# The role of brain monoamine systems in intra-cranial self-stimulation

By

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Thesis presented for the degree of
Doctor of Philosophy in the
University of Edinburgh

Department of Pharmacology
University of Edinburgh Medical School

September 1980

Statement in terms of Ph.D. Regulation 2.4.15 of the Postgraduate Regulations of the University of Edinburgh.

The work presented in this thesis has been composed by myself with the following exceptions:-

A. The estimations of HVA and DOPAC concentrations, described in Section One were performed by Dr. N. Nicolau. The HMPG estimations were performed by Dr. C.M. Yates.

B. The estimations of tyrosine hydroxylase activity in the locus coeruleus as described in Section Three were performed by Dr. R. Zigmond in the University of Cambridge.

C. The estimations of noradrenaline and dopamine concentrations described in Section Four were performed by Mrs. A. Wright.

All experimental design, surgical procedures, behavioural work, tissue preparation, calculations and analysis and subsequent interpretation of results was carried out by myself.

Signed

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Statement in terms of Ph.D. Regulation 2.4.11

The results of some of the experiments in this thesis have been published as follows:-

Section One, initial results:- in

G.W. Arbuthnott, M.J. Mitchell, N.M. Nicolau and C.M. Yates.

J. Physiol (Lond) 267, 36-37P, 1977

Changes in dopamine metabolism after intracranial self-stimulation in area ventralis tegmenti.

Section Three, initial results:- in

G.W. Arbuthnott,

Brain Stimulation Reward, eds A. Wauquier and E.T. Rolls,

North-Holland, Amsterdam, pp 251-260, 1976.

Further evidence for the involvement of noradrenaline in locus coeruleus self-stimulation.

Acknowledgements

I would like to thank the Medical Research Council for their support, and for providing the facilities and communal expertise of the Brain Metabolism Unit. I would like to thank its former director, Professor G. Ashcroft for allowing me to work there, and also the Department of Pharmacology.

Mainly my thanks go to my supervisor, Dr. G.W. Arbuthnott. Help, advice and encouragement can rarely have come in a finer manner - my apologies for "ge'in ye sic ae sair deevin!" My thanks also to my University supervisor Dr. J. Walker, without whose efforts this work could neither have been started nor completed.

My thanks to the staff of the Unit, for much help and friendship. In particular, to Mrs. A. Wright, for both in good measure. To Nicos Nicolau for his time and practical skills.

I remain grateful to Mrs. Jean Hunter and her staff who supplied and cared for my animals, and gave great assistance.

I would also like to thank Fiona Panton who struggled bravely with the manuscript to produce this thesis.

Finally my thanks to my wife Corinne, and daughter Gemma for loving support and encouragement through the prolonged gestation. My apologies for all the time it stole.

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Abstract

The extent of the involvement of the catecholamine systems in intra-cranial self-stimulation (ICSS) in the rat has been investigated. It has been demonstrated that self-stimulation with electrodes situated in the ventral mesencephalon was associated with increased release of dopamine as indicated by the concentrations of the metabolites homovanillic acid and dihydroxyphenylacetic acid. This increase was found only in one terminal region of the mesencephalic dopamine systems, namely olfactory tubercle but not in the nucleus accumbens or corpus striatum. Self-stimulation with electrodes in medial posterior hypothalamus did not affect dopamine release in any of these areas.

A pharmacological study of ICSS from medial posterior hypothalamus using the drugs α-methylparatyrosine, spiroperidol, para-chlorophenylalanine and alaproclate was conducted. This indicated that the catecholamine systems had a considerable involvement in the performance of ICSS, but that the indoleamine systems did not have such a close involvement.

The ability of ICSS from the area of the locus coeruleus to cause enhanced activity of the enzyme tyrosine hydroxylase in that nucleus has been demonstrated. The effect of lesioning the ascending DA systems with the neurotoxin 6-hydroxydopamine on ICSS from an area in the dorsal pons mainly anterior to locus coeruleus has been investigated. No long-lasting effect on ICSS was observed after such a lesion.

The relevance of these experiments to the catecholamine theory of ICSS has been discussed.

Finally, recent experimental developments were discussed and the catecholamine theory of ICSS re-assessed.

## General Introduction

The ability of electrical stimulation of the brain to function as positive reinforcement was first discovered by Olds and Milner in 1954 (Olds J & Milner P, 1954). Reinforcement was a concept introduced by Thorndike (1911) in his "law of effect" which recognised the importance of reward and punishment. It was further defined by Skinner (1953) as:

> "A reinforcer is any stimulus that increases the probability of a response that it follows".

This might be effective in causing an organism either to work for or to avoid the reinforcer (positive reinforcement and negative reinforcement respectively). Behaviour of this type could be called instrumental learning, or operant conditioning (Skinner 1938). Electrical stimulation of the brain in itself was not new. It might be credited to Fritsch and Hitzig (1870) who elicited motor responses in the dog upon galvanic stimulation with an electrode placed on the cerebral cortices thus demonstrating the excitability of brain tissue. This technique was used by Ferrier (1876) using induced or "Faradised" current to produce the first mapping study of the brain using electrical stimulation, in this case the discovery and coarse mapping of motor cortex in the dog. Leyton and Sherrington (1917), in a similar manner, produced a more discrete map of motor cortex in primates by using smaller electrodes and a more refined control of the current used. They also correlated their stimulation map with an anatomical study, using Campbell's (1905) description of cerebral cortex by "cell and fibre lamination". In these points, they laid the foundations for the later investigation of brain stimulation reward.

However, these studies involved surface stimulation only. Studies with electrodes implanted within brain structures were also carried out, for instance by Hess (1929). In this study he produced sleep in cats by stimulating an electrode in the thalamus. With respect to this experiment, although with prophetic implication, he said:

> "In the influence of these deep strata on higher brain parts, in the sense of regulation of the readiness to function, we perceive something fundamental concerning the functional structure of the CNS".

Hess (1949) also noted an arousal effect on stimulating posterior hypothalamus, which he called a 'dynamogenic zone'. This too was to foreshadow later discoveries.

By the time of Olds and Milner's discovery much investigation of the effects of stimulating brain tissue had been done, for example in production of physiological sleep from the thalamus (Akert et al 1952) or in the disruption of some learning tasks by stimulating frontal cortex (Rosvold and Delgado 1953). The ability of electrical stimulation to act as a positive reinforcer or reward which produced electrical self-stimulation behaviour, had major implications and was the start of an intense investigation of the phenomenon.

Electrical self-stimulation, or intra cranial self-stimulation (ICSS) seemed to provide a key to the physiological substrates of reward. There have been three major approaches in the attempt to delinate these substrates. These are firstly, mapping the brain areas which will support ICSS; secondly, the effect of lesions at a site in the brain distal to the stimulating electrode and thirdly the effect of chemical manipulation on ICSS.

## 1. Mapping Studies

Olds extended his initial observation of ICSS in the septum to include the amygdala and anterior hypothalamus, and, although with lower response rates, in hippocampus, cingulate cortex, anterior thalamus and posterior hypothalamus (Olds, 1956). Some hypothalamic areas were shown to be aversive to stimulation, and could produce negative reinforcement (Roberts 1958a). Further investigation of this aversive stimulation indicated that the same area could be both positive and negative. Far lateral and far medial hypothalamus were highly positive, medial was highly aversive, and there were ambiguous areas where they overlapped. The production of reward or punishment in these areas was largely dependent on the stimulus parameters used, long trains of stimulation being the more aversive (Roberts 1958b, Olds and Olds 1963). The occurrence of ICSS in limbic cortex, in entorhinal and cingulate cortex was shown (Stein and Ray 1959). More detailed mapping of the hypothalamus and midbrain led to the discovery of two anatomically distinct groups of positive sites, one passing from septum through medial thalamus to the midbrain and another in a more central aspect through hypothalamus, then turning dorsally into the dorsal tegmentum (Olds et al 1960; Olds and Olds 1963). The ventral group which produced high rate/low threshold ICSS was found to closely follow the medial forebrain bundle (MFB), a compact fibre system extending as far rostrally as the olfactory tubercle and caudally to the ventral tegmental area of Tsai, comprising of ascending and descending fibres, largely uncrossed, which reciprocally connect the limbic forebrain and midbrain (Le Gros Clark 1938: Nauta 1958: Millhouse 1969). There was a qualitative difference between the MFB group and the rhinecephalic

(amyg-hippo-septum-thalamic) group (Olds and Olds 1965). The latter was slower (~ 500 responses/hour as against up to 10,000 responses/hour in MFB), was satiable and did not produce hyperactivity. For the first time, the idea of two distinctive systems underlying CSS was suggested. It should be noted that response rate should not necessarily be equated with reward strength. If a choice was given to the animal to select either septal or lateral hypothalamic stimulation the animal chose the septal even though it responded at a much lower rate (Hodos and Valenstein 1962).

Up till these studies, there was only the simple correlation of anatomy and behaviour. Other work around the mid sixties brought in the question of which specific neurotransmitters were released from the specific neural systems involved in ICSS. This must be mentioned as it was hereon possible to link the anatomical map with a neurotransmitter map.

Two separate experiments linked the catecholamines (CA) to ICSS. The drug, amphetamine, which was known to cause release of NA in the peripheral nervous system was demonstrated to do likewise in the CNS, and simultaneously produced a large increase in responding in lateral hypothalamus (LH) ICSS rates. Amphetamine was not effective if the CAs were depleted, and was stereo specific, the d-isomer being much more potent than the l-isomer (Stein 1964). In addition, the use of monoamine oxidase inhibitors (MAOI) a class of drugs which prevent enzymatic degradation of CA's was also shown to enhance responding in LH ICSS rats (Poschel and Ninteman 1963). Thus the CA's were implicated as being involved in ICSS. The implication was strengthened by the correlation of the map of CA anatomy with the ICSS anatomy. It seems reasonable to delinate the CA anatomy here.

The mapping of catecholamines, noradrenaline (NA) and dopamine (DA) was first done using a histochemical method that involved the formation of fluophores from NA and DA in a reaction with formaldehyde vapour (Falk et al 1962). Using this method the CAs were found to form discrete neural systems, with cell bodies in the pons and mesencephalon and long fibres terminating in the forebrain (Dahlstrom and Fuxe 1964; Fuxe 1965; Anden et al 1966). Using a similar method, a detailed map of the CA systems was produced (Ungerstedt 1971). The introduction of a more sensitive method, the glyoxylic acid method, enabled this map to be considerably extended and more accurately defined (Lindvall & Bjorklund 1974). Based on the latter study, a summarised anatomy of the CA systems was as follows:

The noradrenergic systems

1. The ascending dorsal tegmental bundle (DTB)

It originates in the nucleus locus coeruleus (LC) (A6 in the nuclear designation of Dahlstrom and Fuxe 1964). It then turns rostrally and runs medial to the superior cerebellar peduncle (SCP) and then in the dorsal part of the SCP decussation. Some of the DTB fibres turn rostroventrally along the tegmental radiations, along with some collaterals of fibres continuing with the main bundle. Two branches turn dorsally into the posterior and anterior colliculi. Passing ventrolaterally to the periventricular gray of third ventricle, the bundle turns ventrolaterally between fasiculus retroflexus and the medial lemniscus. Some fibres turn dorsally to innervate thalamus except for a few fibres which turn laterally into the internal capsule, joining with DA nigrostriatal fibres. The rest gradually join the MFB throughout middle hypothalamus. These latter NA fibres may end in the supra optic decussations or ansa lenticularis.

The DTB runs in the dorsomedial part of the MFB. In rostral hypothalamus the axons give off abundant collaterals, the dorsal ones run into the reticular thalamic nuclei, and others more rostral form a bundle which runs on and in stria medullaris to the anterior thalamic nuclei; the ventral collaterals mix with non-DTB axons to run into the supra optic decussation. Further rostrally, fibres run dorsally into the interstitial nucleus of stria terminalis and pass through into stria terminalis hence to amygdala and in the fornix to hippocampus and caudal septum. The remainder of the DTB fibres continue to the level of rostral septum. Some project to the anterior olfactory nuclei but must turn dorso-medio rostrally and run along the septo-hypothalamic tract to the genu of the corpus callosum, where they divide into a caudal and rostral branch. The caudal branch travels along the fornix superior to hippocampus. The rostral branch runs caudally above the corpus callosum within the cingulum, giving off fibres to large areas of neocortex, till it sweeps round to enter hippocampus from the caudal side.

2. The central tegmental tract (CTT)

This originates far caudally in the pons from the CA cell group A1 in the lateral reticular nucleus and A2 in the dorsal vagal nucleus and the commisural nucleus. The axons course rostrally in a dorso-medial direction through the pons, in the CTT which grows in size as axons from A5 cell group (lateral to superior olivary nucleus), A7 the subcoeruleus cell bodies, and possibly also from the dorsal A4. They now run ventrolateral to the DTB, virtually filling this part of the tegmentum. Some fibres from the A6 group run in the CTT dorsal and medial to the main CTT, but seem to pass straight through the tegmental radiations parallel to the DTB and into the MFB. Fibres and collaterals

leave the CTT almost vertically from the CTT to the periventricular gray of the fourth ventricle, including LC, and may give rise to the extensive terminals in this region, and in the dorsal raphe and adjoining ventro-lateral antral gray. The positive and medullary fibres of the CTT run through and ventral to the SCP decussation, then turn rostroventrally to fan out along the tegmental radiations. Some only travel a short way with the radiations to resume a longitudinal course which is more ventrolateral than before. Others continue along the radiations to the ventral tegmentum. Here they join the most ventral CTT fibres to contribute to the MFB. At this level the CTT is thinned out but has a wide mediolateral extension. These fibres bend ventrally through and partly lateral to the medial lemniscus to run into zona incerta and Forels $H_2$ field. They pass along the crus cerebri, which some fibres leave ventrolaterally, while the rest either pass dorsal to the crus cerebri towards the supra optic decussations or through the crus cerebri to join with the internal capsule branch of the DTB to pass into the ansa lenticularis and the supra optic decussations. The fibres of the CTT which join the MFB give off abundant collaterals and innervate

(a) The mediobasal hypothalamic area

(b) The dorsomedial and paraventricular nuclei hypothalamic nuclei (by different projections)

(c) The supraoptic decussations, hence to anterior hypothalamus and periventricular preoptic region. Also to piriform cortex.

(d) Along ansa leticularis to ventral neostratium and amygdala (mainly central amygdaloid nucleus)

(e) To the interstitial nucleus of stria terminalis, thence to septum.

(f) To the medial preoptic area and to the septal nuclei.

3. The dorsal periventricular system.

This system extends through the periventricular and periaqueductal gray of the medulla oblongata, pons, mesencephalon and diencephalon - it can be regarded as the CA component of the dorsal longitudinal fasiculus. It runs sparsely from around the nucleus of the solitary tract to locus coeruleus, and may be either ascending or descending. Rostral to LC, it markedly increases in size gathering fibres from LC or A4, It moves rostrally dorsal and medial to LC in the lateral part of the periventricular gray. CA cell bodies are scattered among the fibres, which could appear as a diffuse rostromedial extension of locus coeruleus. Some of the fibres turn ventrally to either run along the raphe and down the caudal edge of the decussation of the SCP or to turn more sharply ventral to the ventral tegmental nucleus, both ending in the ventromedial tegmentum, and forming part of the tegmental radiations. Fibres carry on rostrally from the raphe complex within the dorsal longitudinal fasiculus to medial and midline thalamic, pretectal and hypothalamic regions. The hypothalamic branch probably contributes to the ventral periventricular system.

4. The ventral periventricular system (VPS)

This system is first seen immediately dorsolateral to the interpeduncular nucleus (IP). The fibres run rostrally central to the supramamillary commisure and then medial to the mamillothalamic tract. The fibres are seen to innervate the medial mamillary nucleus. Fibres from the medial part of the MFB, or collaterals, come rostromedially to join the VPS, thus increase it in size as it passes rostrodorsally into the dense CA terminal area of the dorsomedial hypothalamic nucleus, which also receives fibres from the DPS. The system thus formed is a broad ascending CA fibre system on the lateral aspect of the periventricular nucleus, which connects to the paroventricular hypothalamic nuclei.

Rostral to these nuclei, the VPS becomes dispersed and some fibres turn dorsally along the anterior commisure to caudal septum, and some laterally to the interstitial nucleus of stria terminalis where it joins many other CA fibres from the MFB.

5. Tegmental CA radiations

As already described these form a radial fan of CA fibres in the mesencephalic tegmentum, with contributions from DTB, CPS and mainly CTT to assemble at the mesodiencephalic junction to help form the MFB. In addition to these, there is a median CA fibre flow from the dorsal raphe in a rostroventral direction between the medial longitudinal fasciculi to a region dorsal to the IP nucleus where they turn sharply rostral. These fibres mainly originate from the CA cell bodies distributed within the raphe and this median fibre flow, which may be regarded as a dorsal extension of the A10 cell group. A slightly more lateral group of fibres passes from the dense tangle around the raphe, medial to the DTB, and has a dense terminal network in the ventral tegmentum. The lateral fibres of the radiation are as aforesaid from the DTB and CTT, and mingling with the fibres from the lateral A8 DA cell group then run rostrally, medially dorsal to the medial lemniscus into the ventral tegmental area of Tsai, and between medial lemniscus and SN through zona incerta and $H_2$-field of Forel, to both form part of MFB.

6. Nigro-Striatal DA System

Arising from the A9 DA cell group in SN, zona compacta the fibres run medially, before turning sharply rostral in a well defined bundle that ascends in the $H_2$ field of Forel dorsolateral to MFB. Fibres leave first at the level of the subthalamic nuclei to run laterally and rostrally through the internal capsule to the caudal

parts of the neostriatum. The remaining fibres run rostrolaterally into the medial edge of the internal capsule, then some turn dorsolaterally into the central parts of the striatum, the remaining fibres running more rostrally before turning laterally through globus pallidus to the head of the striatum. Some remaining fibres run rostrally dorsal to MFB and just rostral to the anterior commisure turn into the head of striatum, to the interstitial nucleus of stria terminalis and some to ansa leticularis.

7. The mesolimbic DA system.

Arising from the A10 DA cell group, situated dorsal to the interpeduncular nucleus and possibly diffusing dorsally along the tegmental radiations to the dorsal raphe. The fibres run rostromedially with the converging fibres from the CTT and tegmental radiations to help form the MFB. The fibres run dorsomedially in the MFB till the level of the retrochiasmatic region where fibres, often collaterals, leave laterally to run together with the nigrostriatal fibres in ansa lenticularis towards amygdala, ventrocaudal neostriatum and piriform cortex. The remaining MFB fibres separate, some running dorsally into the nucleus accumbens, and others fan out rostromedial and laterally before turning centrally into the olfactory tubercles. Those still part of MFB continue rostrally, and some fibres turn dorsally into the diagonal band of the septum, giving rise to a dense terminal system in lateral septal nucleus. The final fibres continue rostrally till the anterior nucleus accumbens where they turn dorsally along the lateral edge of the accumbens along the external capsule dorsally and laterally and into the deep layers of the frontal cortex.

Thus the CA systems were shown to be widely distributed throughout the brain. The existence of cortical DA was not shown in the earlier studies using the Falck-Hillarp method. However although it was visualised using the glyoxylic method (Lindvall & Bjorklund 1974), its existence in cortex had already been indicated by biochemical measures which showed it did not simply exist as a precursor to NA (Thierry et al 1973). It was biochemically localised in limbic cortex-cingulate, piriform and entorhinal (Browstein et al 1974), and this was confirmed using pharmacological-histochemical methods (Hokfelt et al 1974, Lidbrink et al 1974). The CA's thus provided very widely distributed neural systems which furthermore often affected different brain areas virtually simultaneously due to the extensive formation of collaterals.

The studies of ICSS sites so far (Olds & Olds 1965), were all situated in areas receiving CA innervation. The MFB was already noted as a major site for ICSS, now also seen as the major pathway for ascending CA systems. Study of all well executed mapping studies revealed that all positive areas were near CA cell bodies, axons or nerve terminals (German & Bowden, 1974). To continue the mapping studies, and consolidate this statement, positive electrode sites in the ventral tegmentum, around the interpeduncular nucleus which corresponded to the A10 group were found, and this was the first anatomical correlation of CA systems and ICSS (Dresse 1966) although it was spoiled by defining them as noradrenergic cells. As this indicated a mesolimbic DA involvement, let us discover if the whole system has been shown to support ICSS, and thence the other major CA systems.

(a) Mesolimbic DA

The cell body area finding was confirmed (Crow 1971, 1972a). The fibre projection was also positive, both in the ventral tegmental area of Tsai (Olds & Olds 1963, Routtenberg & Malsbury 1969) and of course throughout MFB (Olds et al 1960). Areas of terminal projection found positive were nucleus accumbens and olfactory tubercle (Olds et al 1960: Routtenberg 1971), frontal cortex (mainly medial and sulcal regions), (Routtenberg 1971: Routtenberg & Sloan 1972) and entorhinal cortex (Collier et al 1977).

Thus the correlation between ICSS and the mesolimbic system is very high.

(b) Nigrostriatal DA

The cell body area, zona compacta of SN was highly positive (Routtenberg and Malsbury 1969: Crow 1972a), as was the pathway in hypothalamus (Olds et al 1960, Huang & Routtenberg 1971). The terminal areas were also positive, in the neostriatum (Routtenberg 1971: Phillips et al 1976, Wurtz & Olds 1963) and the amygdala (Wurtz & Olds 1963: Routtenberg 1971). Thus again the correlation was very high.

