG, amygdala; AHA, anterior hypothalamic area; PVN, paraventricular nucleus; SCN, suprachiasmatic nucleus; S, septum; DBB, diagonal band of Broca; DMH, dorsomedial hypothalamic nucleus; PH, posterior hypothalamic area; MM, mamillary body

Table 7-1 (cont). Relative metabolic activity of selected brain regions and the pituitary gland in voles

Values are expressed as a ratio (mean ± S.E.M.) of [^{14}C] concentration of selected region to [^{14}C] concentration of the corpus callosum

	Type and number of animal		
	Mated (n=5)	Sham-mated (n=5)	Control unmated (n=6)
IP	1.72±0.21	1.62±0.18	1.59±0.11
PN	0.87±0.09	0.75±0.05	0.86±0.13
PD	0.78±0.07	0.69±0.08	0.68±0.10
LGEN	1.47±0.08	1.52±0.07	1.44±0.04
MGEN	1.65±0.08	1.60±0.06	1.62±0.07
VT	1.53±0.09	1.63±0.11	1.67±0.11
DHIPPM	1.44±0.09	1.49±0.13	1.39±0.05
DHIPPG	1.04±0.03	1.15±0.09	1.00±0.04
VHIPPM	1.29±0.05	1.40±0.13	1.31±0.04
VHIPPG	1.13±0.05	1.17±0.07	1.10±0.02
DG	0.95±0.03	1.03±0.09	0.93±0.02
CS	1.39±0.03	1.59±0.08	1.44±0.07
CI	1.79±0.09	1.90±0.08	1.81±0.12
LLM	1.41±0.10	1.54±0.09	1.48±0.08
LC	1.31±0.06	1.35±0.05	1.28±0.09

IP, interpeduncular nucleus; PN, pars nervosa; PD, pars distalis; LGEN, lateral geniculate bodies; MGEN, medial geniculate bodies; VT, ventral thalamic area; DHIPP M, dorsal hippocampus (molecular layer); DHIPP G, dorsal hippocampus (granular layer); VHIPP M, ventral hippocampus (molecular layer); VHIPP G, ventral hippocampus (granular layer); DG, dentate gyrus; CS, superior colliculus; CI, inferior colliculus; LLM, lateral lemniscus; LC, locus coeruleus

Table 7-2. Relationship between the relative metabolic activities (rma) of central grey (CG) and midbrain reticular formation (RF), lordosis quotient (LQ) and plasma concentration of luteinising hormone (LH) in individual animals.

Means ± S.E.M. are also shown where applicable. The lower limit of detection of the assay for a 20 μl sample was 2.5 ng/ml.

	Animal	rma CG	rma RF	LQ	LH ng/ml
Mated	1	1.14	1.26	100	22.1
	2	1.08	1.16	85	63.2
	3	1.04	1.20	58	56.7
	4	1.04	1.26	95	16.3
	5	0.93	1.10	67	26.2
	±S.E.M.	1.05±0.03	1.20±0.03	81±8	36.9±9.6
Sham-mated	6	1.20	1.41	63	<2.5
	7	1.15	1.34	43	<2.5
	8	1.04	1.19	27	<2.5
	9	1.15	1.26	67	<2.5
	10	0.87	1.10	37	<2.5
	±S.E.M.	1.08±0.06	1.26±0.05	47±8	<2.5

Table 7-3. Numbers of lordoses, mounts, lordosis quotient (LQ), intromissions and ejaculations shown by individual voles

	Animals	Lordoses	Mounts	LQ	Intromissions	Ejaculations
Mated	1	7	7	100	6	3
	2	17	20	85	4	3
	3	48	83	58	5	1
	4	18	19	95	9	4
	5	58	86	67	11	2
Sham-mated	6	12	19	63		
	7	3	7	43		
	8	4	15	27		
	9	14	21	67		
	10	3	8	37		

rma between the three groups in any of the other areas measured in the brain and pituitary gland (Table 7-1).

The lordosis quotient (LQ; $\frac{\text{number of lordoses}}{\text{number of mounts}}$ x 100) and plasma LH concentration for each individual animal in the mated and sham mated groups are shown in Table 7-2. The mean LQ of mated animals was higher than of sham-mated animals ($P < 0.02$). Plasma LH concentration increased dramatically in mated animals, and remained undetectable in sham-mated (Table 7-2) and control, unmated animals. There was no significant correlation between either LQ and the rma of CG and RF (r=0.170 and 0.668, respectively, n=5), or between LQ and plasma LH concentrations (r=0.498, n=5) in the mated animals. There was also no correlation between plasma LH concentration and the rma of the PD (r = 0.373, n=5) in the mated animals. The numbers of lordoses, mounts, intromissions and ejaculations shown by the individual voles are shown in Table 7-3, from which the LQ were calculated.