(c) Dorsal Tegmental Bundle

The cell bodies were shown to be positive (Crow et al 1972 : Ritter and Stein 1973), and the area anterior to A6, around the DTB (Routtenberg & Malsbury 1969: Wolfe et al 1971). The MFB is positive in common with all these systems. Terminal areas which have proved positive include hippocampus (Huang & Routtenberg 1971: Olds et al 1960: Ursin et al 1966), amygdala (Wurtz & Olds 1963): periventricular hypothalamus (Huang & Routtenberg 1971: Atrens et al 1972) interstitial nucleus of stria terminalis (Olds et al 1960: Routtenberg 1971) and dorsal and lateral cortex (Olds et al 1960;

Ursin et al 1966). Thus the correlation for this system was also high.

(d) Central Tegmental Tract

Most of the sites correlated with this tract can be related to the preceding CA systems e.g. in MFB, central tegmentum or where it contains fibres from LC. The other evidence is contradictory. Some evidence exists for the tract to be positive where it is separate from the DTB (Ritter & Stein 1974: Wolfe et al 1971) but still containing fibres from A6, A7 and A4 (Lindvall & Bjorklund 1974).

Attemps to produce ICSS at the cell body sites A1 & A2 have not been successful (Crow 1972b: Anlezark et al 1974). ICSS in the area is possible (Carter & Phillips 1975), although the involvement of A1 or A2 is uncertain.

Thus although the correlation of ICSS with the CTT is fairly good rostral to LC it is not good caudal to the appearance of LC fibres.

(e) The periventricular systems

Again although most of sites corresponding to these systems are common to other CA systems, e.g. stria terminalis, and the pons certain sites may indicate a possible involvement. Firstly, sites in the pons anterior to LC, and medial to the SCP, produce higher rates of responding and more easily obtained ICSS (Routtenberg 1971: Section Four). This could be due to the presence of cell bodies and fibres of the DPS in this area. Also the positive thalamic areas (Olds et al 1960: Olds & Olds 1963) may be partly due to the DPS which passes through thalamus to septum. The often highly positive far medial/periventricular hypothalamic areas (Olds et al 1960: Olds & Olds 1963: Atrens et al 1972) support the possible involvement of the VPS in ICSS.

However, although some correlation exists it is not conclusive. Indeed conclusive proof would be hard to come by due to the diffuse distribution of cell bodies in these systems.

The anatomical correlation of the CA system with ICSS was a very good one. However, with the widespread CA innervation in rat brain, it would be difficult to avoid being in or near a CA system with an electrode which supported ICSS. The demonstration of ICSS from some CA cell bodies was an important factor in the ICSS problem, and has done much to link ICSS with the catecholamines. The indoleamine (5-HT) neural system has also been suggested as a substrate for ICSS. Mapping work has shown ICSS from both the dorsal raphe (Margules 1969: Simon et al 1976) and from the median raphe (Simon et al 1973: St. Laurent 1973).

These nuclei also project into the MFB, projecting ventrorostrally to the ventral tegmentum and then into MFB, where they occupy the ventral part. They innervate hypothalamus, neostriatum, cortex and hippocampus (Ungerstedt 1971a .

However, 5-HT involvement was not certain from the mapping studies, as CA's both follow the same path and terminate in the same structures. In addition the area of the dorsal and medial raphe was thick with CA innervation mainly from the DPS & CTT and also possessed CA cell bodies, probably from A1O. The possibility that they support the ICCS cannot be ruled out in these type of studies.

All the prece ding studies have involved the rat only, and a species specific behaviour might be inferred. This was not the case. ICSS has been produced in fish (Boyd and Gardner 1962), birds (Goodman & Brown 1966: MacPhail 1967), rabbit (Bruner 1967), dog (Stark & Boyd 1963), cat (Roberts 1958b: Wilkinson and Peele 1963: O'Donohue & Hagamen 1967) and man (Heath 1963: Bishop et al 1963) and monkey (Brodie et al 196O: Plotnik et al 1972). The effects in man are varying, ranging from mild pleasure, relaxation and sometimes

sexual feelings to euphoria (Sem-Jacobsen 1968: Heath 1963). However, areas such as the amygdala, caudate, mesencephalic tegmentum and septum were shown to cause self stimulation behaviour (Heath 1963: Bishop et al 1963) which in rat are CA innervated areas and as the principles of organization of the monoamine neurone systems are notably similar in rat and man (Nobin & Bjorklund 1973), it may be possible to assume a CA involvement in man also. More detailed mapping studies of rabbit (Bruner 1967), cat (O'Donohue & Hagamen 1967) and monkey (Plotnik et al 1972) have been carried out and show a similar pattern to the rat studies, with the most positive areas being in ventral tegmentum and MFB, and in cat at least in the LC and area anterior in the pons, Thus a theory based on CA was not confined to the rat.

The data from mapping studies would appear to uphold the involvement of CA's as substrates for ICSS. Derived from mapping studies (and pharmacological studies also) a theory of ICSS based on the A9, A10 DA and A6 NA was evolved by Crow (1972b, 1973). Even the bare mapping studies would seem to indicate that these systems alone are insufficient, viz., the midthalamic area, the olfactory bulb (Phillips & Mogenson 1969) and periventricular hypothalamus. However the theory does allow for such sites by postulating that systems (probably sensory) which feed into these CA systems might also support ICSS. This remained to be proven. As a basic theory, however, a CA theory does have support from the mapping studies (German & Bowden 1974).

Pharmacology of ICSS

In addition to mapping studies of ICSS the use of psychoactive chemicals which alter normal regulation of neurotransmitter function has been employed to characterise the neural basis of ICSS. For this approach to be conclusive, the drug has to have a specific mode of action, and produce a specific effect. This simple aim has proved remarkably elusive in the case of ICSS, since early drug studies with chlorpromazine showed differing effects depending on the electrode site used (Olds et al 1956). This has been a common problem since then, and may be a reflection of the heterogenity of the ICSS systems. Although not definitive, drug studies have suggested a general direction to studies of ICSS, especially with regard to the catecholamines.

This CA involvement was apparent in retrospect in that initial drug study of Olds et al (1956). The drugs reserpine and chlorpromazine known then as tranquillizers, reduced the bar-press rate for ICSS markedly, and these drugs have since been found to interfere with CA transmission, reserpine by releasing and preventing storage in the NA vesicles (Sulser and Sanders-Bush 1971) and chlorpromazine (amongst other actions) to block the CA receptors (Anden et al 1970). This reduction in operant responding was not unique to ICSS as responding for other reinforcers was similarly suppressed (Chance & Silverman 1964). Although animals were shown to be capable of a passive shock avoidance tests after chlorpromazine (Olds, Hogberg and Olds 1964) this generalised deficit in operant responding has remained a problem in the interpretation of drug effects in ICSS.

More direct evidence for CA's as ICSS substrates was produced by drugs enhancing transmission at CA synapses. Amphetamine increased ICSS at low doses, and seemed to act by releasing endogenous amines as

reserpine blocked this effect (Stein 1964). Amphetamines actions are complicated, as it potentiates CA release, inhibits re-uptake and also MAO, an enzyme responsible for breakdown of free amine within the cell (Bloom & Giarman 1968), but the result is an increased amount of CA in the synapse. Drugs which inhibit monoamine oxidase, the MAOI, such as iproniazid, pargyline and tranylcypromine also increased ICSS (Poschel & Ninteman 1964). Another compound, α-methyl-m-tyrosine believed to act as a releasor of CA, increased ICSS (Stein 1966, Crow 1969) especially if given with an MAOI to prevent its breakdown (Poschel & Ninteman 1963). Phenethylamine, which is similar structurally to the amines, also was excitatory if given with a MAOI to prevent its own breakdown (Stein 1964). Cocaine, which is believed to prevent re-uptake of CA, especially NA (Trendelburg 1959) was also facilitatory (Crow 1970). The facilitatory effect of increasing the concentration of CA in the synapse does indicate a role for the CA's in the performance of a reward reinforced behaviour.

A cautionary note must be added here. It has been suggested that the release of CA should be associated with nerve impulses for the generation of reinforcement, both with regard to ICSS (Crow 1970) and free operant behaviour in general (Ahlenius et al 1971). Although it has been shown that amphetamine induced release of DA at least was related to the impulse flow in the neurones (Von Voightlander & Moore 1973) the effect of prolonging the synaptic effect of released transmitter could dissociate the relationship between nerve impulse and post-synaptic effect. In addition amphetamine was found to be rewarding in its own right from self-administration experiments (Pickens & Harris 1968). The interactions between amphetamine and ICSS were clearly more complicated than initially suspected, and the conclusions from such experiments more uncertain.

The use of CA antagonists i.e. drugs which block the receptors also indicated such a role. The neuroleptics, such as haloperidol, pimozide and spiroperidol, which at low doses selectively block DA receptors (Anden et al 1970) severely reduce ICSS, especially in hypothalamus, (Wauquier & Niemegeers 1972: Dresse 1966), as did chlorpromazine (Olds et al 1956). The work with NA antagonists was less conclusive, as α-NA antagonists e.g. phentolamine and phenoxybenzamine did decrease ICSS (Bailey et al 1972: Hastings & Stutz 1973) but did not completely abolish it. However if given intra-ventricularly phenoxybenzamine may achieve complete suppression (Wise et al 1973). However beta-blockade with propanolol might decrease ICSS (Bailey et al 1972) or have no effect (Hastings & Stutz 1973: Wise et al 1973).

The effect of inhibiting CA synthesis was also compatible with a direct role of the CA's in rewarded behaviour. The drug α-methyl-paratyroxine (α-MPT) which blocks the rate limiting enzymes in CA synthesis tyroxine hydroxylate has been shown to decrease brain CA levels dramatically (Spector et al 1965). This decrease in CA was paralleled by a decrease in ICSS (Poschel & Ninteman 1966: Gibson et al 1970: Black & Cooper 1970). The latter used a rate free measure of ICSS and thus indicated a direct role in the reward process for ICSS rather than simply a generalised performance deficit. The blockade of only NA synthesis by using drugs which inhibit dopamine-β-hydroxylase preferentially was claimed to decrease ICSS and thus demonstrated a prime role for NA (Wise & Stein 1969). However, a general sedation effect may have been the cause of the decrement, as replacing the animals on the lever reversed this decrement, (Roll 1970). The use of 6-OHDA, a compound which selectively destroys CA neurones (Breese & Traylor 1970) will be

discussed in much greater depth later, but suffice to say that its initial use indicated that destruction of CA's in whole brain was disruptive to ICSS in a manner related to the extent of CA depletion (Breese et al 1971), and supported the other CA pharmacological evidence.

In addition, not only does CA synthesis inhibition block ICSS, but it appeared to be possible to reinstate the self-stimulation behaviour with CA's. After blockade of NA synthesis by DEDTC or disulfiram, the suppression of ICSS could be reversed only by l-NA given intra-ventricularly, not by d-NA or by DA (Wise & Stein 1969). Also after CA synthesis inhibit by α-MPT, ICSS could be restored by L-DOPA the CA precursor or DOPS i.e. bypassing the blocked tyrosine hydroxylase (Stinus & Thierry 1973) or by methamphetamine (Poschel & Ninteman 1966) presumably either a direct sympathomimetic action or by potentiating the action of small remaining amounts of CA in the neurones. This would seem to be excellent proof for the necessity of CA's at least at some level whether motor, arousal or reward in the maintainance of rewarded behaviour. Facilitation of ICSS has also been produced by intra-ventricular l-NA (Wise et al 1973 , Olds ME 1974) although whether this was directly due to a reward function must be debatable.

It seems highly likely that CA's are involved in the modulation of behaviour, but other neurotransmitters may also be involved. As both MAOI's and reserpine affect the indoleamine 5-hydroxytryptamine in a similar manner to the CA's, it seemed pertinent to examine its function in ICSS.

Attempts have been made to raise levels of 5-HT or stimulate its release as with the CA's. 5-hydroxytryptophan (5-HTP) the immediate precursor of 5-HT was given in combination with an MAOI to raise the 5-HT levels, and this produced a facilitation of ICSS

(Poschel & Ninteman 1968). However, 5-HTP may also release NA, and with a MAOI present this could result in the facilitation by NA already described. The use of p-chloroamphetamine (PCA) a drug believed to cause an initial increase in release of 5-HT by blocking intraneuronal storage (Pletscher et al 1966), caused an immediate decrement in ICSS. Furthermore this decrement was prevented by previously decreasing the 5-HT available by blocking its synthesis with p-chlorophenylalamine (PCPA). PCPA blocked the enzyme tryptophan hydroxylase, and although it has an effect on NA synthesis, this preceded the maximum 5-HT depletion and by waiting a selective effect on 5-HT may be produced (Koe & Weissman 1966). Thus it appeared the decrement in ICSS after PCA was in fact due to increased 5-HT release (Poschel & Ninteman 1971). The administration of 5-HT intraventricularly also caused a decrease in ICSS (Wise et al 1973), thus it appears most likely that increased synaptic 5-HT has an inhibitory effect on ICSS. If an increase in 5-HT was inhibitory it might be expected that a decrease would be excitatory. This has not proved to be generally true, as most studies reported no effect of PCPA at the time of maximum 5-HT depletion (Margules 1969: Gibson et al 1970: Black & Cooper 1970). The Margules study used electrodes near the dorsal raphe nucleus, which contain 5-HT cell bodies, but only reported a decrement after CA synthesis inhibition with α-MPT although this could have been due to the more general sedative and motor disruptive qualities of this drug. An increase in ICSS was seen after a large dose of PCPA (500 mg/kg) (Poschel & Ninteman 1971) but a decrease was seen in both rats and dogs in another study (Stark & Fuller 1972). Thus although not providing any conclusive evidence for a vital role in ICSS for 5-HT there is evidence for some role in the expression of operant behaviour. It has been

suggested that 5-HT mediates a punishment system (Wise et al 1973), but this cannot be certain.

The final major group of drug studies have been based on the cholinergic system. This system may be divided peripherally into two distinct types, nicotinic and muscarinic with pharmacologically distinct receptors and this division seems to apply centrally (Feldberg 1945). The effect of increasing the acetylcholine (ACh) available at the receptor by giving physostigmine, an anticholinesterase (i.e. preventing the breakdown of ACh by inhibiting acetylcholinesterase, the enzyme which destroys ACh), was to depress ICSS (Jung & Boyd 1966: Olds & Domino 1969: Newman 1972). This effect was a CNS one, as neostigmine, an anti-cholinesterase which does not cross the blood-brain barrier, had no effect. The depression of ICSS was blocked by atropine, (Jung & Boyd 1966: Newman 1972), and by scopolamine (Olds & Domino 1969), both muscarinic antagonists. Centrally acting muscarinic agonists also depressed ICSS, though with a shorter duration of action than physostigmine, arec oline (Olds & Domino 1969) and pilocarpine (Newman 1972).

The effects of nicotinic stimulation were however excitatory on ICSS, as nicotine itself increased responding either low response rates only (Pradhan & Bowling 1971): or with all response rates (Newman 1972), although the latter experiment used a different schedule of reinforcement. A biphasic effect, with a short depression followed by a longer excitation was also seen, but the depression may have been due to a local irritation caused by the nicotine injection (Olds & Domino 1969). In all these studies, the nicotine excitation was blocked by mecamylamine, a centrally active nicotinic antagonist. Further evidence for an excitatory nicotinic activity (and conversely for the muscarinic inhibitory system) was found by giving mecamylamine

after physostigmine, and increasing the depression of ICSS (Olds & Domino 1969), presumably by removing the nicotinic excitation. However, the nicotinic excitation may not be a direct action on behaviour, as it seems likely that it acts through the CA's, as pre-treatment with reserpine abolishes nicotine excitation (Pradhan & Bowling 1971).

There was evidence for a muscarinic cholinergic system which is inhibitory on operant behaviour in general, (Morrison 1967) as well as the above studies in ICSS. Some support for the inhibitory system idea may be taken from the increased extinction shown by blocking the cholinergic receptors with scopolamine (Olds ME 1970), thus preventing the inhibitory system from functioning. There was also evidence for a general excitatory effect of nicotine in operant behaviour in general (Morrison 1967) and as mentioned this might be through a CA system. Therefore, although it seemed that the cholinergic system was not the substrate for brain stimulation reward, it may influence it by a more general effect on behaviour, perhaps an an inhibitory system in operant behaviour.

In summary, interpretation of the pharmacological experiments was rendered highly difficult by the apparent ability of catecholaminergic, serotinergic and cholinergic drugs to affect primary motor, sensorimotor or inhibitory systems controlling behaviour. This was in addition to difficulties comparing the individual experiments due to difference in drug dosage, route of administration, electrode site and behavioural parameters studied. However, in the relatively few studies with some control of the behavioural variables, there is some evidence for the involvement of the CA's directly in reward, i.e. the rate free measured effect of alpha-MPT (Black & Cooper 1970) and the

measurement of gross activity as well as rewarded activity, with spiroperidol having a greater effect on reward (Rolls et al 1974). In all the studies it may be seen that the CA's do have a primary function in operant behaviour however, as with a wide variety of treatments increasing the effective levels of CAs facilitates behaviour, and decreasing them depresses behaviour. There seemed little good evidence for a direct cholinergic substrate of reward, but plenty evidence for a role in modulating behaviour. The same might be said of serotinergic systems, except that the evidence for no direct role in reward was less conclusive, and cannot be ruled out. The drug evidence was not incompatible with a catecholaminergic theory of self-stimulation.

Lesion Studies

If ICSS was supported by a discrete neural system, it might be expected that lesions to that system would abolish the ICSS behaviour. This has proved so difficult to do that it has been said:

> "Any search for a critical focus or essential pathway will meet with little success as the neural substrate ..... is characterised by massive redundancy and ..... a plasticity which provides a basis for reorganisation"

(Valenstein 1966). Since the early mapping studies of Olds (Olds et al, 1960; Olds & Olds 1963) indicated the MFB as the critical substrate for ICSS, lesions in this area were the obvious beginning. The techniques available were electrocoagulation or knife cuts. A type of 'reversible' lesion might be induced by intracranial application of local anaesthetics. However the information so obtained was difficult to interpret as MFB contained large numbers of ascending and descending pathways (Gurdjian 1927). A 'mechanical' lesion disrupted them all equally. The discovery of discrete CA systems throughout the brain (Anden et al 1966) led to the possibility of producing highly selective lesions to these systems using the neurotoxin 6-hydroxydopamine (6-OHDA) (Ungerstedt 1971).

Lesions directed at elucidating MFB function could either have involved MFB or LH ICSS and distal lesions, or ICSS from distal areas and MFB lesions. Unfortunately neither approach has produced conclusive evidence. Lesions of septum, fornix and cortex (Ward 1960); amygdala, striatum and cortex (Ward 1961); ventromedial hypothalamus (Hoebel & Teitelbaum 1962; Ferguson & Keesy 1971); cingulate cortex (Coons & Foburg 1963); anterior hypothalamus, reticular formation, ventral tegmentum and central gray (Lorens 1966); fornix (Boyd & Gardner 1967): dorsal and median raphe nuclei (Lorens 1971) and unilateral LC

(Lorens 1973) produced little decrement in LH ICSS. Other studies, with lesions to MFB rostral and caudal to LH (Morgane 1962); to MFB at level of olfactory tubercle (Olds & Hogberg 1964); ipsilateral preoptic area, mammillothalamic tract and ventra-tegmental lesions (Boyd and Gardner 1967); posterior hypothalamus (Olds & Olds 1969) or bilateral parasagittal knife cuts along lateral border of LH (Kent & Grossman 1973) did produce significant decrements to LH ICSS, the most reliable decrements occurring with lesions caudal to LH (Olds & Olds 1969: Boyd and Gardner 1967).

Lesions affecting septal self stimulation were similarly inconclusive. Lesions in midbrain reticular formation reduced septal ICSS (Schiff 1964); bilateral lateral hypothalamic lesions did not affect it (Valenstein & Campbell 1966) and lesions in amygdala increased it (Kant 1969).

However care is needed in interpreting the deficits produced. It has been shown that even with decortication and removal of cortices destruction of hippocampus, amygdala, septum and neostriatum, LH ICSS was unaffected if the operant was simple, e.g. head turning, compared to the complex sequence of motor responses required in bar pressing (Huston and Borbely 1973). This applied to the lateral LH knife cuts, which also disrupted other operant tasks (Kent & Grossman 1973). Thus the posterior hypothalamic lesion deficits could have been due to a non-specific effect.

Experiments with intracranial local anaesthetics have also managed to decrease LH ICSS, with injections into sulcal prefrontal cortex (Rolls and Cooper 1974), lateral hypothalamus and ventral tegmental area (Nakajima 1972; Madryga and Albert 1971). Injections into medial hypothalamus increased LH ss although this might be due to a reduction in any aversive component in LH ss (Olds and Olds 1963). Owing to their

general inactivating effect however few positive conclusions on ICSS substrates could be made.

Thus lesions of MFB could reduce ICSS, although seldom abolish it. Lesions caudal to the stimulation site were generally more effective. In addition the ability to maintain ICSS after decortication suggested the possibility of descending pathways originating at the level of hypothalamus, or intrinsic hypothalamic pathways which could support ICSS. Care must be taken to avoid general performance deficits being interpreted as reward or motivation deficits. The use of an operant task appropriate to the reduced motor capabilities of the animal as after decortication would seem likely to yield more definitive results. Alternatively the ability to maintain appropriate levels of motor performance should be demonstrated. The lack of such controls would make interpretation of a successful lesion experiment difficult. The use of specific 6-OH DA lesions to elucidate the role of the CA's will be discussed in a later section.

These experiments have supported the idea that the ICSS pathways were diffuse and redundantly organised at the level of MFB.

Theories of ICSS

Theories have been constructed to explain the phenomenon of ICSS in more general terms of motivation and reward. From more general rewarded behaviour, Deutsch constructed a theory with separate motivational and reinforcement systems (Deutsch 1960). He considered that it applied to ICSS, as evidence from extinction trials with ICSS, investigating temporal and quantitative factors, fitted with such a theory (Deutsch & Howarth 1963). The further work of Gallistel (1969) separated the motivational and reinforcing aspects of ICSS in a runway situation, by giving both pre-trial primary stimulation and rewarding stimulation after running, and using running speed as the measure. Also, using the pulse pair method to measure refractory period, it was shown that the motivational and reinforcing systems had different refractory periods (Gallistel et al 1969), and thus appeared to be distinct neural systems.

Other workers have described the motivational aspect of ICSS as incentive motivation (Trowill, Panksepp & Gandelman 1969). In various experiments, by manipulating the amount of rewarding stimulation, the timing of the reward, and the length of session used, it has been shown that ICSS can resemble natural reinforcers in both extinction, behaviour and in complex behavioural schedules. In particular ICSS can be most accurately mimiced by conventional reinforcers of high incentive value (e.g. condensed milk) presented without delay after the operant response, with a low drive state of the animal as is normal in ICSS experiments (Panksepp & Trowill 1967). From these observations, these workers have concluded that ICSS produced incentive motivation.

Olds' theories have attempted to relate ICSS to a distinct neural substrate, and the existence of neurones which mediate reward.

The favourite candidate for these special neurones are the pathway neurones in the MFB, in lateral hypothalamus. Positive reinforcement would result from excitation of these LH reward systems, which might act by correlating afferent sensory information (especially olfactory inputs in the rodent) and efferent motor pathways. There would also be motivational (drive) mechanisms in hypothalamus, probably with drive specific areas, which could also be activated by ICSS, but which would normally influence the reward neurones. Olds theory also incorporates a punishment system, in the area aversive to electrical stimulation in medial hypothalamus, which would have an inhibitory input to the reward neurones. Hence, cessation of punishment could be rewarding. The catecholamines (especially NA) could also have a function in this theory, by acting in the reward neurones, possibly by changing their susceptibility to other inputs. The Olds theory thus has a reward system centred in hypothalamus, but which allows for the influence of other systems, and the possibility that they themselves can be rewarding due to this influence.

Stein however attributes the reward function to the NA neural systems themselves on the basis of anatomical and pharmacological evidence. He believes that rewards inherently have incentive properties and thus produce a positive feedback system i.e. rewards facilitate the behaviour that produced the reward through the sensory stimuli that produced the reward. A reward system is sufficient therefore, without an associated motivational one. He also postulates a mutually antagonistic inhibitory or punishment system. However, although initially an attractive theory on the earlier pharmacological and anatomical data, later work has indicated the likelihood of dopaminergic involvement in ICSS, or at least the existence of two distinct neural

systems. It seems unlikely that a theory based solely on NA will be sufficient to explain ICSS.

Crow has evolved a theory that does incorporate both NA and DA. From anatomical experiments he has observed that both mesencephalic DA systems and the locus coeruleus NA system seem to support ICSS, and that the pharmacological evidence in general supported a CA involvement in rewarded operant behaviour. Furthermore, from qualitative behavioural differences observed between apparently dopaminergic or noradrenergic stimulation, he has stated that the DA systems were involved with incentive motivation and the NA system with reinforcement or reward. This difference might also be due to the type of sensory input these systems received. He proposed an olfactory input to the DA system (which could provide anticipatory cues) and a gustatory input to the NA system (which could provide consummatory cues). As the NA and DA systems are part of the MFB, they would both be activated in MFB ICSS which could explain the great responsiveness to ICSS in that area. It could be said that the Crow theory was the most concrete one, in that it predicted both the systems involved and their function, and also that it directly implicated the CA's which accorded with the experimental evidence. In addition it fitted in with the theories based on behavioural observations, in that it possesses two neural systems and it concured with the idea of incentive motivation in ICSS. Therefore, the work in this thesis has been directed at investigating the involvement of the CAs in ICSS. It has included the effects of ICSS on the biochemistry of the CA's; the effect of lesioning CA pathways on ICSS, and the pharmacology of ICSS. The delination of CA involvement in ICSS was necessary to determine the exactness of the Crow hypotheses.

## Section One

# The effect of intra-cranial self-stimulation from sites in the area ventralis tegmenti and posterior hypothalamus on dopamine metabolism

## Section 1

## Introduction

A theory of ICSS based on the specific neural substrates of the CA systems must predict a concomitant release of CA's from the neurones on electrical stimulation. Electrical stimulation of peripheral adrenergic neurones has been shown, for example, to increase the efflux of NA from the dog spleen (Mirkin & Bonnycastle 1954). This approach has been attempted in the CNS, but due to problems with perfusion and small quantities actually released into CSF, direct evidence for release of endogenous amines has been hard to obtain (Portig and Vogt 1969: Vogt 1969). Initially attempts were made to measure amine release by measuring endogenous amine levels.

One such attempt indicated that a decrease in NA was observed as measured histochemically by a decrease in the fluorescence of the NA-terminal areas after self-stimulation of the area ventralis tegmenti (Dresse 1966). However, the introduction of stress by electric foot shock (Maynert & Levi 1964), and the 'emotionality' which accompanied ICSS behaviour had both been shown to reduce NA levels in the brain. A later study indicated that the lowering of brain amine levels was directly related to electrical self-stimulation only, and that neither forced stimulation at rewarding or aversive sites which were more stressful produced such a decrease. In addition, the lowered amine levels were not correlated with other indices of stress measured, such as adrenocorticoid activity (Olds & Yuwiler 1972). Although these experiments have indicated some change in gross amine levels with ICSS, this might not give a reasonable measure of activity in CA neurones, and in addition there could be a lack of a direct correlation between amine levels and function (Carlsson 1966). Indeed in the periphery,

stimulation of adrenergic neurones can lead to an increased level of amine in the tissue (Folkow et al 1967).

In order to obtain a better measure of amine release studies were made on amine turnover after ICSS. A measure of amine turnover can be obtained by either measuring the rate of disappearance of amines after synthesis inhibition (S pector et al 1965) or of radioactive tracer amines (Iversen & Glowinski 1966). The rate of disappearance of the amines could be measured either histochemically or biochemically. Firstly, studies which used synthesis inhibition in order to measure turnover. Using the histochemical method that is measuring the rate of reduction in fluorescence intensity, NA turnover was seen to have increased after both stimulation of anaesthetised (Arbuthnott et al 1970) and conscious self-stimulating animals (Arbuthnott et al 1971) with electrodes in the ventral tegmentum in the area of the ventral NA bundle (Ungerstedt 1971). The NA turnover had increased solely in the projection areas of the ventral bundle such as the hypothalamus. The increases in turnover of NA, and in one case of striatal DA correlated well with the anatomical placement of the electrode though less well with the ICSS behaviour. Due to the inhibiting effect of CA synthesis on ICSS in general, forced stimulation was required to continue the stimulation.

Alternatively, biochemical measurement of increased depletion of amines with stimulation after CA synthesis inhibit also indicated increased utilization. Applied stimulation through electrodes in the area of MFB which had supported ICSS produced decreased NA levels in hypothalamus and the rest of the brain, although not in all the animals stimulated at rewarding sites. There was no change with animals stimulated at non-rewarding sites (Yuwiler & Olds 1973). Similar results were obtained with electrodes placed bilaterally in the area

ventralis tegmenti, with increased NA utilisation in hypothalamus, hippocampus and cortex after applied stimulation (Stinus et al 1973).

However, even measuring utilization of amines might not give a sensitive method for the detection of release. Results from studies of the spontaneous or drug induced release of radicactive DA, synthesised endogenously from radioactive tyrosine (Besson et al 1971) and the biphasic disappearance of both exogenous or endogenously synthesised amine after stress (Thierry et al 1971) have indicated that a unique pool of newly synthetised amines were preferentially released from CA neurones. As this pool was readily labelled using radioactive amines or their precursors (Thierry et al 1971), the rate of disappearance of these tracers might give a better indication of amine turnover. Also with no synthesis inhibition necessary, free self-stimulation was possible, using bilateral electrodes in AVT. Dopamine utilization was increased in the olfactory tubercle, and NA in brain stem, hypothalamus, hippocampus and cortex. No changes in amine levels were detected, and this might have been due to increased synthesis of amine (Stinus et al 1973). More direct evidence of amine release due to stimulation was obtained, by perfusing brain areas using a push-pull cannula and measuring the efflux of radioactive tracer amines which had been previously injected intraventricularly. Applied stimulation of electrodes which had previously been rewarding caused an increased efflux of radioactivity in hypothalamus and amygdala. This increased efflux also contained a larger proportion of CA metabolites (Stein & Wise 1969).

Unfortunately, none of these approaches produced definitive evidence of CA release in ICSS, especially release of a single amine. Most suffer from generalised procedural deficits. Nearly all have had to use imposed stimulation which may produce different results to

free ICSS as in Olds & Yuwiler (1972). In addition some of the studies have examined very discrete brain areas which may or may not be directly involved in ICSS, (Stein & Wise 1969) or others gross whole brain levels in which more discrete effects would be submerged (Olds & Yuwiler 1972, 1973). The use of CA synthesis inhibitors prevented the measurement of CA utilization in self-stimulation by enforcing applied stimulation, and also produced an artificial state in the CA systems which might not reflect normal function. The use of radioactive tracing techniques although more promising does have its own problems. The push-pull cannula technique has been criticised for the trauma it may cause and for causing non-specific release of neurotransmitters (Chase & Kopin 1968). Also, the mechanisms for transport and release of monoamines do not have strict chemical specificity (Kopin 1968), as might be demonstrated by an increased efflux of radioactive DA from hippocampus (Stinus et al 1973), an area apparently without dopaminergic innervation (Lindvall & Bjorkland 1974). The problem has been to demonstrate an increased release of an endogenous amine from an area that normally contains aminergic terminals, after ICSS.

An approach which comes nearer this ideal is to measure the turn-over of an amine by measuring the principle concentration of its final metabolites. The final metabolite of noradrenaline in CNS is 4-hydroxy-3-methoxyphenylglycol, (HMPG), (Mannarino et al 1963). Stimulation of NA cell bodies in locus coeruleus in anaesthetised animals produced an impulse related rise in HMPG levels in cerebral cortex (Walters & Eccleston 1972: Korf et al 1973), and destruction of LC caused a decrease in HMPG levels (Arbuthnott et al 1973: Korf et al 1973), and thus HMPG seemed to be a good measure of NA activity in cerebral cortex. It was found that ICSS in the region of LC also produced an increase in HMPG levels in cortex on the stimulated

side of the brain, although this increase was less than that obtained with applied stimulation from previously rewarding electrodes in the same area in anaesthetised animals (Anlezark et al 1975). However, it was proof of an increase in NA metabolism after ICSS from the LC, without any interference by drugs or from imposed stimulation. Other evidence that NA metabolism was increased after ICSS in the region of LC has been obtained. It was shown that tyrosine hydroxylase (TOH) activity was increased in peripheral sympathetic neurones after electrical stimulation (Ben Ari & Zigmond 1975). TOH was the rate-limiting enzyme for NA synthesis (Levitt et al 1965) and it was suggested that increased TOH activity might reflect increased neuronal activity (Thoenen 1972). It has now been shown that ICSS with electrodes in the area of LC also increased TOH activity (Section 3, this thesis). This was in accord with the HMPG results, and together these experiments implied that the dorsal NA system from LC might be a substrate for ICSS.

It seemed appropriate to use a method to investigate changes in dopamine turnover in ICSS by measuring the major final metabolites of dopamine, which are 3, 4-dihydroxyphenylacetic acid (DOPAC) (Rosengren 1960) and homovanillic acid (HVA) (Sharman 1963). It has been shown that stimulation of the MFB in anaesthetised animals produced a rise in the levels of HVA & DOPAC in the striatum, nucleus accumbens and olfactory tubercle (Korf et al 1976). More directly, stimulation of the nigro-striatal pathway produced on impulse related rise in DOPAC levels in the striatum and stimulation of the mesolimbic pathway produced a similar rise in DOPAC in the olfactory tubercle in anaesthetised animals (Roth et al 1976), these areas being the respective projection areas of these pathways (Ungerstedt 1971). Destruction of the nigrostriatal or mesolimbic pathway produced a decrease in DOPAC levels in the respective terminal areas (Roth et al 1976). The measurement of the levels of DOPAC & HVA thus seemed a relevant measure of activity in these dopaminergic pathways.

An anatomical map of the ventral mesencephalon had indicated a close correlation of sites positive for ICSS with the dopamine cell bodies, in cell groups A8, A9 and A10 (Crow 1972). It was important to discover if dopamine was released from the nerve terminals by the electrical stimulation of conscious animals during ICSS. With electrodes situated in the area of the A10 cell group, the origin of the mesolimbic pathway (Ungerstedt 1971), changes in dopamine metabolism as measured by metabolite concentrations were looked for in the main projection areas of that pathway, the olfactory tubercle and nucleus accumbens. Changes in DA metabolism in the striatum were also looked at in case of a more generalised increase in DA release during this behaviour. Finally, the concentration of HMPG in cerebral cortex and hippocampus was measured to discover whether increased activity in the dorsal NA system accompanied ICSS from dopaminergic cell body areas. It had been shown using the radioactive labelling technique that stimulation in AVT, near the mesolimbic pathway, produced increased utilization of NA from cortex and hippocampus (Stinus et al 1973). It was pertinent to discover whether NA release occurred without pre-loading with radioactive tracers in view of the already mentioned problems with such a technique.

Methods

1. Implantation of electrodes

Bipolar electrodes were constructed from 100um stainless steel wire coated with Teflon for insulation (Thermal Wire of America). The wire was twisted together, then cut to produce two uninsulated tips, approximately 200um in maximum diameter. The other ends of the electrode were also cleared of insulation at their tips and crimped into gold pin sockets (ITT Canon electrics) with a crimping tool (Buchanan). The electrode sockets were cemented together using dental acrylic cement (Simplex-Howmedica Int. ) to provide a stable assembly. This was then mounted in a Kopf electrode holder for implantation.

Male Wistar rats (180 - 220 gm) were anaesthetised with halothane (ICI) and fixed in a David Kopf stereotaxic frame using blunt guinea-pig ear bars. The tooth bar of the frame was set -2.4 mm below ear bar zero. The skull was exposed by a medial incision, and viewed with a binocular operating microscope (Zeiss) the periosteum scraped from the surface to make the sutures clearly visible. In these experiments, bregma was used as the stereotaxic reference point. The electrodes were aimed dorsal to the interpeduncular nucleus, using the following co-ordinates:

Anterior posterior (AP) - 4.3 mm

Lateral (L) - 0 mm

Vertical (V) - 8.3 mm (measured from the skull surface)

A second group aimed at the posterior hypothalamus were inserted at:

Anterior posterior (AP) - 4.0 mm

Lateral (L) - 0 mm

Vertical (v) - 8.1 mm

A burr hole was drilled at the site of these co-ordinates, taking care not to damage the cortex, then filled with bone wax (Ethicon). The electrode was then lowered to the appropriate depth using the stero-taxic electrode holder. Previous to inserting the electrode three plastic screws had been inserted into holes drilled in the skull, and the electrode was made secure to these using dental acrylic cement. The skin was then drawn together around the electrode assembly and stitches inserted. The rats were allowed to recover from the operation for one week before screening for ICSS behaviour, and were maintained on ad lib water and diet of lab chow.

2. Stimulation

In the initial part of this experiment, a Campden brain stimulator (Campden 522) was used which produced 50Hz sine wave current. The stimulus parameters used were 200 msec trains, with intensity being in the range 20 - 50 μA.

The second part of the experiment used Neurolog 700 series stimulators (Digitimer) producing monophasic square wave pulses. The stimuli used were 200 msec trains,.15 msec pulses, 50 pulses/sec. The current was typically 150 - 250 μA. This was checked and routinely monitored on an oscilloscope (Telequipment).

3. Behavioural equipment and training procedures

All experiments were carried out in Campden rodent test chambers, housed in Campden sound attenuating boxes. The test chamber was made of aluminium, measuring 8" x 9⅝" x 8½" with a perspex side door. The lever was 2" wide and protruded ¾" into the test chamber at a height of 2½" from the floor. It required a force of 10 gm. The test chamber was continually illuminated, and could be viewed through a one way window

in the box. The stimulator was connected to the electrode assembly via a mercury slip ring which allowed free movement of the rat.

The rats were run on a continuous reinforcement schedule (CRF). This was controlled by electro mechanical logic modules (Colne Instruments) which allowed one train of pulses for each lever press. A delay control was inserted in the programming circuit so that at least 200 msec elapsed between trains. The number of lever presses and the number of pulse trains given in each session of ICSS were counted on an electro-mechanical counter. In addition the rate of pressing throughout a session was displayed with a cumulative pen recorder (Campden).

After the one week post-operative recovery period, the rats were placed in the test chamber and the electrode connected to the cable from the slip ring in the roof of the chamber. The rat was shaped for lever pressing by the experimenter manually giving a pulse train whenever the rat approached the lever. The current was progressively increased until a reaction was elicited, either a positive one indicated by the increased time spent near the lever or conversely for an aversive one. Occasionally a neutral response occurred, when the rat was simply disinterested in the electrical stimulation. Those rats in which the electrical stimulation was positively reinforcing were trained to press the lever to receive the stimulation, after at most three thirty minute training sessions on separate days. Thereafter the rats were allowed one 30 minute session for five consecutive days. At the end of the last session, the rats were quickly removed from the box and killed by stunning and decapitation.

4. Dissection procedure

The rats were rapidly decapitated and the brains removed and immersed in ice-cold saline, then dissected over ice. The brain was placed ventral side up, and the olfactory tubercles pinched off with a curved forceps. A coronal cut was made 2 mm anterior to the optic chiasma, followed by a similar cut through the optic chiasma itself. The resulting tissue slice was placed horizontally and a vertical cut at $90^{o}$ to the first cut made through the anterior commissure. The part ventral to the commissure was removed, and tissue lateral to the lateral olfactory tract was cut away. The remaining tissue contained the nucleus accumbens. The dorsal section of the slice contained the septum, striatum and frontal cortex. Septum and striatum were picked out with curved forceps, as was the striatal tissue remaining in the rest of the brain caudal to optic chiasma. The cortex and hippocampus were removed. Immediately after dissection the tissue was frozen in aluminium foil amongst solid $CO_2$, before longer term storage in liquid $N_2$. The hypothalamus and pons were snap frozen to be cut into thin sections in a cryostat for histological evidence of electrode placement.

5. Histological procedures

The staining technique used was a modified version of the one reported by Kluver Barrera, 1953. A tissue slice about 3 mm containing posterior hypothalamus and the ventral tegmentum was frozen onto a cryostat chuck in solid $CO_2$. Sections of 20 µm were cut in a cryostat and every fourth one in the region of the electrode tract was collected. The sections were melted on to glass microscope slides in preparation for staining.

The sections were treated as follows:

(a) Washed in 95% ethanol for 5 minutes.

(b) Stained for 20 minutes at room temperature in a filtered 0.1% Luxol Fast Blue in 95% ethanol solution containing 5 ml of 10% acetic acid in every 1000 ml.

(c) Washed in distilled water to remove excess stain.

(d) Differentiated by brief immersion (15 seconds) in 0.05% Lithium Carbonate solution.

(e) Differentiation continued in 70% Ethanol for 30 seconds.

(f) Washed in distilled water

Steps (d) - (f) were repeated until only the white matter in the section was stained light blue against a clear background.

(g) Stained for 10 minutes in cresyl violet solution (0.2%) containing 5 drops of 10% acetic acid to every 30 mls of solution.

(h) Differentiated in 95% Ethanol by frequent brief (5 seconds) immersion.

(i) Dehydrated in absolute Ethanol, cleared in Xylene and mounted in D.P.X. ready for microscopic examination.

6. Estimation of 3, 4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) in rat brain by Gas-liquid chromatography and Electron Capture Detection

This method was based largely on that of Pearson and Sharman (1975), with modifications in the extraction procedure (Nicolau N, Ph.D. Thesis, Edinburgh).

(a) Simultaneous estimation of HVA & DOPAC concentrations in rat brain samples

Tissue samples were homogenised in 0.8 ml of 0.4N perchloric acid (PCA), then centrifuged for 4 minutes (at low speed).

The supernatant was transferred to an Eppendorf tube and 0.5 ml of toluene added. After 30 seconds of mixing, the tube was centrifuged at low speed. The organic phase was discarded, and after adding 0.5 ml of ethyl acetate (Reeve Angel Scientific Ltd) the tube was shaken for 1 minute. The tube was centrifuged then for 4 minutes, and the ethyl acetate layer transferred to a reaction vial. This ethyl acetate extraction procedure was repeated twice, taking the aqueous layer. The combined ethyl acetate sample extracts were evaporated to dryness under a stream of $N_2$.

In the derivatisation step, 0.2 ml of twice redistilled trifluoroacetic anhydride (Aldrick Chemical Co. Inc.) and 0.1 ml of redistilled hexafluoroisopropanol (BDH), were added to the dried residue and reacted at 100°C for 1 hour. Following this step the reaction vial was allowed to cool to room temperature before opening and the contents were evaporated just to dryness under a stream of dry $N_2$. The oily residue was dissolved in 1 ml of dry ethyl acetate, containing 100 ng of pentafluorophenyl benzoate used as an internal standard. 2 µl of this solution was injected into the gas chromatograph.