7.4 DISCUSSION

The main findings of this study are that in *Microtus agrestis* the CG and RF are involved in the lordosis response to mounting, but neither the metabolic activity of these or other areas of the brain are selectively changed in relation to the reflex surge of LH. As expected from the study by Charlton et al. (1975), mating evoked a massive surge of LH in the vole; however, voles in which intromission was prevented did not show an increase in plasma LH concentration, although they were mounted and showed lordoses. Mechanical cervical stimulation was also unsuccessful in increasing plasma LH concentration in the vole (Charlton et al., 1975). This

suggests that the complete copulatory stimulus, including intromission and cutaneous stimulation is necessary for LH release in the vole, and that the stimulus of mounting alone and vaginal stimulation alone is not sufficient. The stimulus necessary for LH release therefore varies between species; in the LL rat mounting alone was effective in causing LH release and ovulation (Brown-Grant et al., 1973; Davidson et al., 1973), while in the oestrous rat mating caused an acute rise in plasma LH concentration but cervical stimulation with a glass rod did not affect pituitary LH concentration, as assessed by the ovarian ascorbic acid depletion assay (Taleisnik, Caligaris and Astrada, 1966). The oestrous cat will ovulate in response to stimulation of the vagina with a glass rod (Greulich, 1934), but in the oestrous rabbit mechanical stimulation of the vagina will only induce ovulation if the animal has been previously primed with oestrogen (Sawyer and Markee, 1959).

In LL rats, sexual receptivity, as assessed by LQ, was not correlated with the magnitude of the LH surge induced by mating (Davidson et al. 1973), and similarly, in the present study on the vole there was also no correlation between the magnitude of the LH surge induced by mating and the LQ.

The higher mean LQ in the group of mated voles compared with the sham-mated group could be due to experimental bias, in that highly sexually receptive females tended to be placed in the mated group. However, the higher mean LQ could also be as a result of a greater stimulus from the male; in the rat the strength of the lordosis response increases with cutaneous pressure exerted by the male, hence thrusting associated with successful intromission causes a

larger lordosis response than mounting alone (Kow, Montgomery and Pfaff, 1979). In the sham-mated group of voles the males were prevented from thrusting by the tape on the female, and so the magnitude of the resulting lordoses may have been too small to have been noticed.

The fact that the rma of the CG and RF increased in both the mated and sham-mated animals provides further support for the view that the CG and RF are important components of the neuronal pathway involved in lordosis, and that they probably comprise the essential 'midbrain module' of the reflex (Conrad and Pfaff, 1976b; Kow, Grill and Pfaff, 1978; Sakuma and Pfaff, 1979a,b). In the rat, vaginal stimulation is unnecessary for lordosis to occur (Hardy and Debold 1971; Pfaff, Montgomery and Lewis, 1977), cutaneous stimulation by the male is relayed by primary sensory neurones to pressure sensitive inter-neurones in the spinal cord (Kow, Zemlan and Pfaff, 1980). There was no difference in the rma of the CG and RF between mated and sham-mated voles. If the rma of the CG and RF are related to increased activity in the neural pathway involved in lordosis, then it would appear that, as in the rat (Pfaff et al., 1977) and hamster (Kow, Malsbury and Pfaff, 1976), vaginal stimulation is unnecessary for lordosis in the vole.

The lordosis pathway is not a simple reflex arc, since rats with spinal cord transections do not show lordosis, suggesting that a supraspinal facilitation is needed (Kow, Montgomery and Pfaff, 1977). Electrical stimulation of the VMH facilitates lordosis, while lesions of the VMH decrease the lordosis response (Pfaff and Sakuma, 1979a,b; Mathews, Donovan, Hollingsworth, Hutson and

Overstreet, 1983). The mechanism by which the VMH affects lordosis is thought to be due to an action of oestrogen on the firing rate of the VMH cells. Many oestrogen-concentrating cells are present in the VMH and POA (Pfaff and Keiner, 1973; McEwan, Davis, Parsons and Pfaff, 1979), and oestrogen has been shown to increase the firing rate (Bueno and Pfaff, 1976) and the biosynthetic activity (as assessed by ultrastructural changes, Cohen and Pfaff, 1981) of VMH cells. Morrell and Pfaff (1982) have estimated that 26 to 36% of oestrogen-concentrating cells of the VMH send projections to the CG, and these oestrogen-concentrating neurones and their projections to the midbrain may be involved in the facilitation by oestrogen (Beach, 1948) of the lordosis reflex. Oestrogen-induced progestin receptors have been shown to be maximal in the POA and MBH when sexual receptivity is maximal following oestrogen treatment (Parsons, MacLusky, Krey, Pfaff and McEwen, 1980). Electrical stimulation of the POA reduced lordosis behaviour in the hamster (Malsbury, Pfaff and Malsbury, 1980) and lesions of the POA slightly increased, or had no effect on lordosis behaviour in the rat (Numan, 1974; Raisman and Brown-Grant, 1977a). Oestrogen has been shown to reduce firing in the POA (Bueno and Pfaff, 1976). The POA appears, therefore, to exert a tonic inhibition on the lordosis reflex, and this inhibition is counteracted by oestrogen.

Lordosis behaviour is also facilitated by a subcutaneous injection of LHRH in ovariectomised rats treated with oestrogen (Moss and McCann, 1973) and in ovariectomised, hypophysectomised rats treated with oestrogen (Pfaff, 1973). The facilitation of lordosis by LHRH appears to be due to a direct action of LHRH on CG