(b) Gas-liquid chromatography

This was performed with a Hewlett-Packard model 5710A gas chromatograph, fitted with $^{63}Ni$ electron capture detectors, maintained at a temperature of 250°C. The carrier gas was argon containing 5% methane. It was delivered at a flow rate of 50 ml/min which corresponded to a gas pressure of 40 pounds/sq. in. (psi). The chromatograph column consisted of a 2% SE 52 liquid phase coated on Chromosorb Q (Hewlett Packard) used at an oven temperature of 115°C.

The relative retention times of the trifluoroacetic anhydride and hexafluoroisopropanol derivatives of DOPAC and HVA, with respect

to the internal standard, were 0.35 and 0.52 respectively. The areas of the DOPAC, HVA derivatives peaks were measured in each case and the ratio of metabolite peak area to internal standard peak area was calculated. These ratios were compared with those in a standard curve and the amounts of HVA and DOPAC in each sample was calculated. With this method the recovery of both HVA and DOPAC was typically 90% and the values given are uncorrected for recovery.

7. Estimation of 4-hydroxy-3methoxyphenylglycol (HMPG) in the cortex and hippocampus by gas-liquid chromatography

The tissue samples were homogenised in 5.0 ml chilled 0.4N perchloric acid (PCA) and the homogenate transferred to a centrifuge tube, washing it out with another 1.0 ml of 0.4N PCA. After high speed centrifugation (10,000 r.p.m. for 10 minutes) of the homogenate, the supernatant was decanted into a test tube and the pH adjusted to about 5 with KOH. The solution was poured into a chilled C14 tube, washing out with a few drops of distilled water. The sample was placed in a deep freeze for 15 - 60 minutes to ensure the maximum precipitation of potassium perchlorate. After thawing, it was centrifuged (3,000 r.p.m. for 5 - 10 minutes) and 2.5 ml supernatant decanted into a C14 tube. In some of the samples a duplicate was taken from the supernatant to which was added standard amounts of HMPG. To all tubes were added 100 µl 1M sodium acetate buffer pH 5.0, 50 µl helicase (50 mg/ml) and a drop of chloroform. The sample was incubated overnight at 37°C.

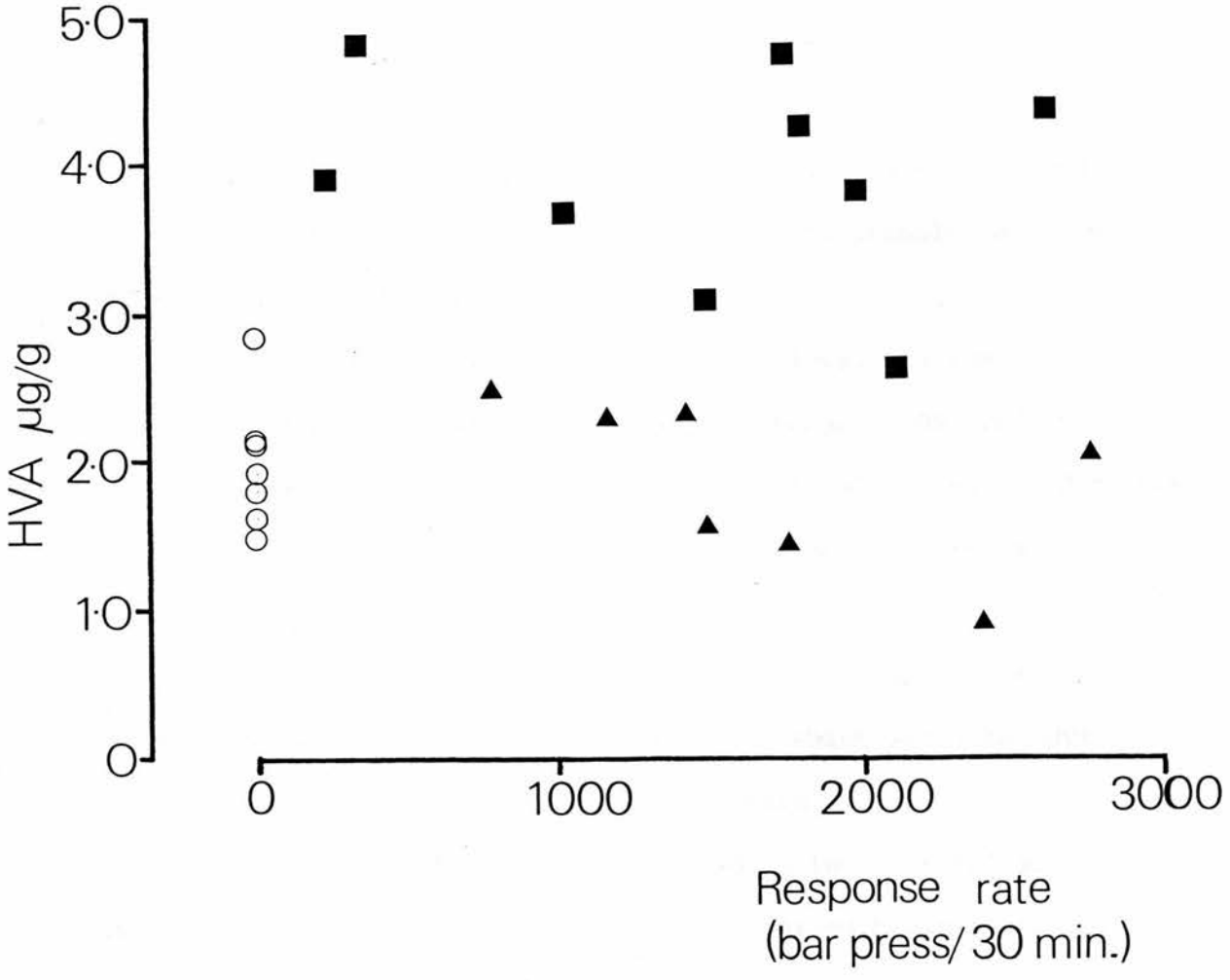
The sample was then shaken twice, for 5 minutes each time, with 4 ml and 3 ml of ethyl acetate. After centrifugation, 3.5 and 3 ml respectively of the ethyl acetate layer, which contains the glycols, was removed. The pooled ethyl acetate extracts were evaporated to dryness under $N_2$ in heated block at 56°C. The residue was taken up in

0.4 ml water and acetylated by adding 50 µl of acetic anhydride (redistilled) and 0.6 ml $KHCO_3$ (16.5 g/100 ml). The solution was agitated, and the reaction proceeded for 30 minutes. The sample was then agitated for 1 minute with 1.6 ml dichloromethane and centrifuged. 1.3 ml of the lower organic layer (containing the acetylated derivatives of the alcohol metabolites) was shaken with a little anhydrous $Na_2SO_4$. The dichloromethane extract was decanted into a 2.5 ml test-tube and evaporated under $N_2$ at 56°C. The residue was taken up in 0.6 ml trifluoroacetic anhydride mixture (1 part trifluoroacetic anhydride + 5 parts ethyl acetate) and the tube was stoppered and heated at 56°C for 15 minutes (to convert the acetylated HMPG into its fluorinated derivative). The solution was evaporated to dryness under $N_2$ at 56°C and the residue was taken up in 0.2 ml ethyl acetate containing 30 ng/ml benzene hexachloride as an internal standard. This solution was transferred by Pasteur pipette into a GLC microvial. 4 µl of this solution was injected into the column.

GLC conditions

A Perkin-Elmer 900 gas liquid chromatograph was used, with a column containing 2.5% silicone gum rubber E301 on a support of chromosorb. The carrier gas was argon/methane (90/10), at a pressure of 70 lbs/sq.in. The column temperature was 170°C and the temperature of the electron capture heads was 290°C. This gave retention times of 1.8 min for HMPG and 2.8 minutes for benzene hexachloride.

The height of each HMPG peak was expressed as a proportion of the peak height of the internal standard. The amount of HMPG in the sample was calculated by comparing the sample ratio with the ratio obtained from standard amounts of HMPG taken through the method.




Section One Fig 1

The relationship in individual rats between response rate (bar presses/30 minutes) and HVA concentration in olfactory tubercle after ICSS (Experiment A). Open circles (○) represent unstimulated controls (n = 7); square symbols (■) represent rats with electrodes in area ventralis tegmenti (AVT group, n = 9); and triangles (▲) represent rats with electrodes in medial posterior hypothalamus ($PH_A$ group, n = 7).

## Results

### HVA and DOPAC levels

#### Experiment A

The concentrations of HVA and DOPAC were measured in three forebrain areas, the olfactory tubercle and nucleus accumbens and the corpus striatum. There were two experimental groups, one of 16 rats which had self-stimulated as described above, and a control group of 7 rats which had had electrodes implanted but had proved negative for self-stimulation. The control groups were placed in the test boxes for an equivalent period of time to stimulated group, but received no stimulation.

Initial examination of HVA and DOPAC levels in the tissues of the stimulated group revealed a slight increase in HVA and DOPAC in the olfactory tubercle compared with control values, but no difference in nucleus accumbens or corpus striatum. However there was a large variance within the stimulated olfactory tubercle group, with some individual increases in HVA and DOPAC of 200 - 300% of the mean control values. This can be seen in Fig 1 which shows the HVA levels in olfactory tubercle of the individual rats.

The exact locations of the electrodes in the rats' brains were found by microscopic examination of the stained brain sections. These were mapped onto an atlas of the rat brain (Konig and Klippel 1963) and hence the location was defined. It was discovered that some of the electrodes were in the area dorsal to the interpeduncular nucleus, in the area ventralis tegmenti, in which the A10 dopamine cell bodies and their axons are to be found. However, some electrodes were misplaced, and were in a more rostral aspect of the brain, in the posterior hypothalamus dorsal to the mamillary bodies. There appeared

Section One Table One

The Concentration of HVA and DOPAC in forebrain areas after ICSS in the ventral mesencephalon

Experiment A

Olfactory Tubercle

| | HVA (µg/gm) | p vs control | DOPAC (µg/gm) | p vs control |
|---|---|---|---|---|
| Control (7) | 1.97 ± .15 | - | 2.47 ± .23 | - |
| AVT (9) | 3.93 ± .23 | $p < .001$ | 4.95 ± .53 | $p < .005$ |
| $PH_A$ (7) | 1.85 ± .22 | n.s. | 1.60 ± .27 | $p < .05$ |

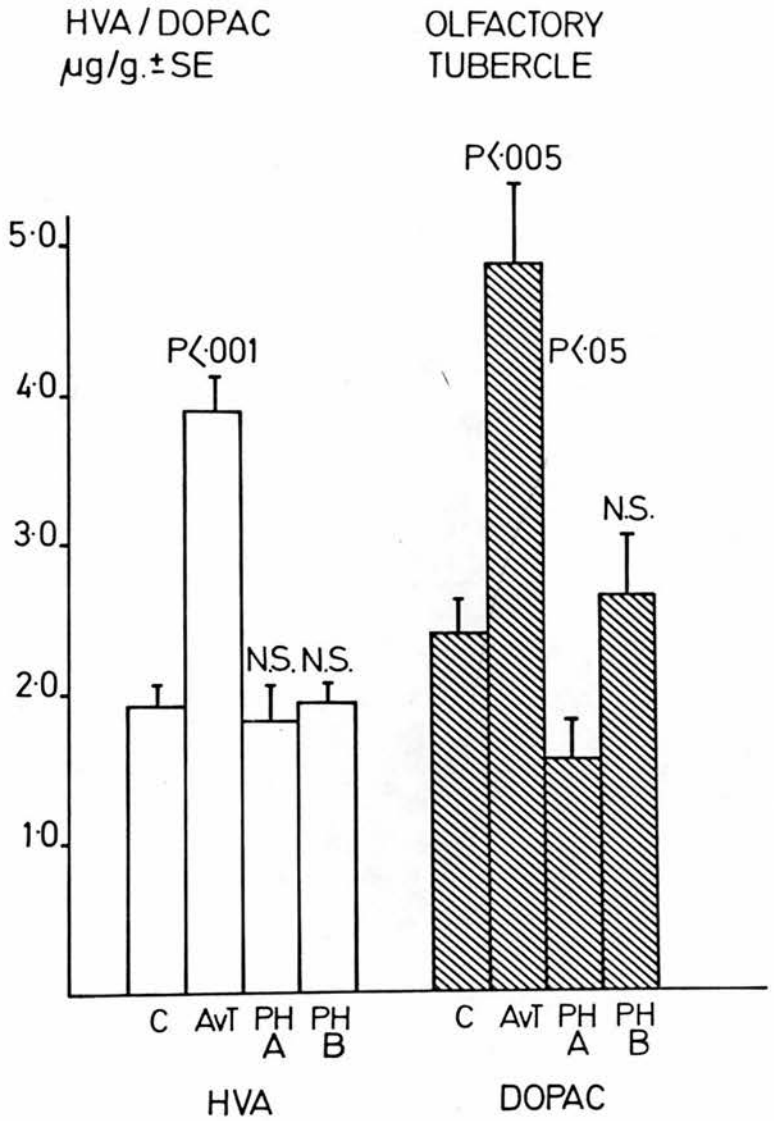
Nucleus Accumbens

| | | | | |
|---|---|---|---|---|
| Control (7) | 1.42 ± .26 | - | 1.44 ± .16 | - |
| AVT (9) | 1.96 ± .20 | n.s. | 2.31 ± .40 | n.s. |
| $PH_A$ (7) | 1.45 ± .18 | n.s. | 1.05 ± .18 | n.s. |

Corpus striatum

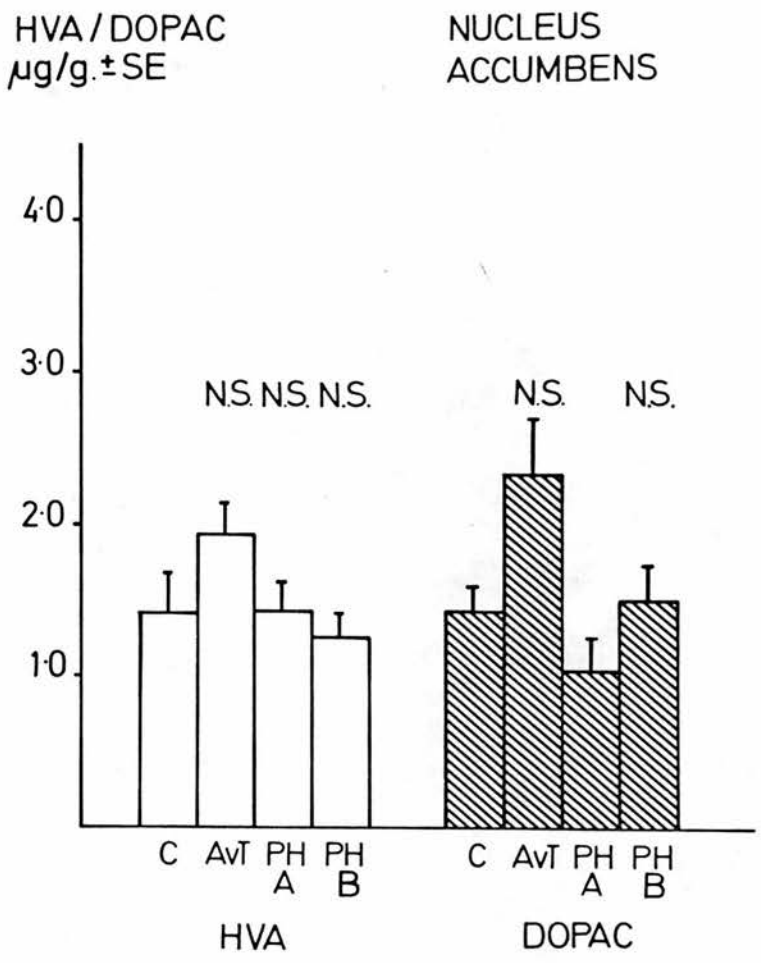
| | | | | |
|---|---|---|---|---|
| Control (7) | 1.09 ± .09 | n.s. | 1.13 ± 0.13 | - |
| AVT (9) | 1.01 ± .10 | n.s. | 1.05 ± .24 | n.s. |
| $PH_A$ (7) | 0.94 ± .08 | n.s. | 0.80 ± .27 | n.s. |

Results expressed as mean concentrations in µg/gm ± SEM. AVT-area ventralis tegmenti group. $PH_A$ - medial posterior hypothalamus, experiment A. The groups were compared with the unstimulated control using the student t-test, unpaired.



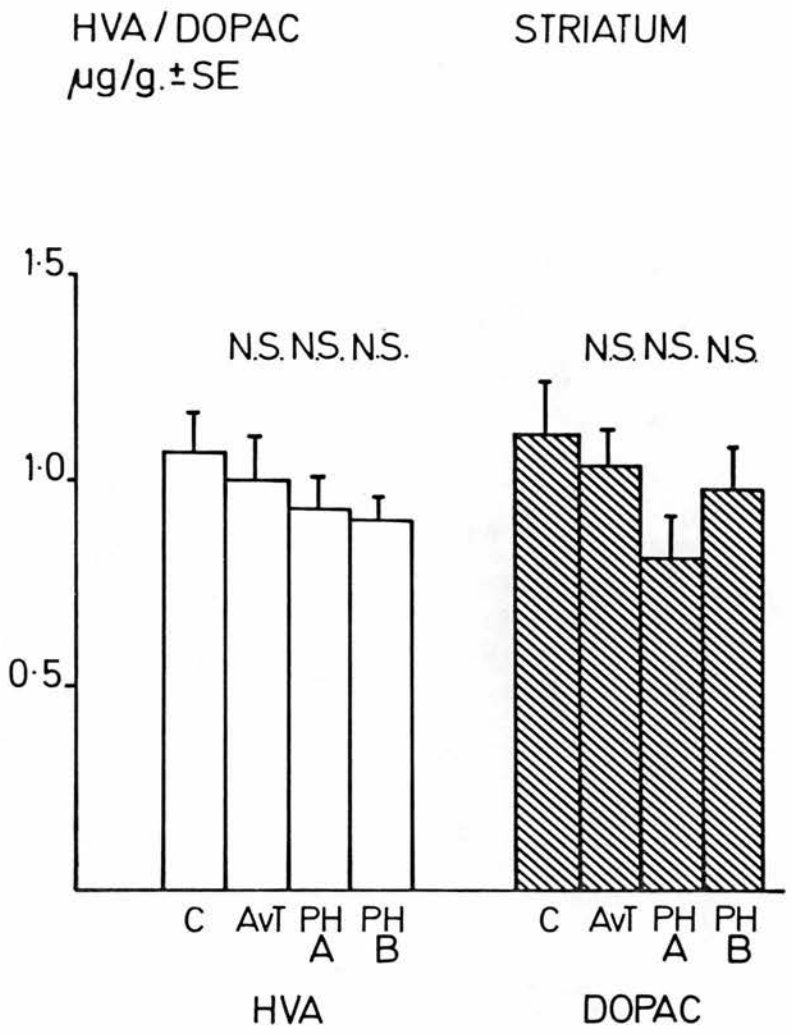

Section One Fig 2

The concentration of HVA and DOPAC in olfactory tubercle after ICSS. The groups were unstimulated control (c), n = 7; electrode location area ventralis tegmenti (AVT), n = 9; posterior hypothalamus, experiment A ($PH_A$) n = 7; and posterior hypothalamus ($PH_B$) n = 5. Group comparisons using student t-test, unpaired.




Section One Fig 3

The concentration of HVA and DOPAC in nucleus accumbens after ICSS. The groups were unstimulated control (c), n = 7; electrode location area ventralis tegmenti (AVT), n = 9; posterior hypothalamus, experiment A ($PH_A$) n = 7; and posterior hypothalamus ($PH_B$) n = 5. Group comparisons using student t-test unpaired.




Section One Fig 4

The concentration of HVA and DOPAC in the striatum after ICSS. The groups were unstimulated control (c), n = 7; electrode location area ventralis tegmenti (AVT), n = 9; posterior hypothalamus, experiment A ($PH_A$) n = 7; and posterior hypothalamus ($PH_B$) n = 5. Group comparisons using student t-test, unpaired.

to be two anatomically distinct subgroups of self-stimulating rats. Rats with electrodes placed rostral to A2420 were assigned to the PH group, those caudal to this in the AVT group.

The biochemical data was arranged in corresponding groups to investigate the correlation between electrode location and changes in dopamine metabolite levels. The levels of HVA and DOPAC as found in these groupings was as follows, (see Table 1).

The concentration of HVA in the olfactory tubercle of the AVT group 3.93 µg/gm was elevated significantly compared with the non-stimulated control group 1.97 µg/gm ($P < .001$, student t-test, two-tailed), while the level of the PH group 1.85 µg/gm, was no different to the control value. DOPAC concentrations were increased similarily, the AVT groups containing 4.95 µg/gm; the controls 2.47 µg/gm and the PH group 1.60 µg/gm (AVT to control, $P < .005$), PH to control, $P < .05$ (see Fig 2).

In the nucleus accumbens, the concentration of HVA in the AVT group was 1.96 µg/gm, in the controls 1.42 µg/gm and the PH group 1.45 µg/gm. The DOPAC concentrations were 2.31 µg/gm from AVT group, 1.44 in controls and 1.05 µg/gm in the PH group. Neither metabolite was significantly elevated in either of the stimulated groups compared to control, although the highest concentrations were seen in the AVT group (Fig 3).

The concentration of HVA in the striatum was 1.01 µg/gm in the AVT group, 1.09 µg/gm in controls and 0.94 µg/gm in the PH group. DOPAC concentrations were 1.05 µg/gm, 1.13 µg and .80 µg/gm for AVT, control and PH groups respectively. None of these metabolite concentrations were significantly different from control values (Fig 4).