neurones, since infusion of LHRH into the CG increased lordosis and infusion of an antiserum to LHRH into the CG disrupted lordosis in ovariectomised rats treated with oestrogen (Sakuma and Pfaff, 1980a; 1983). Infusion into the CG of either an anti-LHRH serum or an antagonist to LHRH also reduced significantly the increased LQ produced by infusion of naloxone into the CG of ovariectomised rats primed with oestrogen (Sirinathsinghji, Whittington, Audsley and Fraser, 1983). The content of LHRH in the MBH is increased two days after oestrogen treatment in the rat (Kalra, 1976), and some nerve processes in the CG stain with antiserum to LHRH (Silverman and Krey, 1978). Oestrogen replacement in ovariectomised rats increases LHRH release in the CG (assessed by quantitative immunohisto-chemistry, Shivers, Harlan, Morrell and Pfaff, 1983a) but there has been no evidence that the oestrogen-concentrating cells in the VMH projecting to the CG contain LHRH (Shivers, Harlan, Morrell and Pfaff, 1983b). However, it is probable that the LHRH-containing VMH neurones alter the excitability of CG neurones (Sakuma and Pfaff, 1980b) through the action of oestrogen, either directly or through interneurones, and so facilitate the lordosis reflex. The CG and RF may therefore act as integrating units, relaying the descending hormonally-dependent hypothalamic influence on the lordosis reflex (Pfaff, 1980). The lordosis pathway is completed by axons leaving the CG and RF, relaying in the medullary reticular formation, and continuing in the reticulospinal and vestibulospinal tracts to activate the relevant muscles for lordosis (Modianos and Pfaff, 1976).

Since the pathways mediating lordosis and the control of reflex

ovulation both involve the POA and MBH it is surprising that no change in rma was seen in these areas. If, however, the activity of some POA neurones decreased to facilitate lordosis while the activity of a different set of POA neurones increased to allow LHRH release (Everett, 1964, 1965; Dyer, Pritchett and Cross, 1972; Fink and Jamieson, 1976), it is conceivable that the overall rma of a diffuse area such as the POA would remain unchanged. The rma of the POA did increase after vaginocervical stimulation in the ovariectomised rat treated with oestrogen (Allen, Adler, Greenberg and Reivich, 1981). However, since in the study by Allen et al. (1981) the control group of animals did not have the vaginal probe inserted, and no hormonal data were presented to determine whether the stimulus was effective in causing LH release, the increase in rma of the POA seen in the study by Allen et al (1981) may have been due to non-specific neuronal activation by the stimulus (Barraclough and Cross, 1962; Lincoln, 1969). In the present study on the vole, mating and mounting accompanied by lordosis caused an increase in rma of only the CG and RF. It is not clear whether the differences between the results of the present study and those of Allen et al. (1981) for the POA were due to species or methodological differences.

In the vole and in the LL rat, the amount of LHRH released after mating is likely to be large, as assessed by the decrease in hypothalamic content of LHRH (Smith and Davidson, 1974; Versi et al., 1982). In addition, the LH released from the PD after mating in the LL rat probably involves most of the readily releasable pool of LH (Fink, 1975). No change in rma was seen in either the VmArc and POA, or in the PD in the mated group of voles. This suggests

that the increase in metabolic activity associated with the synthesis and release of LHRH and LH after mating is not sufficient to be detected by the 2DG method. In the case of LH this is perhaps not surprising, since, at least in the rat (Denef et al., 1978), the gonadotrophs make up only a relatively small proportion of the cells in the PD.

CHAPTER VIII

Comparison of Rat, Mouse and Vole Brains

8.1 INTRODUCTION

The 2DG technique has been used to demonstrate alterations in local cerebral metabolism in a variety of animal species, in response to physiological and pharmacological stimuli. The species used have included the rat (e.g. Sharp et al., 1975; Sokoloff et al., 1977; McCulloch, Savaki, McCulloch and Sokoloff, 1979; Greer, Stewart, Teicher and Shepherd, 1982; Savaki, Kadekaro, McCulloch and Sokoloff, 1982), mouse (e.g. Mikoshiba et al., 1981), monkey (e.g. Kennedy et al., 1975; Kennedy, Des Rosiers, Sakurada, Shinohara, Reivich, Jehle and Sokoloff, 1976; Des Rosiers, Sakurada, Jehle, Shinohara, Kennedy and Sokoloff, 1978; Caveness, Kato, Malamut, Hosokawa, Wakisaka and O'Neill, 1980), cat (Webster, Serviere, Batini and La Plante, 1978), guinea pig (Silverman, Hendrickson and Clopton, 1977) and ground squirrel (Kilduff et al., 1982).

Comparisons of the metabolic activity of brain areas in different mutant strains have focussed on the brain areas associated with the nature of the genetic defect; thus 2DG studies have demonstrated differences in the metabolic activity of components of the visual system in albino and pigmented rats (Miyaoka et al., 1979; Batipps et al., 1981), of cerebellar structures in weaver and nervous mice (Mikoshiba et al., 1981) and of the hypothalamo-neurohypophysial system in the Brattleboro rat (Sutherland et al., 1983).

Although Sokoloff (1981) compared rates of local cerebral glucose utilisation in the rat and monkey using data obtained from Sokoloff et al. (1977) and Kennedy et al. (1978), there have been no other comparisons made on the metabolic activity of brain areas in

different animal species. The aim of the present chapter, therefore, was to compare the rma of areas of the brain and pituitary gland in the rat, mouse and vole already estimated in earlier chapters, and also determine the rank order of functional activity throughout the brain in each species.

8.2 EXPERIMENTAL DESIGN

The data used in these studies were that obtained for untreated Wistar rats (Chapter III), untreated normal house mice (Chapter IV) and unmated voles *Microtus agrestis* (Chapter VII). The rma of areas of the brain and pituitary gland were compared by analysis of variance and Duncan's multiple range test, and were also ranked in order of decreasing rma.

8.3 RESULTS

8.3.1 Comparison of rat and mouse brain and pituitary gland

The rma of the following areas of brain were significantly greater in rats compared with mice: S, POA, MFB, VmArc, AMYG, AHA, MM, VHIPPM, LGEN, MGEN, CI and CG (Table 8-1).

8.3.2 Comparison of rat and vole brain and pituitary gland

The rma of rat brain was significantly greater than vole brain in the following areas: S, POA, SCN, PVN, MFB, VmArc, AMYG, AHA, MM, IP, VHIPPM, LGEN, MGEN, CS, CI and CG (Table 8-1).

8.3.3 Comparison of mouse and vole brain and pituitary gland

The rma of the following areas of brain were significantly greater in mice compared with voles: S, MFB, MM, VHIPPM, CS, CI and CG (Table 8-1).

There were no significant differences between the 3 species in the rma of the IC, PN or PD.

Table 8-1. Relative metabolic activity of selected areas of brain and the pituitary gland in Wistar rats, Normal house mice and voles.

Values are expressed as a ratio (mean ± S.E.M.) of [^{14}C] concentration of selected region to [^{14}C] concentration of the corpus callosum.

	Wistar rat (n = 6)	Normal house mouse (n = 5)	Vole (n = 6)
S	2.08 ± 0.14	1.62 ± 0.08	1.23 ± 0.02
POA	1.46 ± 0.09	1.09 ± 0.06	0.83 ± 0.04
SCN	2.12 ± 0.08	1.56 ± 0.21	1.01 ± 0.06
PVN	1.29 ± 0.06	1.00 ± 0.07	0.86 ± 0.04
MFB	1.94 ± 0.06	1.41 ± 0.06	1.12 ± 0.04
VmArc	1.37 ± 0.08	0.94 ± 0.03	0.83 ± 0.05
AMYG	1.45 ± 0.07	1.11 ± 0.07	0.99 ± 0.04
AHA	1.63 ± 0.07	1.17 ± 0.05	0.95 ± 0.03
MM	2.70 ± 0.14	2.74 ± 0.19	1.92 ± 0.11
IP	2.46 ± 0.10	2.13 ± 0.13	1.59 ± 0.11
VT	2.20 ± 0.17	1.90 ± 0.12	1.67 ± 0.11
VHIPPM	2.30 ± 0.15	1.69 ± 0.06	1.31 ± 0.04
LGEN	2.15 ± 0.13	1.71 ± 0.08	1.44 ± 0.04
MGEN	2.60 ± 0.11	1.93 ± 0.08	1.62 ± 0.07
CS	2.11 ± 0.06	2.05 ± 0.16	1.44 ± 0.07
CI	3.81 ± 0.21	2.66 ± 0.23	1.81 ± 0.12
CG	1.61 ± 0.03	1.26 ± 0.07	0.92 ± 0.02
IC	1.05 ± 0.04	1.04 ± 0.07	0.90 ± 0.02
PN	1.00 ± 0.07	0.77 ± 0.06	0.86 ± 0.13
PD	0.80 ± 0.05	0.61 ± 0.04	0.68 ± 0.10

S, septum; POA, preoptic area; SCN, suprachiasmatic nucleus; PVN, paraventricular nucleus; MFB, medial forebrain bundle; VmArc, ventromedial-arcuate hypothalamic area; AMYG, amygdala; AHA, anterior hypothalamic area; MM, mamillary body; IP, interpeduncular nucleus; VT, ventral thalamic area; VHIPPM, ventral hippocampus (molecular layer); LGEN, lateral geniculate bodies; MGEN, medial geniculate bodies, CS, superior colliculus; CI, inferior colliculus; CG, central grey; IC, internal capsule; PN, pars nervosa; PD, pars distalis

Table 8-1 (cont). Significance of differences in the relative metabolic activity of the brain and pituitary gland in Table 8-1 (Duncan's multiple range test).

P < 0.05

Region	Comparison
S	Rat v Mouse Mouse v Vole
POA	Rat v Mouse
PVN	Rat v Vole
MFB	Mouse v Vole
AMYG	Rat v Mouse
MM	Rat v Mouse Mouse v Vole
VHIPPM	Mouse v Vole
LGEN	Rat v Mouse
CS	Rat v Vole Mouse v Vole
CI	Mouse v Vole

P < 0.01

Region	Comparison
SCN	Rat v Vole
VmArc	Rat v Mouse
AMYG	Rat v Vole
MM	Rat v Vole
IP	Rat v Vole
MGEN	Rat v Mouse
CI	Rat v Mouse

Table 8-1 (cont). Significance of differences in the relative metabolic activity of the brain and pituitary gland in Table 8-1 (Duncan's multiple range test).

$P < 0.005$

POA	Rat v Vole
VmArc	Rat v Vole
VHIPPM	Rat v Mouse
LGEN	Rat v Vole
CG	Rat v Mouse Rat v Vole

$P < 0.001$

S	Rat v Vole
MFB	Rat v Mouse Rat v Vole
AHA	Rat v Mouse Rat v Vole
VHIPPM	Rat v Vole
MGEN	Rat v Vole
CI	Rat v Vole
CG	Rat v Vole

Table 8-2. Comparison of the relative metabolic activity of selected areas of the brain and pituitary gland in Wistar rats, Normal house mice and voles.

The rma values are expressed in order of decreasing magnitude within a species.