Section One Table Two

The Concentration of HVA and DOPAC in forebrain areas after ICSS in medial posterior hypothalamus

Experiment B

| | HVA (µg/gm) | p vs control | DOPAC (µg/gm) | p vs control |
|---|---|---|---|---|
| Olfactory tubercle (5) | 1.87 ± .13 | n.s. | 2.67 ± .40 | n.s. |
| Nucleus accumbens (5) | 1.25 ± .13 | n.s. | 1.53 ± .18 | n.s. |
| Striatum (5) | 0.90 ± .05 | n.s. | 1.08 ± .16 | n.s. |

Results expressed as mean concentrations in µg/gm ± SEM. $PH_B$ - medial posterior hypothalamus group, experiment B. Groups were compared with the unstimulated controls using the student-t test, unpaired. Control values as in Table One.

Section One Table Three

The Concentration of HMPG in cortex and hippocampus

Experiment A

| | | p vs control |
|---|---|---|
| Control (7) | 61.7 ± 5.7 | - |
| AVT (9) | 64.6 ± 6.2 | n.s. |
| $PH_A$ (6) | 62.8 ± 5.8 | n.s. |

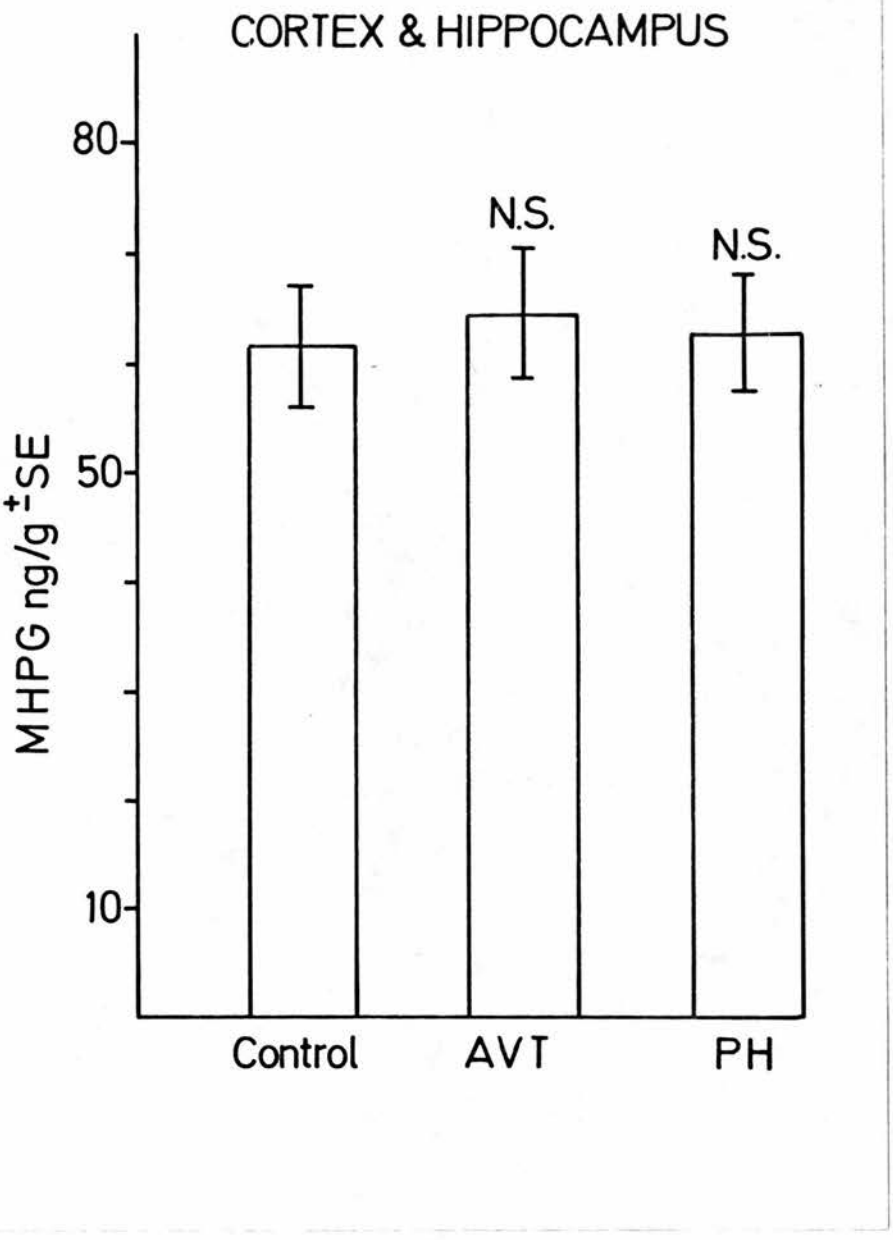
Results expressed as mean concentrations in ng/gm ± SEM. AVT - area ventralis tegmenti group. $PH_A$ - medial posterior hypothalamus, experiment A. The groups were compared using the student t-test, unpaired.

Experiment B

Although there were increased concentrations of dopamine metabolites in the olfactory tubercle only in the group of rats with electrodes actually in the AVT, discrimination between AVT electrode sites and those in the posterior hypothalamus was made on a post-hoc basis. The experiment was repeated therefore with electrodes aimed deliberately at the medial posterior hypothalamus. A group of 5 rats was used and the same procedures were followed. Histological examination confirmed the location of the electrodes in medial posterior hypothalamus, dorsal to the mamillary bodies. They were mostly found dorsal to the supramamillary decussations, medial to the mamillo-tegmental tracts. Some were on the edge of the periventricular gray matter. The amount of HVA and DOPAC in the same forebrain areas were obtained (Table 2).

In this posterior hypothalamic group, $PH_B$, the concentrations of HVA in olfactory tubercle was 1.87 µg/gm, in nucleus accumbens 1.25 µg/gm and in striatum 0.90 µg/gm. None of these values were significantly different to control values. The concentration of DOPAC in olfactory tubercle was 2.67 µg/gm in nucleus accumbens 1.53 µg/gm and in striatum 1.08 µg/gm and again none were significantly different to controls. These can be seen in Figs 2, 3 and 4.

In this experiment therefore, there were no increases in the concentrations of the DA metabolites HVA and DOPAC. It seems likely that the anatomical discrimination in Experiment 1 was in fact justified.




Section One Fig 5

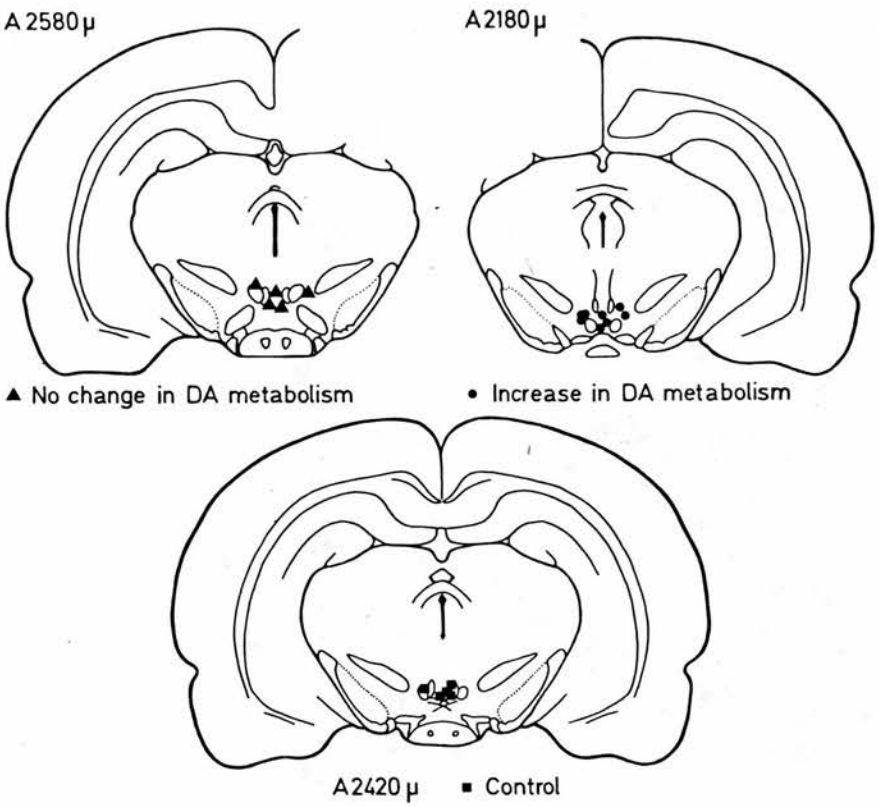
The concentration of HMPG in parietal cortex and hippocampus after ICSS. The groups were unstimulated control (c) n = 7; area ventralis tegmenti (AVT) n = 9; and posterior hypothalamus from experiment A (PH) n = 6.

MHPG

The concentration of a metabolite of NA, MHPG, was measured in the parietal cortex and hippocampus of the rats in the first experiment. The results were grouped on the basis of the anatomical distinction between electrodes in AVT and those in PH, (Table 3). Control tissues contained 61.7 µg/gm, those from the AVT groups 64.5 µg/gm and those from the pH group 62.8 µg/gm. There were no significant differences between any of the groups (Fig 5).

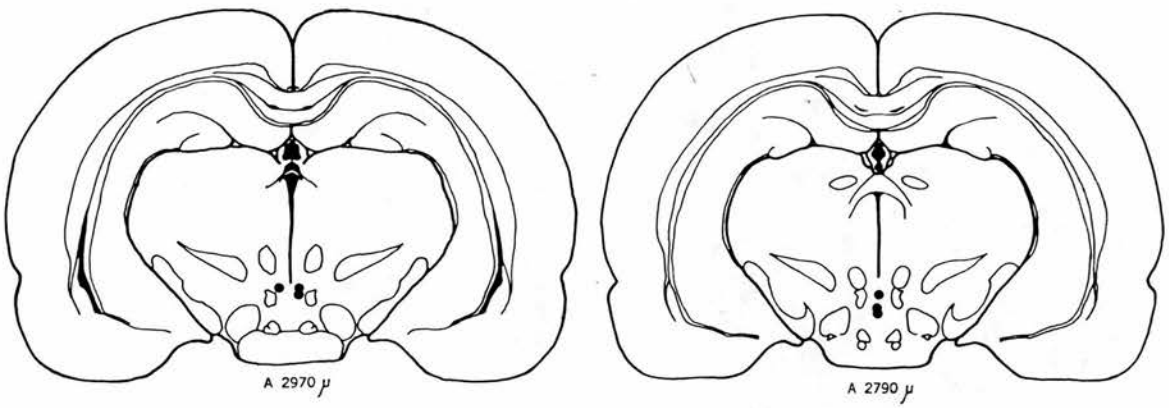
Statistics

The student t-test was used to estimate the statistical significance of the experimental variations, as described by Snedecor and Cochran (1967).




Section One Fig 6

The location of electrodes in Experiment A, plotted on coronal sections from the atlas of Konig and Klippel. The relationship to changes in DA metabolism is indicated - group AVT, increase in DA metabolism; group $PH_A$, no change in DA metabolism, and unstimulated control.




Section One Fig 7

The location of electrodes in experiment B, diagrams based on the atlas of Konig and Klippel. All electrodes in group $PH_B$ were found in medial posterior hypothalamus, dorsal to the mamillary bodies.

Discussion

The catabolism of dopamine in the brain is effected by the enzymes catechol-O-methyl transferase (COMT) monoamine oxidase (MAO) and aldehyde dehydrogenase (Sharman 1973 and Duncan and Sourkes 1974). The major end products are the acidic metabolites, 3, 4-dihydroxy-phenylacetic acid (DOPAC) (Rosengren 1960) and homovanillic acid (HVA) (Sharman 1963), and measurement of the concentrations of these metabolites can give a measure of dopamine turnover. Furthermore, changes in the firing rate of dopamine neurones may be reflected in the concentration of these metabolites in terminal areas. The level of DOPAC increases after administration of chlorpromazine (Walters and Roth 1972), a phenothiazine which increases the firing rate of DA neurones (Bunney et al 1973). Electrical stimulation is also efficacious in raising DA metabolite levels. Electrical stimulation of the rat nigro-striatal pathway enchances release of dopamine in the caudate (McLennan 1964), and enhances the output of HVA into ventricular perfusates in the cat (Portig and Vogt 1969). Stimulation of the nigro-striatal pathway produced rises in DOPAC in the corpus striatum on the stimulated side (Roth et al 1976). This experiment used pooled tissue and so exact proof of DA neurone stimulation causing increased DOPAC concentration in the individual animal is lacking. In another experiment, electrical stimulation of the medial forebrain bundle (MFB), a nerve bundle with which DA neurones are associated (Ungerstedt 1971A, produced rises in the concentration of both DOPAC and HVA. These rises are found in the olfactory tubercles and nucleus accumbens part of the terminal area of the meso-limbic DA system, and in the corpus striatum, the terminal area of the nigro-striatal DA system (Korf et al 1976). In both sets of experiments though, anaesthetised animals

are stimulated by the experimenter. In a different type of experiment, stimulation in the area ventralis tegmenti, in conscious rats near the A10 DA cells, increases the rate of utilization of radioactivity labelled DA, $^{3\text{-H}}$DA, with which the brains had been previously loaded (Stinus et al 1973).

It seems reasonable therefore to conclude that electrical stimulation of dopamine neurones can cause release of dopamine from the neurones. This release may be measured by determining the concentration of the dopamine metabolites DOPAC and HVA. Indeed, for conscious animal studies this may be the only reasonable method. Other methods of measuring DA turnover using the rate of disappearance of DA after synthesis inhibition are unusable as CA synthesis inhibitors cause physical impairments in the animal. Pre-loading of radioactive label has also disadvantages, mainly that the label may be taken up into non-dopaminergic neurones, and thence released, for example from the hippocampus (Stinus et al 1973), an area not know to contain DA terminals (Lindvall & Bjorkland 1974). For the purpose of examining DA release in self-stimulating animals, the method used in the present study would seem to be appropriate.

The results from the present study show that electrical self-stimulation from electrodes placed in the area ventralis tegmenti (AVT), in close proximity to the DA neurones from the A10 cell group, causes large increases in the concentration of both DOPAC and HVA in the olfactory tubercle. The size of these rises (approximately 200% increase) is in close agreement with that obtained with enforced stimulation of the MFB in anaesthetised animals (Korf et al 1976), which were believed to be maximal (Korf et al 1976b). There is also agreement with other studies on changes in DA metabolism after electrical

self-stimulation of the AVT. An increase in the rate of disappearance of pre-loaded $^{3-H}$DA from the olfactory tubercle (Stinus et al 1973) and an increase in the DA concentration from the olfactory tubercle (St. Laurent et al 1976) are reported.

All these measures indicate an increase in the dopaminergic activity in olfactory tubercle, and the DA release study and the present metabolite study indicate an increased release of DA on stimulation of the DA neurones.

The absence of a significant rise in HVA or DOPAC in the nucleus accumbens is unexpected, as the DA neurones of the A10 cell group project to this area of the brain (Ungerstedt 1971). Although there are no changes in DA concentration after electrical self-stimulation (St. Laurent et al 1976), large increases in DOPAC are found after MFB stimulation in the ipsilateral accumbens only (Korf et al 1976a). In the present experiment, both HVA and DOPAC concentrations were greater in the nucleus accumbens in the AVT stimulated group than in the control group. The ipsi-and contralateral accumbens are pooled before assay, thus any unilateral rise in accumbens may have been masked by the pooling, as the stimulating electrodes are found mostly in the lateral aspects of the A10 area.

There was no change in HVA or DOPAC in the corpus striatum. The corpus striatum has a different DA innervation, from the substantia nigra pars compacta DA cell group, A9 (Ungerstedt 1971). In the ventral tegmentum the A9 DA neurones run lateral to those from A10, and hence are not so likely to be stimulated from electrodes situated near the midline. The lack of a rise in DA metabolites in this area also suggests that there is no non-specific rise in DA metabolism due to increased motor activity.

In the animals with electrodes located in medial posterior hypothalamus (the PH groups) there are no increases in HVA or DOPAC in any of the DA terminal areas examined compared with control. As the stimulation parameters are identical to those of the AVT group, and electrical stimulation of dopaminergic pathways has previously been shown to increase DOPAC (Roth et al 1976) and HVA (Korf et al 1976), it seems likely that electrodes in this loci are not stimulating the mesolimbic or nigro-striatal dopamine pathways. There appears to be no other study of changes in dopamine metabolism after electrical stimulation of medial posterior hypothalamus with which to corroborate the present results.

It has been suggested that ICSS from at least some brain areas is dependent on NA release (Stein 1966: Crow 1969). Anatomical mapping studies have indicated that the dorsal noradrenergic system, originating in the locus coeruleus (A6) pontine cell group (Fuxe et al 1965 and Ungerstedt 1971) may support ICSS (Crow et al 1972: Ritter & Stein 1973). The dorsal noradrenergic bundle projects rostrally into the hypothalamus, in the medial forebrain bundle (Ungerstedt 1971), and hence it may be possible that stimulation of the pH group in fact activated this system.

The main metabolites of noradrenaline in brain are 4-hydroxy-3-methoxyphenylglycol (HMPG) and its sulphate conjugate (MHPG-$SO_4$) (Mannarino et al 1963: Schanberg et al 1968), with HMPG-$SO_4$ being the major metabolite in rat brain (Walter and Eccleston 1973). Fluorescence histochemistry suggests that the main NA innervation of the hippocampus and cerebral cortex originates in the locus coeruleus (Ungerstedt 1971). Lesions of the locus coeruleus markedly decrease both the NA concentration (Anzelark et al 1973) and the metabolite MHPG (Arbuthnott et al 1973: Korf et al 1973) in the ipsilateral

cerebral cortex. Electrical stimulation of one locus coeruleus produced significant increases in the concentration of MHPG-$SO_4$ in the ipsilateral cerebral cortex (Walter and Eccleston 1973: Korf et al 1973). In the rat brain, MHPG-$SO_4$ concentration gives a measure of noradrenaline turnover. Furthermore, electrical self-stimulation with electrodes placed in or near locus coeruleus is shown to increase MHPG-$SO_4$ concentration most markedly in the cerebral cortex ipsilateral to the electrodes (Anzelark et al 1975). The measurement of HMPG-$SO_4$ concentration in cerebral cortex and hippocampus as a measure of electrical activation of the dorsal noradrenergic system in the present study seems justified.

There are no increases in HMPG in these areas after stimulation in the AVT or PH compared with the unstimulated control group. This indicates that stimulation at these sites does not produce an increase in noradrenaline metabolism in cerebral cortex, and hence activation of the dorsal noradrenergic pathway does not seem to have occurred. This result is not unreasonable as the sites of stimulation do not correspond with the location of the dorsal bundle in the mesencephalon and hypothalamus (Ungerstedt 1971). Although no other study is known of the effects of medial posterior hypothalamic stimulation on noradrenaline metabolism, previous studies examine the effect of AVT stimulation. After pre-loading with $^{3-H}DA$, utilization of $^{3-H}DA$ and $^{3-H}NA$ in cortex and hippocampus increased a self-stimulation with bilateral electrodes in AVT (Stinus et al 1973). However, with unilateral stimulation in AVT, and measuring the disappearance of amine fluorescence after α-MPT administration, no change is found in the levels of cortical noradrenaline (Arbuthnott et al 1971). The latter experiment is in accord with the present results. Whether the results

of the other experiment are due to the bilateral stimulation or to the pre-loading technique remains uncertain. Pre-loading of amines can be criticised on the grounds of non-specific uptake and release (Chase & Kopin 1967), and the descending pathway which is surmised to activate the dorsal noradrenergic system is not yet verified experimentally. In the light of the present study it seems likely that the dorsal noradrenergic system is not activated by electrical stimulation of the AVT.

Unfortunately the present study does not provide evidence for activation of the ventral noradrenergic system, which passes through the AVT (Ungerstedt 1971). Both the previous studies suggest that such an activation takes place, and this seems a reasonable supposition in view of the close proximity of the ventral bundle to the stimulation sites (Arbuthnott et al 1970: Stinus et al 1973). It may be possible that activation of the ventral noradrenergic pathway supports self-stimulation behaviour. There is conflicting evidence for the ability of this pathway to support self-stimulation. Self-stimulation can be produced in the rostral pons, in the area of the ventral noradrenergic bundle (Ritter & Stein 1974). However, attempts to produce self-stimulation from the principal cell bodies of origin of the ventral noradrenergic system, A1 and A2 (Fuxe et al 1965) are not successful (Anlezark et al 1974: Clavier & Routtenberg 1974). As the ventral noradrenergic system is joined by other ascending NA fibres in the rostral pons to form the central tegmental tract (Lindvall and Bjorkland 1974) there remains the possibility that self-stimulation in the ventral mesencephalon and/or the posterior hypothalamus has a noradrenergic component.

The ventro-medial areas in the hypothalamus are often described as sites in the brain which produce aversive behaviour on electrical

stimulation (Olds 1960, Olds & Olds 1963). Sites in the ventro-medial posterior hypothalamus in the present study support high rates of self-stimulation. This area has been shown previously to be positive for self-stimulation (Atrens and Von Vietinghoff-Riesch 1972). Furthermore there exists a series of points throughout the ventro-medial hypothalamus which support self-stimulation (Atrens et al 1972: Ball 1972), with one area, around the paraventricular nucleus producing very high reward with a very weak aversive component (Atrens et al 1972). The behavioural aspects of self-stimulation in this area are similar to those in lateral hypothalamus, with excitation, sniffing and "searching" or exploratory behaviour (personal observations; Atrens et al 1972), although it may require a longer shaping procedure (personal observations: Ball 1972). It is tempting to speculate that there might exist a medial hypothalamic system which supports self-stimulation as well as the better documented one in lateral hypothalamus. Intriguingly there existed a noradrenergic pathway in this area, the ventral periventricular system (Lindvall & Bjorklund 1974) which could perhaps support the self-stimulation, although this must be considered pure speculation.