	Wistar rat (n = 6)	Normal house mouse (n = 5)	Vole (n = 6)
CI	1	2	2
MM	2	1	1
MGEN	3	5	4
IP	4	3	5
VHIPPM	5	8	8
VT	6	6	3
LGEN	7	7	6
SCN	8	10	11
CS	9	4	6
S	10	9	9
MFB	11	11	10
AHA	12	13	13
CG	13	12	14
POA	14	15	18
AMYG	15	14	12
VmArc	16	18	18
PVN	17	17	16
IC	18	16	15
PN	19	19	16
PD	20	20	20

CI, inferior colliculus; MM, mamillary body; MGEN, medial geniculate bodies; IP, interpedencular nucleus; VHIPPM, ventral hippocampus (molecular layer); VT, ventral thalamic area; LGEN, lateral geniculate bodies; SCN, suprachiasmatic nucleus; CS, superior colliculus; S, septum; MFB, medial forebrain bundle; AHA, anterior hypothalamic area; CG, central grey; POA, preoptic area; AMYG, amygdala; VmArc, ventromedial-arcuate hypothalamic area; PVN, paraventricular nucleus; IC, internal capsule; PN, pars nervosa; PD, pars distalis

The rank order of the rma of the different areas of the brain and pituitary gland (Table 8-2) showed that the rma of the same area of the brain and pituitary gland differed by 2 or less rank positions between the 3 species in 12 of the 20 areas investigated. In 7 of the remaining 8 areas, the rma differed by 2 or less rank positions in 2 of the species, but differed by more than 2 rank positions from the third species. The rma of the CS varied by 2 or more rank positions in all 3 species.

8.4 DISCUSSION

In general, the rma of the areas of brain investigated were greatest in the rat, lowest in the vole and intermediate in the mouse. When analysed statistically, the rma values for the rat brain were significantly greater than for the vole brain in all the areas of brain measured, except for the IC, and were significantly greater than for the mouse brain in 12 of the 18 brain areas measured. The rma values for mouse and vole brains were more similar to each other than to those for the rat, and there was a significant difference in only 7 areas between the two species.

The greater rma of areas in the rat brain compared with mouse or vole brain could have been due to more 2DG being available for uptake by the brain, since the 2DG was given i.v. to rats (3.2) and i.p. to mice and voles (4.2 and 7.2). However, the rma of the IC did not differ significantly between the 3 species, and this suggests that the grey matter areas may, indeed, be more active in rat brain than in mouse or vole brain. Alternatively, the high rma of the rat brain may reflect species differences in the density of cellular packing of the brain, since this has been suggested as the

reason for the lower (one-third to one-half) rates of local cerebral glucose utilisation in the monkey compared with the rat (Sokoloff, 1981).

The rank order of the rma values suggests that although the rma of brain areas differed between the 3 species, the ordinal ranking remained similar. In the present study, the areas of brain with the highest rma in rat, mouse and vole brain were primarily sensory, especially auditory, as has been found in other studies on the rat, cat, monkey (Sokoloff, 1978) and ground squirrel (Kilduff et al., 1982), and the areas with the lowest rma were hypothalamic areas, the pituitary gland and the white matter IC.
Comparisons made using ordinal ranking must be interpreted with caution, however, since large differences in rank position can be accounted for by several areas having very similar rma. Thus, the different positions of the rma of the POA, AMYG, IC and PN in the 3 species can be explained by the large number of areas with similar rma at the lower end of the rank order in mouse and vole brain. The areas with the most interesting differences in ordinal ranking of rma, therefore, were the VT, which was greatest in the vole, the SCN and VHIPPM, which were greatest in the rat, and the CS, which was greatest in the mouse. The difference in the ordinal ranking of the CS may have been due to the fact that the mice and voles were pigmented, rather than due to a species difference. The rates of glucose utilisation in the cerebral components of the visual system (CS, LGEN and CX) have been shown to be significantly lower in albino rats compared with pigmented rats (Batipps et al., 1981) and decrease with increasing intensity of light above 7 lux (Miyaoka et al., 1979).

Whether the differences in the ordinal rank position of the SCN, VHIPPM and VT reflect species differences or were due to other factors is not known.

CHAPTER IX

Summary

The 2DG technique for metabolic mapping has been used to measure the rma of structures throughout the brain and pituitary gland caused by, or associated with various neuroendocrine events.

Water deprivation led to a significant increase in the rma of the PVN as well as the PN in Wistar rats. Increased metabolic activity of the hypothalamo-neurohypophysial system was measured in the homozygous Brattleboro rat which has a genetic deficiency of vasopressin. The activity of the PVN and PN in the Brattleboro rat could be increased further by water deprivation, and the increased 2DG uptake by the PVN was found to be localised around the perikarya, possibly in terminals of afferent projections mainly to the magnocellular divisions of the PVN. The rma of the PN in the Brattleboro rat could be reduced by the administration of dDAVP, and was accompanied by a significant decrease in plasma osmolality. There was no difference in the rma of the SON between the two strains or following osmotic stimulation. The rma of the PD was increased in the Brattleboro rat, and was reduced by either water deprivation or the administration of dDAVP.