There remains the problem of the neurological substrate of the self-stimulation produced in ventro-medial posterior hypothalamus, which appears not to involve the main catecholamine systems hypothesised to be responsible for this behaviour. As well as the ventral periventricular system mentioned above, 5-HT containing fibres are found in the supramamillary decussation (Bobellier et al 1975), and there are nerve fibres which pass from lateral hypothalamus to this decussation (Huang & Routtenberg 1971). Pharmacological experiments to investigate the neurochemical characters of this "ventromedial reward system" are detailed in the next section.

In conclusion, it has been demonstrated that in vivo electrical activation of the dopaminergic mesolimbic system in the self-stimulating rat produces an increase in the dopamine metabolites HVA and DOPAC in a terminal area of the system viz the olfactory tubercle. However, such an increase in dopamine metabolites is not a necessary concomitant to electrical self-stimulation in the rat as demonstrated by the results from the posterior hypothalamic group of rats. Furthermore electrical self-stimulation in the ventral mesencephalon or ventromedial posterior hypothalamus does not appear to activate the dorsal noradrenergic system, as measured by an increase in the NA metabolite, MHPG.

## Section 2

## Pharmacological evaluation of medial posterior hypothalamic ICSS

### Introduction

The existence of an area of medial posterior hypothalamus (MPH) which supported ICSS but which did not appear to be mediated by the mesencephalic dopaminergic systems has been demonstrated (Section One). Early mapping studies of ICSS in the brain have shown that this area can support ICSS (Olds et al 1960). Furthermore the ICSS from medial sites in caudal hypothalamus has been shown to be almost purely positive with very little aversive effects (Atrens et al 1972), and more especially in the area dorsal to the supramamillary decussations (Olds and Olds 1963). The neural systems involved here have remained undefined, and these areas have not been investigated thoroughly, especially the MPH.

A number of pathways which have an identified neurotransmitter traverse this area, in particular the monoamine containing pathways. The ventral periventricular NA system has been found to run rostrally through this area ventral to the supramamillary commisure and medial to the mamillothalamic tract (Lindvall and Bjorklund 1974). In the more dorsal aspects of this area the A11 cell group (Fuxe et al 1969) has been found, which appeared to contain both NA and DA cells, with ventral projections towards the mamillary body (Bjorklund and Nobin 1973). The central tegmental tract has multiple projections to this area, corresponding in part to the former ventral NA system (Lindvall and Bjorklund 1974). The MPH thus has a considerable catecholaminergic innervation of various origin, which could be supporting ICSS.

An indolaminergic innervation of this area has been described. The mamillary nuclei were found to have a moderately dense innervation from the raphe nuclei in an autoradiographic study (Moore et al 1978).

There have also been descriptions of fibres from the raphe crossing above the mamillary bodies (Bobillier et al 1975). The possibility of this serotoninergic system supporting ICSS would have to be considered.

The identity of the pathways supporting ICSS in this area might be elucidated by pharmacological investigation. The effect of pharmacological manipulations in ICSS has been studied extensively, and in particular the effect of drugs acting on the monoaminergic systems. In general drugs which increase the availability of CA's at the synapse have potentiated ICSS. The amphetamines, which have been shown to enhance NA and DA release, inhibit their re-uptake and block MAO (Sulser and Sanders Bush 1971), enhance ICSS response rates (Stein 1964; Crow 1969). The enhancement produced was most marked at near threshold current intensities (Stein 1964). In addition, amphetamine has been shown to reduce these thresholds (Stein and Ray 1960). Drugs which inhibit monoamine oxidase (MAO) and hence increase the concentration of amine in the nerve terminal have been shown to facilitate ICSS (Poschel and Ninteman 1964). These MAOI prevent the intraneuronal breakdown of both CA's and 5-HT, hence these results do not differentiate between the monoamines. Cocaine, which enhances the release of the CA's (Trendelenburg 1959) and may also inhibit their re-uptake enhances ICSS (Crow 1970). There has been considerable support for an involvement of CA's in ICSS from these experiments with enhanced CA release.

In experiments in which CA transmission was either blocked or reduced, decreases in responding for ICSS were found. CA synthesis was found to be inhibited by α-methyl-p-tyrosine (αMPT), αMPT inhibited the enzyme tyrosine hydroxylase which catalysed the rate-limiting step

in CA synthesis (Nagatsu et al 1964), and thus led to a reduction in brain CA concentrations (Spector et al 1965). A corresponding reduction of ICSS was seen after α-MPT (Poschel and Ninteman 1966: Black and Cooper 1970), using the bar-press response. However, using rate-free measures a reduction of ICSS was also obtained with α MPT (Black and Cooper 1970). This indicated that a non-specific impairment of the animal was not responsible for the reduction in ICSS, which suggested a vital role for CAs in ICSS.

A different approach was possible using reserpine, which was believed to deplete CA transmitter stores by interfering with the intraneuronal storage of the transmitter (Bloom and Giarman 1968). Reserpine markedly reduced ICSS response rates (Olds et al 1956) and also elevated the current threshold for responding (Stein 1962). Another drug which also depletes intraneuronal CA's, tetrabenazine also reduced ICSS response rates (Stein 1966). There seemed to be good evidence for an essential role of the CA's from the evidence of these depletion studies also.

As well as interfering with the pre-synaptic neurone it was possible to reduce CA neurotransmission by blocking the post-synaptic receptors. The neuroleptics, which block DA receptors, severely reduced ICSS (Olds et al 1956: Wauquier and Niemeegers 1972). With NA antagonists, the alpha-blockers phentolamine and phenoxybenzamine did decrease ICSS, but at doses which could have caused a general impairment (Bailey et al 1972: Hastings and Stutz 1973). The beta-blocker propanolol had little effect at doses which caused pronounced blockade (Hastings and Stutz 1973: Wise et al 1973). These experiments strongly suggested an essential role for DA, but were less conclusive for NA.

The effect of drugs on the 5-HT system has been contradictory. When p-chloroamphetamine was given a decrease in ICSS was observed (Poschel and Ninteman 1971). This compound was believed to increase the release of 5-HT, but it also similarily affects CA systems (Sulser and Sanders-Bush 1971). If 5-hydroxytryptophan (5-HTP) was given in conjunction with a MAOI, an increase in responding was seen. However, 5-HTP not only increases brain 5-HT levels, but it may displace NA. Furthermore, MAOI themselves may increase ICSS (Poschel and Ninteman 1968). The use of drugs which deplete 5-HT in the brain has not supported a crucial role for this amine in ICSS. P-chlorophenylalamine (PCPA) has been found to inhibit tryptophan hydroxylase, and lower brain 5-HT levels significantly (Koe and Weissman 1966). Maximum 5-HT depletion occurred around seventy-two hours after administration of PCPA, and numerous studies have failed to show an effect on ICSS responding (Black and Cooper 1970: Margules 1969). Any effect of PCPA on ICSS has been in the first 24 hours after dosing (Stark et al 1970: Crow and Deakin 1977) when a marked effect on NA levels was seen (Koe and Weissman 1966). There has been little conclusive pharmacological evidence on the role of 5-HT in ICSS behaviour.

The localisation of monoamine containing neurones in medial posterior hypothalamus suggested that the initial experiments to investigate the pharmacology of ICSS from this area should involve drugs with specific actions on these systems. The investigation of catecholaminergic involvement was based on the use of α-MPT, the tyrosine hydroxylase inhibitor, and a dopamine antagonist, spiroperidol. The possibility of 5-HT systems being involved was investigated using PCPA, a tryptophan hydroxylase inhibitor and alaproclate, a specific inhibitor of 5-HT uptake.

Methods

Male Wistar rats (180 - 200 gm) were implanted with bipolar electrodes as in Section I. The electrodes were aimed at medial posterior hypothalamus, and the co-ordinates used relative to bregma were:-

A - P - 4.0 mm

L - 0

V - 8.2 mm

After a one week recovery period all animals were tested for ICSS behaviour as previously described. Animals which showed no evidence of positive self-stimulation behaviour after three half hour training sessions were discarded. The stimulation used was biphasic square wave pulses, produced by a Neurolog stimulator (Digitimer). Stimulation parameters were as follows:- 200 msec trains of 0.2 msec pulses, with an inter pulse interval of 4 msec. A continuous schedule of reinforcement was used, although any lever press occurring during a stimulus train was ineffective.

All rats positive for ICSS were allowed to self-stimulate for 15 minutes/day until stable rates of responding were achieved. During this period current levels were varied to obtain an approximate current-response rate relationship. A current level was chosen for each rat which produced a response rate of approximately 60% of maximal. The rat was then stabilised at this rate.

After stabilisation response rates were obtained, the drug tests began. When a drug vehicle control was obtained drug sessions were performed two days later if the response rate remained constant on the interceding day. At least one week elapsed before a second drug treatment was given to any individual rat.

Drugs acting on CA systems

The tyrosine hydroxylase inhibitor α-methyl-p-tyrosine methyl ester (Sigma) was administered i.p. at a dose of 150 mg/kg. It was suspended in hydroxy propyl methyl cellulose (HMPC) (ICI), in a volume of 4 ml/kg. Vehicle alone was given on the control day. After drug administration the animals were returned to their home cage for 4 hours. They were then placed in the test chamber for 15 minutes.

The dopamine antagonist spiroperidol (Janssen) was administered i.p. at a dose of 0.05 mg/kg. It was dissolved in a few drops of 0.1N Tartaric Acid and made up with distilled water to a volume of 2 ml/kg. The drug was given immediately after the first 15 minute ICSS session which served as the baseline. After a period of 30 minutes the animal was returned to the test chamber for a 15 minute test session. There was then a 15 minute time out, and the test repeated. This pattern was repeated until the final session, 2 hours post drug administration.

All bar-press responses were counted for the test period on electro-mechanical counters as previously described. Visual records were sometimes obtained on cumulative pen recorders.

Drugs acting on 5-HT systems

The tryptophan hydroxylase inhibitor parachlorophenylalanine (PCPA) (Sigma) was administered orally at a dose of 400 mg/kg. It was suspended in HPMC in a volume of 4 ml/kg. This was done after a baseline test session. Subsequent test sessions of 15 minutes were performed 24, 48 and 72 hours after drug administration.

The 5-HT uptake inhibitor alaproclate hydrochloride monohydrate (GEA 654) (Astra) was administered i.p. at doses of 10 and 20 mg/kg. It was dissolved in distilled water at a volume of 2 ml/kg. The drug was

given immediately after a baseline test session, and subsequent 15 minute sessions were performed 30, 75 and 120 minutes after drug administration.

The responses were measured as before.

Histology

At the end of the experiment the rats were killed and their brains removed. The procedures followed were exactly as in Section One, and electrode location was determined from the stained sections using a light microscope.

Section Two Table One (a)

The effect of α-methyl-para-tyrosine on ICSS from medial posterior hypothalamus

| Mean Responses/15 min ± S.E. (bar presses) n = 8 | Control | α-MPT + 4 hrs | % of Control |
|---|---|---|---|
| | 894 ± 108 | 518 ± 81.0 ** | 58% |

Drug was administered i.p., suspended in HPMC. Response rates, expressed as mean ± S.E., were measured before and four hours after drug administration. The difference in response rates was statistically significant, ** -P < .01, paired student t-test.

Section Two Table One (b)

The Changes in response rate within sessions before and after α-MPT

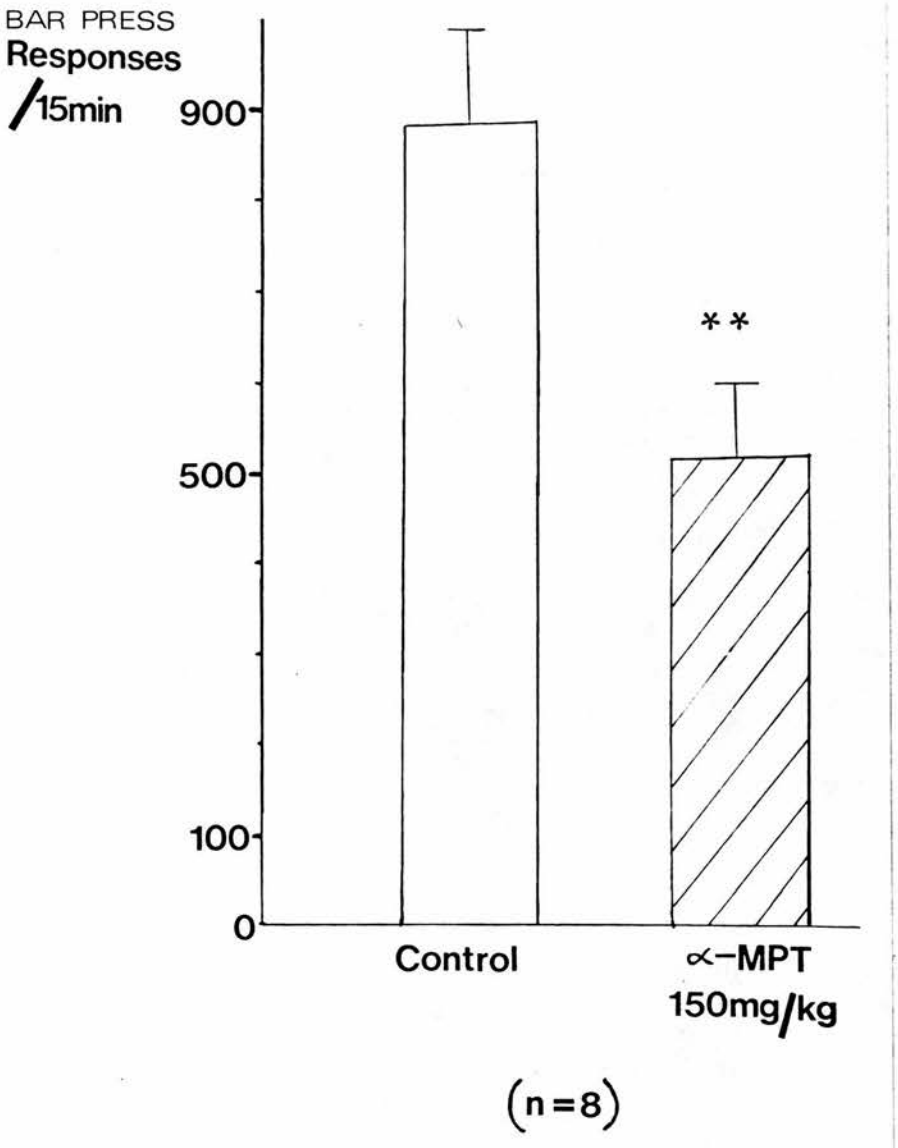
| | Control | α-MPT | |
|---|---|---|---|
| Rate/min first 10 min | 60 | 41 | n=8 |
| Rate/min last 5 min | 58 | 22 | |

Section Two Table One (c)

The effect of α-MPT on ICSS from lateral hypothalamus (initial results)

| LH ICSS | Control | α-MPT + 4 hrs | % of control |
|---|---|---|---|
| (n = 4) | 597 ± 74 | 134 ± 39 | 22% |

α-MPT, 150 mg/kg i.p. as above. Mean bar presses/15 min.




Section Two- Fig 1

The effect of α-methyl-para-tyrosine 150 mg/kg i.p. on ICSS from medial posterior hypothalamus. The test session was four hours after drug administration. Response rates expressed as mean ± SE (n = 8) The difference was statistically significant, * P < .01, student paired t-test.

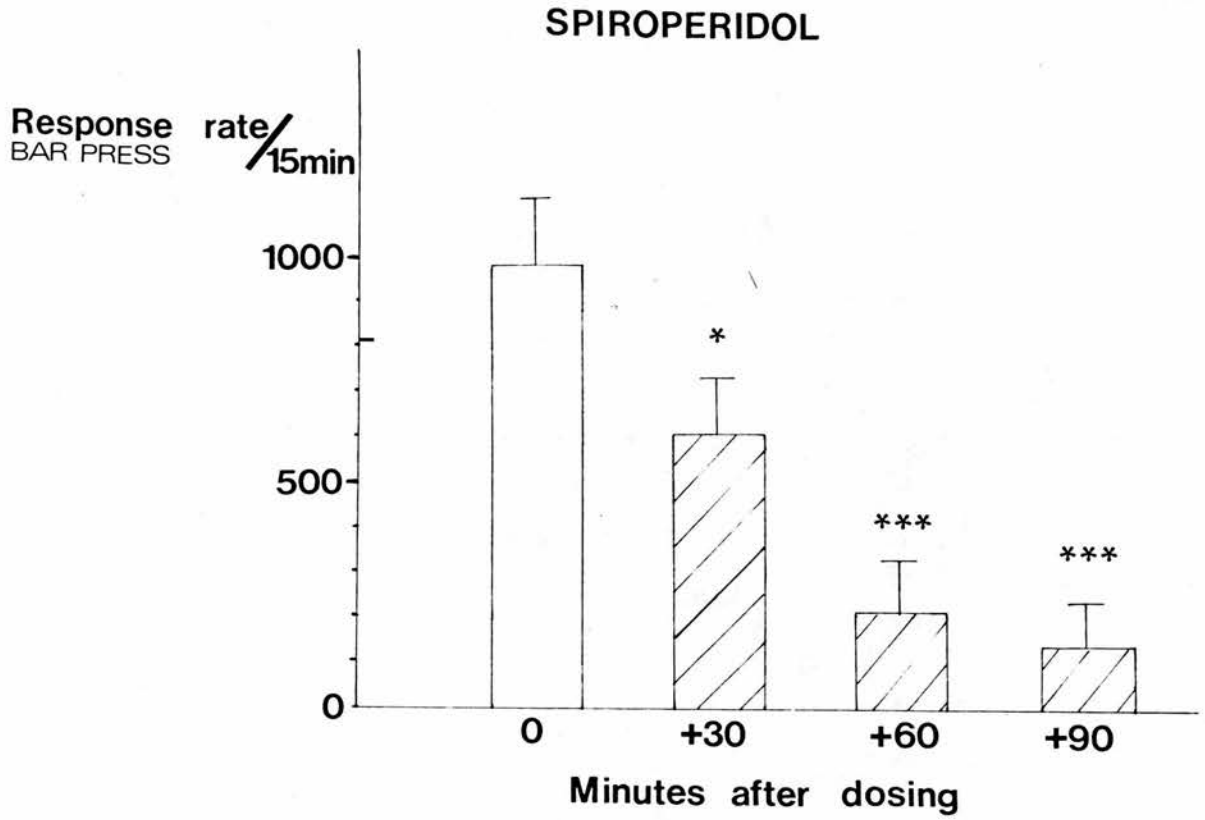
Section Two Table Two

The effect of spiroperidol on ICSS

from medial posterior hypothalamus

| Time after dosing | + 0 (pre-drug control) | + 30 | + 60 | + 90 | + 120 min |
|---|---|---|---|---|---|
| Responses/15 min | 975 | 610 | 207 | 135 | 80.0 |
| | ± 143 | ± 135 | ± 102 | ± 74.2 | ± 55.9 |
| | n = 8 | n = 8 | n = 8 | n = 8 | n = 5 |
| Difference from control | | P < .02 | P < .001 | P < .001 | P < .01 |
| % control | 100 | 63% | 21% | 14% | 8 |

Drug was administered i.p. at a dose of 0.05 mg/kg. It was dissolved in a few drops of 0.1N Tartaric Acid, and made up to volume with distilled water. Thirty minutes after drug administration, 15 minute test sessions commenced, with 15 minute time-outs in between sessions. Response rates expressed as mean ± S.E. There was a statistically significant difference compared to pre-drug control rates at 30 minutes, P < .02, 60 and 90 minutes, P < .001 and at 120 minutes, P < .01 - paired student t-test.




Section Two Fig 2

The effect of spiroperidol, 0.05 mg/kg i.p. on ICSS from medial posterior hypothalamus at various times after drug administration. Response rates expressed as mean $\pm$ SE (n = 8). The differences were statistically significant: *-P $<$ .02, *** P $<$ .001, student paired t-test.

## Results

### CA pharmacology

The effect of α-MPT was to reduce although not abolish ICSS. The control response rate of 894 ± 108 (S.E.) was reduced to 518 ± 81.0 and this difference was statistically significant ($P < .01$, Student paired-t test, two-tailed). This reduction was most marked in the last five minutes of the test session (Table One). The mean response rate/minute was the same throughout the control session, 60/minute during the first ten minutes and 58/minute in the next five minutes. After α-MPT the response rate was reduced to 41/minute and 22/minute respectively (Table One).

Spiroperidol caused a decrease in ICSS response rates which became progressively more pronounced throughout the sessions. The initial rate of 975 ± 143 was reduced to 135 ± 74.2 in the session commencing 90 minutes after drug administration. Most of the animals were tested at 120 minutes and responding had decreased to 80 ± 55.9 ($n = 5$). All these reductions were statistically significant at least $P < .02$ (paired t-test, two-tailed) (Table Two).

### 5-HT pharmacology

There was no observable effect of PCPA on ICSS (Table Four). The response rates at all times observed were not significantly different from the pre-drug baseline session. The animals were observed to be hyperactive and hyperirritable compared with their normal behaviour, which was typically seen after PCPA treatment in other experiments.

The effect of alaproclate was complex (Table Three). At a dose of 10 mg/kg it produced an increase in response rates that was statistically significant at both 75 minutes ($P < .05$) and 120 minutes ($P < .01$) after drug administration ($n = 10$, paired-t test, two-tailed).

Section Two Table Three

The effect of alaproclate on ICSS

from medial posterior hypothalamus

| Time after dosing (minutes) | Response rates/15 min<br>Alaproclate 10 mg/kg i.p. n = 10 | | Alaproclate 20 mg/kg i.p. n = 5 | |
|---|---|---|---|---|
| 0 (pre-drug control) | 803 ± 137 | | 665 ± 276 | |
| 30 | 878 ± 164 | n.s. | 78 ± 47 | n.s. |
| 75 | 1073 ± 183 | * | 631 ± 151 | n.s. |
| 120 | 1077 ± 163 | ** | 968 ± 386 | n.s. |

Alaproclate administered i.p., dissolved in distilled water. Response rate expressed as mean ± S.E. Comparison with pre-drug control rates using paired student-t test, * $P < .05$, ** $P < .01$

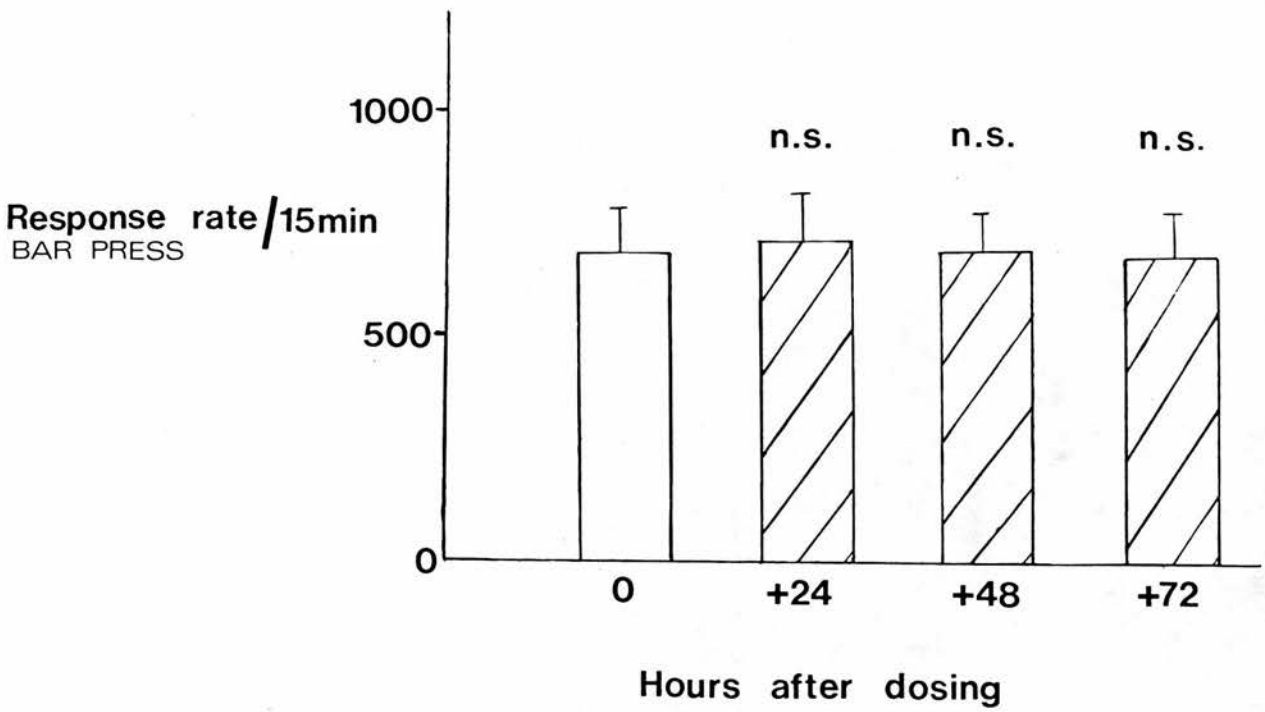
Section Two Table Four

The effect of p-chlorophenylalanine on ICSS

from medial posterior hypothalamus

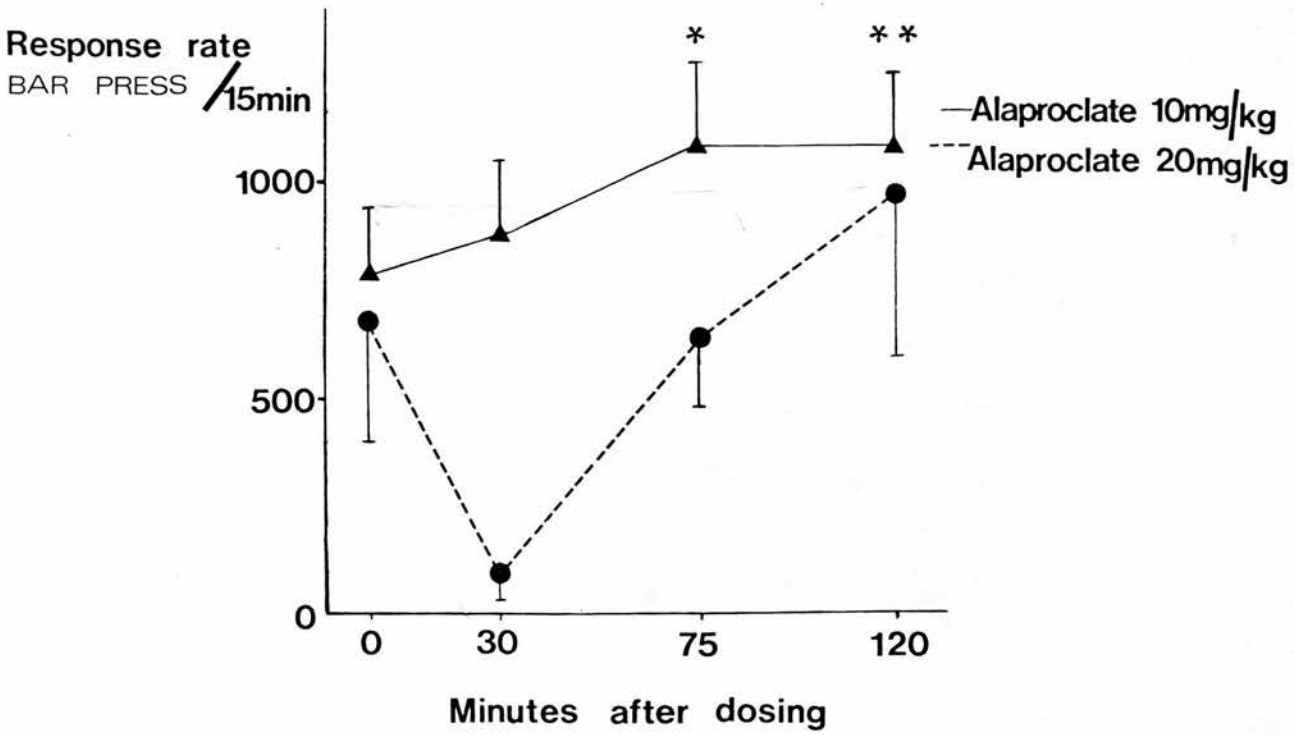
| Time after dosing (hrs) | Mean response rate/15 mins ± S.E. n = 8 | |
|---|---|---|
| 0 (pre-drug control) | 680 ± 86.3 | |
| +24 | 695 ± 96.5 | n.s. |
| +48 | 680 ± 80.6 | n.s. |
| +72 | 670 ± 92.3 | n.s. |

The drug was administered p.o., suspended in HPMC. Dosage was 400 mg/kg. Response rate was expressed as mean ± S.E. The results were compared with pre-drug controls using the paired student t-test. There were no differences at any point.




Section Two Fig 3

The effect of para-chlorophenylalanine, 400 mg/kg p.o. on ICSS from medial posterior hypothalamus. Response rates expressed as mean ± SE (n = 8). There was no significant change in response rates.



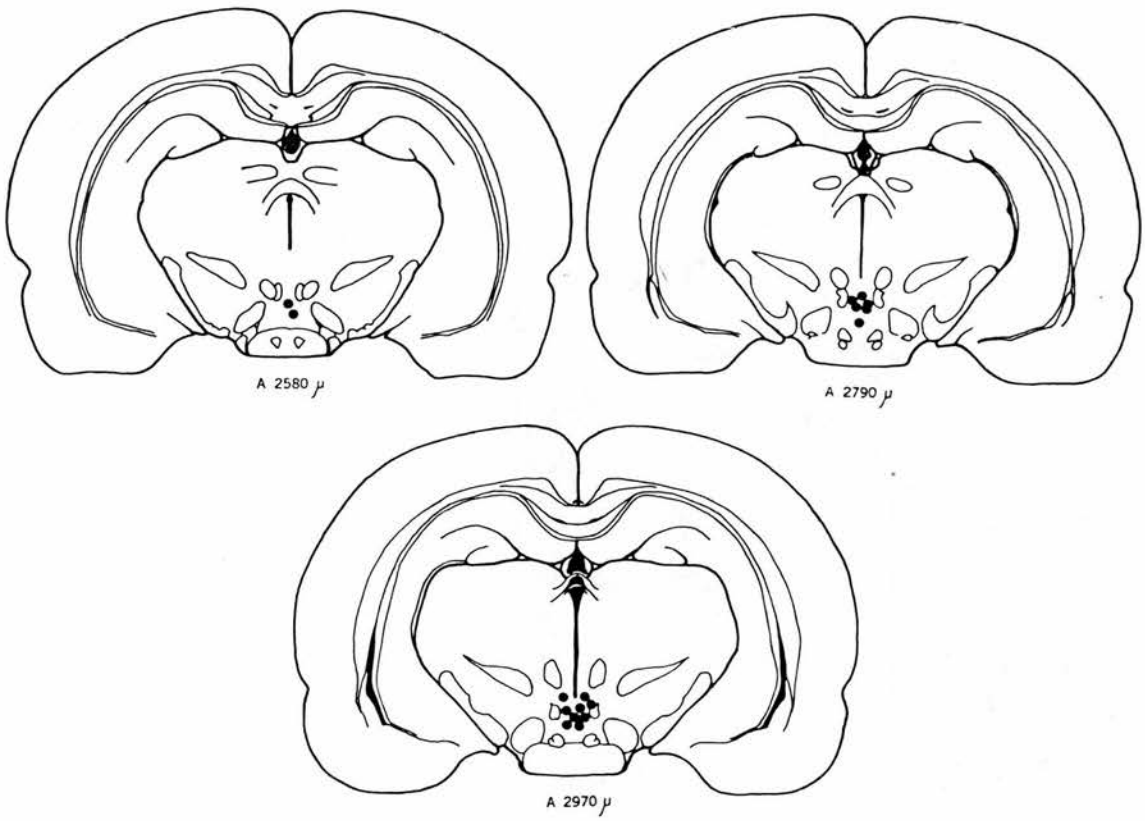

Section Two Fig 4

The effect of alaproclate at doses of 10 and 20 mg/kg i.p. on ICSS from medial posterior hypothalamus. Response rates expressed as mean ± SE. The 10 mg/kg dose produced a statistically significant increase, * $P < .05$,** $P < .01$, $n = 10$, student paired t-test. The 20 mg/kg dose produced no statistically significant changes ($n = 5$).

The effect of 20 mg/kg was variable. None of these variations in response rate were statistically significant (paired-t test, two-tailed), but there was a small group size in this part of the experiment (n = 5).

Electrode placements

The electrodes of all the rats used in these experiments were found to be located in medial posterior hypothalamus, dorsal to the mamillary body. They were mostly found on the dorsal aspects of the supramamillary decussations, medial to the mamillo-tegmental tracts. Some positive sites extended dorsally along the edges of the periventricular gray up to the ventral aspect of the third ventricle, parallel to medial lemniscus. Two electrodes were placed in the supramamillary decussations between the medial forebrain bundles (Fig Five).




Section Two Fig 5

The location of electrodes in medial posterior hypothalamus as plotted on coronal sections from the atlas of Konig and Klippel. All electrodes were located in a region dorsal to the mamillary bodies, mostly dorsal to the supramamillary decussations.

## Discussion

### Drugs acting on CA systems

These two experiments have both indicated that the CA systems have a function in ICSS from medial posterior hypothalamus (MPH). α-MPT has been shown to markedly reduce the concentration of both NA and DA, and that this was observed 4 - 8 hours after administration (Spector et al 1965). This has been shown to cause a marked reduction in lever-pressing for ICSS (Poschel and Ninteman 1966; Cooper et al 1971). It has also been shown to increase the electrical threshold for ICSS (Gibson et al 1970) and to decrease ICSS when a rate-free measure of reward was used (Black and Cooper 1970). These were of considerable importance to the relevance of the α-MPT effect as it had been shown to adversely affect a number of tasks involving motor co-ordination (Rech et al 1966) and it was suggested that such an effect on ICSS might be related to non-specific effects (Roll 1970). A technique which relates reward strength and running speed in a runway test of ICSS, which in part is a sophisticated measure of reward threshold, has been used to determine the nature of the α-MPT effect. This has shown that α-MPT did affect the performance aspects, but at some electrode locations it also reduced the reward strength (Edmonds and Gallistel 1977).

Unfortunately there was no way of determining the precise effect on ICSS in the present experiment. However in view of the performance debilitating aspects of α-MPT it would not be wise to attribute the present result to a direct effect on a CA pathway mediating reward. It was possible that the decreased rate of responding in the latter part of the session after α-MPT was a reflection of an effect on the ability to perform the motor task, perhaps increased fatigueability or weakness for example. However an alternative explanation must be pointed out.

It has been shown that prior depletion of the reserpine-sensitive CA storage pool enhanced the abolition of ICSS by synthesis inhibitors (Franklin and Herberg 1975). It could be suggested that due to impaired synthesis the release of CA by the electrical stimuli caused depletion of CA from the nerve terminals in the initial part of the test session. Hence in the later part of the session the release of CA was reduced and thus also the magnitude of the reward. However in view of the evidence of the effect of α-MPT on performance this seems a less likely explanation.

The extent of the effect of this dose of α-MPT on ICSS from MPH would also seen to indicate a non-essential role for CA at this site. This was of the order of a 40% decrease. In a similar experiment ICSS from the AVT and from LH was reduced to a much greater extent by the same dose of α-MPT, by approximately 90% (Stinus and Thierry 1973). In a recent experiment initial results indicated that this dose of α-MPT caused an effect on ICSS from lateral hypothalamus similar to this with a 80% decrease (Table One, initial results). It thus seems that at other sites this dose of α-MPT has a much more severe effect on ICSS. In view of the experiment which showed directly that α-MPT affected reward at only some sites (Edmonds and Gallistel 1977) it must be considered possible that ICSS in MPH has a non-CA component.

The result of the spiroperidol experiment however indicated that DA systems had an essential role in ICSS from MPH. Spiroperidol, a potent and specific DA antagonist (Anden et al 1970) has been shown to be a very potent inhibitor of ICSS from lateral hypothalamus (Wauquier 1976), with an $ED_{50}$ of 0.02 mg/kg. In the present experiment a dose of 0.05 mg/kg produced 86% inhibition and this would seem to support these earlier findings. Another experiment which used spiroperidol has shown a 91% inhibition of ICSS from LH with a dose of 0.06 mg/kg (Mora et al 1975). These results would seem to show considerable unanimity, and suggested

that ICSS from the MPH and LH was mediated by DA systems.

There has been considerable controversy over the effect of DA antagonists on ICSS. It has been suggested that they disrupt a variety of operant tasks (Wauquier and Niemegeers 1972). They have been shown to disrupt complex motor responses such as bar-pressing for various reinforcers including ICSS (Fibiger et al 1976; Rolls et al 1974) and other learned motor responses (Fibiger et al 1975). It may be possible that the low doses of DA antagonists which severely disrupt ICSS might not affect motor responses so severely (Mora et al 1975; White et al 1978). Furthermore some experimental designs have indicated that the reward and performance aspects of DA blockade may be separated. Experiments using a rate-free measure of ICSS in a shuttle-box (Liebman and Butcher 1974), a runway test (White et al 1978) or the measure of the reward summation function in the runway (Franklin 1978) have all indicated that low doses of specific DA antagonists could decrease the rewarding effect of ICSS. Of considerable relevance was the runway test which showed a differential effect at sites in far-lateral or medial-lateral hypothalamus. At both sites the bar-press response for ICSS was similarly attenuated (White et al 1978). It would seem fair to say that DA antagonists have a disruptive effect on complex learned motor tasks such as bar-pressing and that the measure of their effects on average rates of such responses did not differentiate between reward and performance effects. The results of the present experiment must be considered open to either interpretation.

The possibility that a DA system supported ICSS in MPH would not seem to fit with the results of Section One. However only the mesolimbic and nigro-striatal DA systems were investigated in that experiment and in view of the possible involvement of DA in ICSS from frontal cortical areas (Clavier and Gerfen 1979; Robertson and Mogenson 1978) it was

possible that mesocortical fibres were stimulated in MPH as cortical DA metabolites were not measured. The anatomical evidence would not seem to support this supposition, especially the more anterior sites (see Fig. 10, Lindvall and Bjorklund 1974). Alternatively the DA system which was found in caudal hypothalamus, from cell groups A11 and A13 and which projected into this area (Bjorklund and Nobin 1973) could have supported ICSS. This would explain the spiroperidol result on the basis of a reward effect.

In conclusion the effects of α-MPT and spiroperidol have indicated that CA systems, in particular in the DA system have a critical role in ICSS from MPH. The α-MPT effect could in fact be explained solely by its effect on DA systems, but of course no evidence was presented to discount a role for NA. The significance of CA involvement was not determined, as it could have reflected an effect either on performance or the operant response or on the rewarding aspects of the stimulation. A more refined approach to these problems, either by more sophisticated behavioural controls or the use of more direct measures of reward strength, would have been required to answer these problems.

Drugs affecting 5-HT systems

The effect of pharmacological manipulations of 5-HT systems has not indicated that these have a crucial role in ICSS from MPH. The complete lack of effect of PCPA would suggest that a 5-HT system did not support ICSS in this area. PCPA has been shown to cause a large decrease in brain 5-HT levels with maximum depletion around 72 hours after drug administration (Koe and Weissman 1966). The present dose of 400 mg/kg given orally has been shown to cause a similar pattern of depletion (Van der Kooy et al 1977). A disruption of NA synthesis has been noted but the effect had a much shorter time span of about

24 hours (Welch and Welch 1967). No consistent effect of PCPA on ICSS has been found. At the time of 5-HT depletion PCPA was shown to have no effect on ICSS from lateral hypothalamus (Stark et al 1970; Black and Cooper 1970), or to facilitate it (Phillips et al 1976b; Poschel and Ninteman 1971) and in one study to inhibit it (Gibson et al 1970). It must be emphasised that these were the effects which correlated with 5-HT depletion as inhibition of ICSS at early times after dosage did not.

The effect of PCPA on ICSS from the 5-HT cell bodies in the dorsal and median raphe has also been contradictory. In the region of the dorsal raphe it has been shown to facilitate (Simon et al 1976), to have no effect (Margules 1969) and to inhibit (Van der Kooy et al 1978) ICSS. In the median raphe it has been shown to inhibit (Miliaressis et al 1975) or to have no overall effect (Van der Kooy et al 1978). Reasons for such disparate results might be found in different experimental procedures. It has been suggested that long test sessions were more sensitive to the disruptive effects of PCPA (Miliaressis et al 1975) and the demonstrations of inhibitory effects in the raphe have used this technique. Even median raphe ICSS has shown some decremental effect of PCPA in the latter part of a two hour session (Van der Kooy et al 1978). However the use of long test sessions might also emphasise any deleterious effects on motor performance, as has been discussed previously. Experiments which differentiated reward and performance effects should be carried out to determine the role of 5-HT.

It has been possible to demonstrate inhibitory effects of PCPA using short test sessions as used in the present experiment. A marked inhibition of ICSS in the striatum has been shown and most interestingly a facilitation was seen with LH placements using the same design (Phillips et al 1976). It thus seems possible that in some areas an inhibitory effect of PCPA can be demonstrated using a similar exper-

imental design to the present one. The lack of effect of PCPA in the present experiment might be said to indicate that 5-HT systems were not responsible for the mediation of ICSS in MPH. In one of the previous experiments although facilitation was seen after PCPA with electrodes in LH, no effect was seen with some electrodes in medial PH (Poschel and Ninteman 1971). It would have been interesting to have observed the effect on long test sessions to perhaps confirm these observations. The total lack of effect might suggest that this would also be negative. The lack of effect at 24 hours which might reflect CA inhibition has however been seen in other experiments with PCPA (Margules 1969; Stark et al 1970), and thus need not suggest a general ineffectiveness of PCPA administration in this experiment. The effects on activity and irritability were as previously reported (Fibiger and Campbell 1974) and hence the PCPA treatment was likely successful.