Studies on the effects of water deprivation and the administration of dDAVP were extended to include the DI +/+ Severe mouse which has hereditary nephrogenic diabetes insipidus. In these animals the rma of the hypothalamo-neurohypophysial neurones was not greater than in control mice, presumably because unlike the Brattleboro rat, the Severe mouse does not have an increased plasma osmolality. A short period of water deprivation increased the rma of the PVN and PN in the Severe mice, and had no effect on the rma of any brain area or the pituitary gland in Non-severe mice. The

increased rma induced by water deprivation could not be reversed by the administration of dDAVP, indicating that in this study dDAVP had no significant central action on the metabolic activity of the hypothalamo-neurohypophysial system. As in the Brattleboro rat, the rma of the PD was increased in Severe mice and could be reduced by both water deprivation and administration of dDAVP, possibly as a result of reduced blood flow following either of these treatments. The rma of the SON was unchanged following osmotic stimulation in all the strains of mice studied.

The changes in rma throughout the brain and pituitary gland associated with milk ejection and PRL release were investigated in conscious and anaesthetised suckled animals and following mammary nerve stimulation. In these studies the conscious animals showed no change in rma of any area of the brain or pituitary gland measured, although plasma PRL concentrations increased. Since the pups had obtained milk, milk ejections probably occurred either before the 2DG was injected, or in the later part of the experimental time period. Anaesthetised suckled animals, however, showed an increase in the rma of the SON and PVN. Since the rma of the PN did not increase in these animals, the 2DG method probably showed increased activity in afferent terminals to the PVN and SON, rather than of the magnocellular neurones themselves. Mammary nerve stimulation caused an increase in the rma of the SON, PVN and PN, indicating that in these animals the activity of the hypothalamo-neurohypophysial neurones was increased. Although the rma of the TSTH in the region of the pons increased following mammary nerve stimulation, it was not possible to trace an increase in rma between

the pons and the PVN and SON, suggesting that beyond the brainstem the milk ejection pathway is too diffuse to be measured by the 2DG technique. In animals in which plasma PRL concentration increased following suckling (conscious and the second group of anaesthetised animals) no change was found in the rma of any area of the brain or pituitary gland measured. This suggests that the release of PRL does not involve a detectable increase in cerebral metabolic activity.

Since an increase in rma of the SON was measured following suckling and mammary nerve stimulation in anaesthetised animals, the absence of a change in the rma of the SON following water deprivation (Chapter III) cannot be attributed to practical difficulties in the measurement of the rma of the nucleus. Since the 2DG method is thought to measure mainly terminal activity, it seems likely that the absence of a change in rma of the SON following water deprivation reflected a difference in the activity of afferent projections to the SON compared with the PVN.

Studies on the effects of electrical stimulation of areas of the 'neuroendocrine brain' provided information on functional connections between areas of the brain, and information concerning the interpretation of results obtained using the 2DG method. Although data from previous chapters in this thesis suggest that the 2DG method measures mainly terminal activity, activity of the perikarya was probably demonstrated in Chapter VI following antidromic neuronal firing. For instance, stimulation of the ME caused an increase in the rma of the PVN, SON and ST. In the electrical stimulation studies the 2DG method demonstrated changes

in the metabolic activity of many brain areas shown by neuroanatomical tracing techniques to be connected with the site of stimulation. Thus, for example, stimulation of the POA and AHA resulted in increased rma of most hypothalamic nuclei, S, CG and AMYG. However, the 2DG method does not trace all anatomical pathways but rather measures functionally active pathways. No change in the rma of the POA-m, VMH or ARC was found following AMYG stimulation, or in the MDT or MM following POA stimulation. Since these areas have been shown to be anatomically connected with the AMYG and POA respectively, the 2DG method demonstrates that antatomical connections are not always active, or if they are, are sometimes too diffuse to be detected by the 2DG method. Similarly, the HIPP is known to have complex intrinsic connections, yet only stimulation of the DHIPP caused an increase in the rma throughout the HIPP.

Brain areas which have not previously been shown to be connected with the site of stimulation were also shown to have increased rma. For example, stimulation of the AHA caused increased rma of areas including the PYRCX, RF and HIPP, and stimulation of the PVN increased the rma of areas such as the MM, IP, PYRCX and thalamic nuclei. These increases in rma may indicate anatomical connections which have not been previously demonstrated, but equally may reflect activation of secondary neurones or fibres of passage. For this reason the 2DG technique is useful in tracing functional multisynaptic pathways which cannot be followed easily using conventional tracing techniques.

The 2DG technique can also give some information on whether a

functionally active pathway is mainly ipsilateral or contralateral. Thus, for example, the rma of the c SCN increased to a lesser degree than the i SCN following VLGEN stimulation, whereas the rma of the c S-1, DHIPPG and VHIPPG were not significantly different from those on the i side following DHIPP stimulation.

Studies on the pathways associated with reflex ovulation in the vole demonstrated that the reflex release of LH was not accompanied by a significant change in the rma of the brain or pituitary gland. However, the rma of the CG and RF were increased in voles showing lordoses. These results further indicate that a brain area can usually only be shown by the 2DG method to be active in a given situation depending upon the activity within and anatomical structure of the afferent input to the area.

Although the rma of brain areas was shown to differ between anatomical species (Chapter VIII), possibly indicative of differences in functional activity or in the cellular packing within the brain, the rank order of the rma values for each species were remarkably similar. The rma of sensory brain areas (primarily auditory) were always highest, while the rma of areas of the hypothalamus, pituitary gland and white matter were always lowest. These results were in agreement with other studies on the rat, cat, monkey and ground squirrel.

The modified 2DG technique has therefore provided information on cerebral metabolic activity in situations where the measurement of LCGU was impractical. As explained in the Introduction, the measurement of rma makes no assumptions as to the stability of the plasma glucose concentrations or cerebral glucose utilisation, and

therefore avoids a potential source of error.

The relationship between OD ratios and LCGU, and between OD and LCGU have been shown to be linear within a single animal, including in areas of high activity (Sharp, Kilduff, Bzorgchami, Heller and Ryan, 1983). The OD ratios or OD alone, however, do not provide a good index of LCGU when compared in animals in quite different physiological states, such as conscious and anaesthetised animals (Sharp et al., 1983b). Therefore, in order to provide valid information on cerebral glucose utilisation, OD ratios and rma should only be compared in animals under similar physiological conditions.

Other criticisms of the modified technique have included the fact that plasma concentrations of [^{14}C]2DG and glucose are not measured throughout the experimental time period (Kelly and McCulloch, 1983a). Provided (1) the animal has been feeding properly and has been allowed to recover sufficiently from any surgery so that blood pressure and glucose concentrations are likely to be normal, and (2) a period of 45 min is allowed to elapse after the injection of 2DG before the animal is killed, these criticisms should not interfere with the validity of the technique in most experiments. Another potential source of error in the modified technique is the length of exposure time of the X-ray film to the sections of brain (Kelly and McCulloch, 1983b). The relationship between [^{14}C] concentration and OD is not linear (Fig. 2-A), and the degree of curvature varies with the duration of the exposure time used in the preparation of the autoradiograph (Kelly and McCulloch, 1983b). However, the [^{14}C] concentrations of most

brain areas fall on the linear part of the curve, and provided the exposure time of the autoradiographs is kept constant within any one experiment, this source of error is kept to a minimum. In Chapter VI the rma of brain areas following electrical stimulation were extremely high, and were not on the linear part of the curve. For this reason the results were interpreted rather more qualitatively than quantitatively, since errors in the values of the rma may have been present.

Thus, for the majority of experimental situations where the measurement of LCGU is impractical, the use of the modified technique to measure rma or relative 2DG uptake is perfectly valid, and has provided useful information on a variety of cerebral functions.

The resolution of the 2DG technique has been improved to the cellular level by a variety of techniques. The uptake of 2DG has been localised in nerve perikarya, glia and terminals in different situations and using different techniques. The resolution of the 2DG technique has been improved to the ultrastructural level in investigations of the invertebrate visual system (Buchner and Buchner, 1982), although there are still practical difficulties to overcome before studies on vertebrate systems can use electron microscopy coupled with 2DG. If the resolution can be improved to this level easily, the 2DG method will provide an even more powerful tool with which to investigate metabolic, and therefore functional, activity throughout the brain.

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Appendix 1. Chromium gelatine coated slides

Reagents

Gelatine (B.D.H.)	2 g
Chromic potassium sulphate (AnalaR)	0.2 g
Distilled water	400 ml

Gelatine dissolved in water at 60°C. Chromic potassium sulphate added.

Cooled to room temperature. Slides immersed in the solution and dried overnight. Stored at 4°C.

Appendix II. Perfusion fixative for [^{3}H] 2-deoxyglucose autoradiography (Maclean and Nakane, 1974)

Reagents

Lysine hydrochloride	0.1 M
di-Sodium hydrogen orthophosphate	0.1 M
Paraformaldehyde	2%
Sodium periodate	0.01 M

1. Lysine and di-Sodium hydrogen orthophosphate dissolved in 900 ml distilled water
2. pH adjusted to 7.4 with 1 M Sodium hydroxide
3. Paraformaldehyde dissolved in distilled water by gentle heating
4. 3 parts lysine buffer added to 1 part paraformaldehyde
5. Solid sodium periodate added

Appendix III. Celestine blue and Mayers Haematoxylin stains for autoradiographs

Reagents

Celestine blue (Hopkin and Williams Ltd., England)	2.5 g
Ferric ammonium sulphate	25 g
Glycerin	70 ml
Distilled water	500 ml

Iron alum dissolved in water. Celestine blue added, boiled for 3 min. Filtered and cooled, glycerin added.

Haematoxylin (B.D.H.)	1 g
Sodium iodate	0.2 g
Potassium alum	50 g
Citric acid	1 g
Chloral hydrate	50 g
Distilled water	1000 ml

Haematoxylin, alum and sodium iodate dissolved overnight. Chloral hydrate and citric acid added and brought to the boil. Boiled for 5 min, and cooled.

Method

1. Sections rinsed in distilled water
2. Stained in celestine blue 3-5 min
3. Washed in distilled water
4. Stained in Mayers haematoxylin 3-5 min
5. Washed in running tap water 30 min
6. Dehydrated in graded alcohols 50, 70, 90, 95, 100%,5 min in each
7. Xylene 10 min
8. Mounted in DPX

Appendix IV. Luxol fast blue and cresyl violet stains (Kluver and Barrera method for myelin)

Reagents

Luxol fast blue (Gurr B.D.H.)	0.5 g
10 acetic acid	2.5 ml
95% alcohol	500 ml
Filtered	

Cresyl fast violet (Fluka)	0.5 g
Distilled water	500 ml
Filtered	

Lithium carbonate	0.25 g
Distilled water	500 ml

Method

1. Cryostat sections rinsed in 95% alcohol for 30 min
2. Luxol fast blue 30 min
3. Differentiated by alternating between lithium carbonate 10 s and 70% alcohol 30 s until the blue stain remained only in white matter
4. Washed in distilled water
5. Counterstained in cresyl fast violet for 1-2 min
6. Washed in distilled water
7. Differentiated in 95% alcohol for 5-10 min
8. Rinsed in 100% alcohol for 10 min
9. Xylene 10 min
10. Mounted in DPX

Appendix V. Protocol for Radioimmunoassay of Prolactin

a) Stock solutions

0.01 M Phosphate buffered saline (PBS)

8.17 g Nacl
0.10 g sodium methiolate
0.25 g $NaH_2PO_4.2H_2O$
1.193 g Na_2HPO_4
anhydrous
per litre distilled H_2O
pH 7.6

Antiserum buffer (AS)

400 ml PBS
9.306 g EDTA dissolved by warming
pH adjusted to 7.0 with 5N NaOH before adding
1.65 ml normal rabbit serum

Assay buffer (0.01 M PBS/1% BSA)

10.0 g bovine serum albumin (BSA)
per litre 0.01 M PBS

0.1M Borate buffer

0.618 g boric acid
0.1 g sodium methiolate
11.8 ml 0.1 M NaOH
made up to 1 litre with
distilled H_2O
pH 8.6

0.05 M Phosphate buffer (PB)

12.5 g $NaH_2PO_4.2H_2O$
59.65 Na_2HPO_4
anhydrous per litre
distilled H_2O

0.01 M $NaHCO_3$

1.14 g $NaHCO_3$
per 100 ml distilled H_2O

Rinse for iodination (KI/sucrose)

0.1 g KI
0.8 g sucrose
per 10 ml distilled H_2O

b) Iodination

Column

25 x 1 cm Sephadex G50

coated with 200 µl human
albumen (4.5% in 0.01 M
PBS)

Hormone	Rat PRL (rat-PRL-1-5, NIADDK) 100 μg/400 μl $NaHCO_3$ 5 μg/20 μl aliquots stored at -40°C
$Na^{125}I$	1 mCi in 10 μl
Chloramine T	1 mg/ml 0.05 M PBS; 15 μl used
Sodium metabisulphide ($Na_2S_2O_5$)	2.4 mg/ml 0.05 M PBS; 50 μl used

Protocol

i) Hormone mixed with $Na^{125}I$

ii) Chloramine T added and mixed

iii) Reaction time 20 sec

iv) Reaction stopped with $Na_2S_2O_5$

v) Transferred to column; PRL eluted with 0.1 M borate buffer at pH 8.6. Container rinsed twice with KI/sucrose, and the rinses eluted on the column

vi) Fractions for PRL collected in 0.5 ml PBS/1% BSA

vii) Radioactivity in fractions estimated and fractions with high radioactivity retained

c) <u>Standards</u>

Rat PRL (rat-PRL-RP-1, NIADDK) 0.5, 1.2, 2.0, 4.0, 8.0, 16.0, 32.0 and 64.0 ng/ml 0.01 M PBS/1% BSA, stored as 200 µl aliquots at -40°C.

d) <u>Assay Protocol</u>

Day 1 200 µl standard/sample
200 µl assay buffer
200 µl antiserum diluted in AS

Day 2 200 µl ^{125}I-rat PRL (~ 10000 cpm) diluted in assay buffer

Day 4 200 µl ARGG diluted in 0.01 M PBS

Day 5 Tubes centrifuged for 45 min at 2000 x g, supernatant aspirated and pellet counted.
Assay incubated at 4°C

Appendix VI. Protocol for Radioimmunoassay of luteinising hormone

a) Stock solutions

0.01 M PBS	see V a)	
AS	see V a)	
Assay buffer	see V a)	
0.01 M PBS/5% egg white		5 g egg albumen per 100 ml 0.01 M PBS, centrifuged at 2000 x g for 5 min before use

b) Iodination

Column	12 x 1 cm Biogel P60; coated with 1.5 ml 0.01 M PBS/5% egg white
Hormone	ovine LH (LER-1056-C2, NIADDK) 500 μg/ml 0.01 M PBS 10 μl aliquots stored at -40°C
$Na^{125}I$	1 mCi in 10 μl
Chloramine T	5 mg/ml 0.01 M PBS; 10 μl used
$Na_2S_2O_5$	5 mg/ml 0.01 M PBS; 25 μl used

Protocol

i) Hormone mixed with $Na^{125}I$

ii) Chloramine T added and mixed

iii) Reaction time 2 min

iv) Reaction stopped with $Na_2S_2O_5$

v) Transferred to column; LH eluted with 0.01 M PBS

vi) 1.0 or 0.5 ml fractions collected into tubes containing 0.5 ml 0.01 PBS/5% egg white

vii) Radioactivity in fractions estimated and fractions with high radioactivity retained

c) Standards

Ovine LH (NIH-LH-S18, NIADDK) 0.25, 0.50, 0.75, 1.2, 2.0, 4.0, 8.0 and 16.0 ng/ml 0.01 M PBS/1% BSA, stored as 200 μl aliquots at -40°C.

d) Assay Protocol

Day 1	200 μl standard/sample
	200 μl assay buffer
	200 μl antiserum diluted in AS
Day 2	200 μl ^{125}I-ovine LH (~ 10000 cpm) diluted in assay buffer
Day 4	200 μl ARGG diluted in 0.01 M PBS

Day 5	Tubes centrifuged for 30 min at 2000 x g, supernatant aspirated and pellet counted

Assay incubated at 4°C