The other part of the experiment produced a complicated pattern of results. Alaproclate (2-(4-chlorophenyl)-1, 1-dimethylethyl 2-aminopropanoate) has been shown to be a potent and specific inhibitor of brain 5-HT uptake (Lindberg et al 1978). The effect of increasing the level of 5-HT in the synapse which such a drug should cause might have indicated the role of 5-HT in ICSS in this area. The results obtained were somewhat confusing. A slight but significant increase in responding was seen after 10 mg/kg, but 20 mg/kg produced an initial fall in response rates, which then recovered and in fact increased at a later time. The latter results were not statistically significant but the increase over baseline was seen in all of the small group of animals. The indication was that alaproclate slightly facilitated ICSS from this hypothalamic area. In other experiments with 5-HT uptake inhibitors, fluoxetine has been shown to inhibit ICSS (Katz and Carroll 1977) as has Lu 5-003 (Atrens et al 1977). The effect of altering brain 5-HT

levels might help explain these conflicting results. The administration of 5-hydroxytryptophan (5-HTP) has been shown to facilitate ICSS at low doses (Poschel and Ninteman 1968) and to inhibit it at high doses (Bose et al 1974). The results with PCPA, the 5-HT synthesis inhibitor have also shown both facilatory and inhibitory effects (as discussed previously). No clear conclusion has been reached on the role of 5-HT in ICSS, but the duality of effects would seem to suggest both inhibitory and excitatory roles.

In a different approach, the injection of 5-HT into the lateral ventricles was shown to inhibit ICSS (Wise et al 1973). The distribution of ventricular injections of monoamines (Fuxe and Ungerstedt 1960) would suggest that cortical structures might be particularly affected by such a procedure. Interestingly alaproclate has a regional selectivity on 5-HT uptake, with a more pronounced activity on hypothalamic and hippocampal uptake, and much less activity in cerebral cortex (Astra; unpublished results). This selectivity would have been apparent at the 10 mg/kg dose, but not at 20 mg/kg.

The possibility that an action on cortical 5-HT systems caused inhibitory effects was an intriguing possibility. Of possible relevance to this speculation was the fact that zimelidine, a less selective 5-HT uptake inhibitor without this pattern of regional activity, has been found to inhibit learning in a conditioned avoidance task, whilst doses of alaproclate similar to those used here had no inhibitory effect (T. Archer, Astra - personal communication). It would be exceedingly interesting if fluoxetine or Lu 5.003 did not demonstrate such regional effects, especially with regard to their effect on cortical 5-HT uptake.

This experiment has suggested that the neuronal systems which mediated ICSS in MPH might have interacted with a facilatory 5-HT system at some level.

These two experiments taken together have not presented a definitive picture of the role of 5-HT systems in MPH. The effect of PCPA has indicated that 5-HT systems did not play a direct role in ICSS in this area. There was a possibility that an indirect interaction with a 5-HT system at some level was present but the evidence for this was equivocal. The use of longer test sessions in the PCPA experiment, and the effect of alaproclate at other sites supporting ICSS would have thrown some light on these problems, and might have reinforced these tentative conclusions.

General conclusion

This limited pharmacological investigation has indicated that ICSS in medial posterior hypothalamus was not mediated by the 5-HT systems in this area. The involvement of the CA systems was suggested, and as described in the introduction a variety of NA and DA neurones could have been involved. These included the ventral periventricular NA system, the A11 and A13 NA and DA systems and the ventral NA system. The very potent inhibition of this ICSS by spiroperidol has indicated the importance of the DA systems. It has been pointed out that an involvement of DA at the cortical level of reward mediation would not negate the results of section one which indicated that mesolimbic and nigrostriatal DA systems were not stimulated by MPH ICSS. The possibility that they were an essential part of a ICSS "loop" and trans-synaptically activated could be suggested. However, the effect of CA blockade and depletion on the performance of bar-press tasks renders such speculation unecessary. The present results could be explained on such a basis. A more critical experimental approach would be required to determine the exact role of CA systems in MPH self-stimulation.

Section 3

The effect of ICSS on the activity of tyrosine hydroxylase in the locus coeruleus.

Introduction

The enzyme tyrosine 3-hydroxylase (EC 1.14.16.2) has been shown to catalyse the conversion of L-tyrosine to 3, 4, -dihydroxy-L-phenylalanine, the first step in the conversion of L-tyrosine to noradrenaline (Nagatsu et al 1964). This synaptic step was found to be the rate limiting one for this pathway (Levitt et al 1965).

Early experiments had shown that stimulation of sympathetic nerves caused a large increase in the catecholamine content of the venous effluent from adrenergically innervated tissues, with little or no reduction in the catecholamine content of these tissues (Von Euler 1955), and hence it was suggested that increased catecholamine synthesis had occurred. Later it was shown that electrical stimulation of the sympathetic innervation to the guinea pig vas deferens in vitro caused an increased conversion of radiolabeled tyrosine to noradrenaline (Roth et al 1966). Furthermore, stimulation of the sympathetic nerve to the submaxillary glands in vivo was shown to increase the rate of conversion of radiolabelled tyrosine, but not radiolabelled Dopa, to noradrenaline (Sedvall & Kopin 1966). This indicated that the increase in noradrenaline synthesis due to nerve stimulation was effected at or before the tyrosine hydroxylation step. The exposure of the intact rat to cold or exercise also caused an increase in noradrenaline synthesis from labelled tyrosine in adrenergically innervated organs (Gordon et al 1966).

An increase in the activity of tyrosine hydroxylase was induced by similar treatments. Such an increase in central and peripheral adrenergic neurones was demonstrated after cold stress (Thoenen 1970). Long lasting increases in TOH activity in the superior cervical ganglion have been shown after stress (Hanbauer et al 1973; Zigmond & Mackay 1974). The drug reserpine also induced increased TOH activity in the SCG (Mueller et al 1969). Both reserpine and cold stress were shown to increase TOH activity in the brain stem, specifically in the area around locus coerule us, and with a similar time course to the changes seen in SCP (Zigmond, Schon & Iversen 1974).

The prolonged depolarisation of SCG cells in vitro has been found to increase TOH activity (Mackay & Iversen 1972). In addition, electrical stimulation of the SCG caused an increase in ganglionic TOH activity (Ben-Ari and Zigmond 1975). Electrical stimulation of the guinea pig vas deferens preparation in vitro also produced increased TOH activity (Morgenroth et al 1974).

It has been suggested that the increase in TOH activity might be an indication of increased neuronal activity (Thoenen 1972; Zigmond et al 1974) and hence a measure of long term changes in activity in adrenergic neurones. The prece ding experiments have suggested that increased noradrenergic synthetic activity in response to direct stimulation of the neurones and external stressors has been paralleled an increase in the activity of TOH in those neurones. It would seem that this activation of TOH was common to both peripheral and central adrenergic neurones (Thoenen 1970; Zigmond et al 1974).

There has been considerable evidence to suggest an increase in metabolism in central adrenergic neurones after electrical stimulation. An increased rate of disappearance of radiolabelled noradrenaline was seen after stimulation in the area ventralis tegmenti, without changes in tissue levels of NA (Stinus et al 1973). Electrical stimulation of the dorsal noradrenergic bundle has produced rises in the concentration of 4-hydroxy-3-methoxy phenylglycol (HMPG) the final metabolite of NA in brain (Mannarino et al 1963), in the terminal areas of this pathway both in unconscious (Walter & Eccleston 1972) and conscious (Anlezark et al 1975) rats. These experiments suggested an increased release of NA after electrical stimulation of central noradrenergic neurones.

There thus seemed to be reasonable evidence to suggest that electrical stimulation of noradrenergic neurones could produce increased TOH activity and increased release of NA. It has been shown that electrical self-stimulation produced from electrodes in the area of the cell bodies of origin of the dorsal noradrenergic system, locus coeruleus, caused an increase in the concentration of HMPG in cerebral cortex, which indicated increased release of NA (Anlezark et al 1975). This present study was initiated to discover whether electrical self-stimulation from the area of locus coeruleus, the cell bodies of origin of the dorsal noradrenergic system (Dahlstrom & Fuxe 1964), produced an increase in TOH activity in these cells. The in vivo electrical stimulation of TOH in central noradrenergic neurones had not previously been demonstrated.

It has been shown that stress can induce increased TOH activity both centrally (Zigmond et al 1974) and in the periphery (Thoenen 1970). In order to control for a general increase in TOH activity which could

be induced by any stress inherent in the behavioural procedures, TOH activity was measured in the superior cervical ganglion, as stress has been shown to increase TOH activity in this ganglion (Hanbauer et al 1973: Zigmund & Mackay 1974).

The experiment involved two groups of rats. All of the rats were to be implanted with electrodes aimed at locus coeruleus in the dorsal brain stem, but those which did not demonstrate self-stimulation behaviour would serve as a control group for the study. The control group would indicate whether chronic self-stimulation produced an increase in TOH activity over and above any increase which might be caused by the implantation procedure itself.

In summary, the experiment was conducted to determine whether the electrical self-stimulation behaviour produced with electrodes in the vicinity of locus coeruleus was associated with increased TOH activity in the noradrenergic neurones. If in fact this was the case then the experiment could also indicate that in vivo electrical stimulation of central noradrenergic neurones induced an increase in TOH activity.

Methods

1. Implantation of electrodes

The materials used and procedures followed were as in Section I. The electrodes were aimed at the dorsal brain stem, in the area of locus coeruleus. The lambdasuture was used as the stereotaxic reference point. The co-ordinates relative to lambdawere as follows:

Anterior-posterior (AP) - 1.7 mm

Lateral (L) - 1.1 mm

Vertical (V) - 7.3 mm (from skull surface)

2. Stimulation

A Campden brain stimulator (Campden 522) was used. The parameters used for the stimuli were 200 msec trains of 50 Hz sine wave, with the current being in the range of 20 - 50 μA.

3. Behavioural equipment and training procedures

All equipment was as described in Section I. A continuous reinforcement schedule was used.

After a one week post-operative recovery period the rats were placed in the test chamber and shaped for lever pressing as previously described. It was usual for multiple training sessions to be required before self-stimulation was initiated, and at least five individual sessions were undergone with each rat before it was rejected as being negative for ICSS.

Rats which produced ICSS behaviour were allowed to self-stimulate for 30 minute sessions on five consecutive days. At the end of the last session the rats were removed from the box and killed by stunning and decapitation.

4. Dissection procedure

The brains were removed, immersed in ice-cold saline, then dissected over ice. The dissection followed the procedure of Zigmond, Schon and Iversen (1974). The brain was placed dorsal side upwards, and the cerebellum removed by first transecting it, then cutting the cerebellar peduncles at the lateral border of the pons. An imaginary line can be drawn at the level of the cerebellar peduncles through three deep indentations on the dorsal surface of the pons. A coronal cut was made through the identations and a second coronal cut made 1 - 1.5 mm rostrally. The resulting coronal section was placed with its caudal surface up. A horizontal cut was made 1 mm from the dorsal surface of this pontine section. The portion between the two sulci limitantes was removed, and the remaining pieces of tissue were taken as "left and right locus coeruleus".

The pieces of tissue were then weighed and stored in liquid $N_2$ until they could be assayed.

5. Estimation of tyrosine hydroxyase activity

The activity was estimated using a radioenzymatic assay based on the method of Hendry & Iversen 1971.

Tissue preparation

The brain tissue samples were added to 100 µl of 5mM $PO_4$ buffer, pH 6 and homogenised. Two 10 µl aliquots were taken for the assay procedure. The ganglion samples were added to 30 µl of buffer, homogenised, and 10 µl aliquots taken.

Incubation Mix

This was made up in two parts:-

(A) 0.15 ml $^{3}H$ tyrosine (11.5 Ci/mmol)

+ 0.25 ml 0.005M Tris buffer pH 8.6

+ 0.15 ml alumina

This mixture was agitated then allowed to stand for 20 minutes to absorb out the impurities in the $^{3}H$ tyrosine.

(B) 1 mg pteridine co-factor ($DMPH_4$)

100 µl β-Mercaptoethanol

1.0 ml PO4/NSD 1055 buffer pH 6.0

350 µl of the supernatant from mixture A were added to 350 µl of mixture B just before using to produce the incubation mixture. This was kept on ice before usage.

Reaction

The 10 µl samples of tissue homogenate were added to 10 µl of the incubation mixture in Eppendorf tubes. These were then incubated at 37°C for 20 minutes, on an Eppendorf heating block.

The reaction was stopped by adding 0.25 ml of 0.4N perchloric acid containing 2 µg/ml cold dopa carrier.

The mixture was washed out into glass stoppered tubes by approximately 4 ml of neutralising solution containing 0.1M Tris pH 8.6, 0.2M EDTA and 0.3N NaOH in the ratio 2:1:1.

Separation and extraction

The mixture was separated using small glass columns packed with alumina. The alumina was freshly prepared by immersing it in K-P buffer 0.5M pH 7.4.

The mixture was decanted from the tube into the column and the

tube washed out with 0.005M Tris buffer, pH 8.6. The columns were then washed out with approximately 40 mls of the same solution.

Once this was completed, the Dopa was eluted with 3 mls 1N Acetic Acid. 1 ml of this was removed and added to 10 mls Triton scintillant. The scintillant mixture was 0.4% butyl PBD in toluence containing 2:1 (vol:vol) Triton X-100.

Blanks

Two aliquots of 10 µl 5mM $PO_4$ buffer.

Recovery

10 µl of $^{3H}$-DOPA solution was taken through the assay in duplicate from the incubation stage onwards. These were compared with 10 µl of $^{3H}$-DOPA counted directly. Recovery was typically around 65%.

Results

Tyrosine hydroxylase activity, as measured by the rate of conversion of tyrosine to dopa, was estimated in the locus coeruleus and superior cervical ganglion in two groups of rats. The first group, seven in all, had shown evidence of self-stimulation behaviour and been allowed to stimulate themselves throughout 30 minute sessions for five consecutive days. The mean rate of responding in the last three sessions was 574 $\pm$ 68.5 (S.E.M.) responses/30 minutes. The second group contained eight rats which had had electrodes implanted as in the first group, but had not demonstrated self-stimulation behaviour. These rats had not been placed in the behavioural equipment for at least one week prior to killing, and thus had no response rates.

TOH activity was measured individually in left and right locus coeruleus. The electrodes had been aimed at the left locus coeruleus, which was thus presumed to be the one which was stimulated. All

Section Three Table One

Tyrosine hydroxylase activity in rat locus coeruleus after chronic ICSS

| | Self-stimulated (n = 7) | Un-stimulated (n = 8) |
|---|---|---|
| Left locus coeruleus | 495.5 ± 52.3 | 344.0 ± 34.6 *(L.v.L.) p < .05 |
| Right locus coeruleus | 451.9 ± 49.1 n.s. (L.v.R.) | 344.1 ± 34.8 n.s. (R.v.R.) n.s. (L.v.R.) |

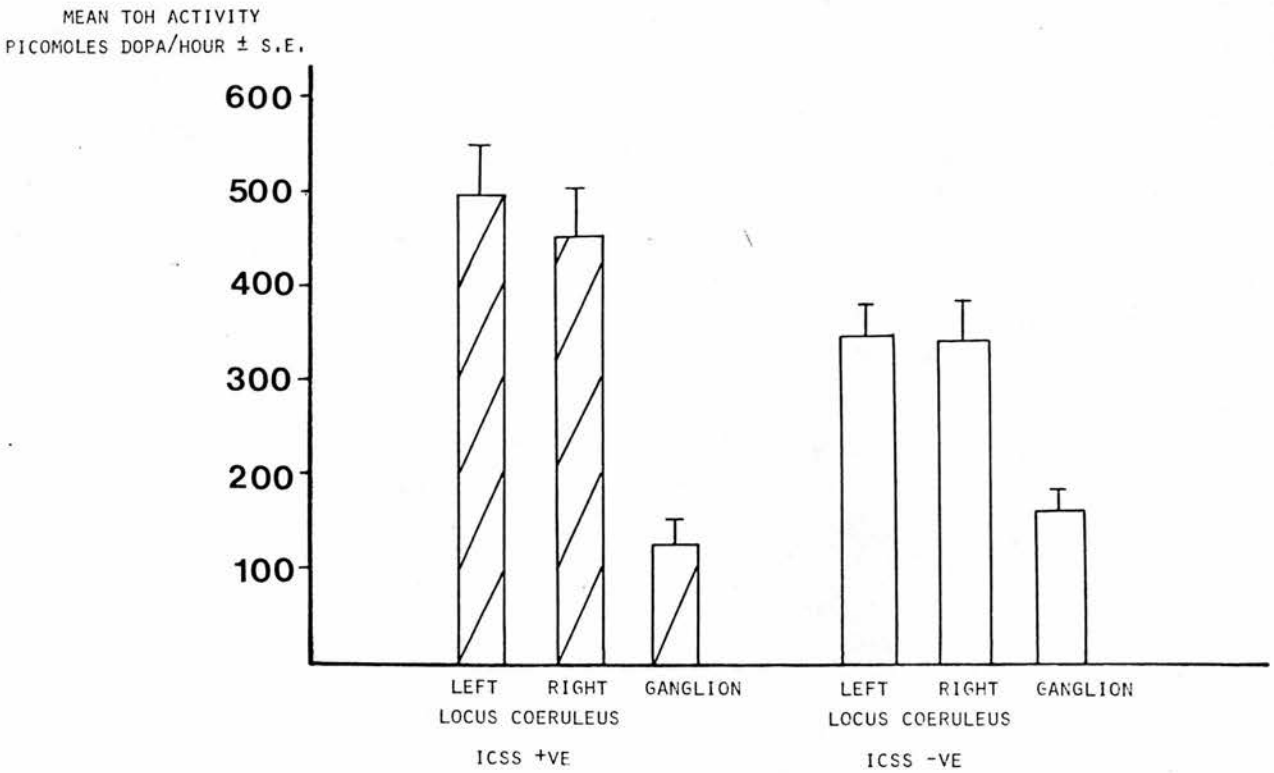
Electrodes were in the region of this nucleus. Mean TOH activity was expressed as picomoles DOPA/hour/locus coeruleus ± S.E.M. Statistical significance was assessed using the student t-test, the paired test for intra-group comparisons and unpaired for inter-group.

Section Three Table Two

Mean TOH Activity in locus coeruleus (left and right combined) after ICSS

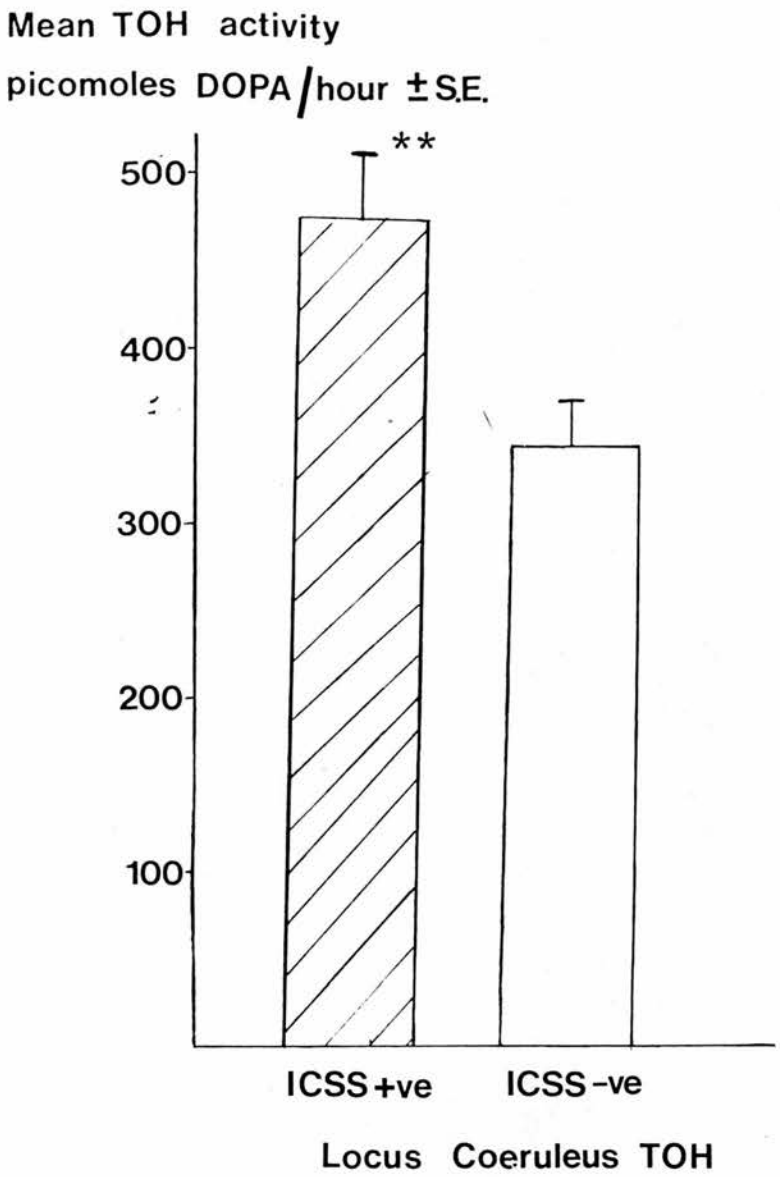
| | Self-stimulated (n = 14) | Unstimulated (n = 16) |
|---|---|---|
| Mean locus coeruleus TOH activity ± SE | 473.6 ± 35.0 | 344.1 ± 23.7 ** p < .005 |

TOH expressed as picomoles DOPA/hour/locus coeruleus ± SEM (significance was assessed using the student t-test, unpaired).




- Section Three Fig 1

Mean TOH activity (± S.E.) in locus coeruleus and in paired superior cervical ganglion after chronic self-stimulation in the area of locus coeruleus and in unstimulated controls. The activity in left locus coeruleus in the stimulated group was significantly higher than in the unstimulated group, $P < .05$, student t-test, two-tailed.




Section Three Fig 2

Mean TOH activity (± S.E.) in the locus coeruleus (both left and right) after chronic self-stimulation in the area of locus coeruleus, and in unstimulated controls. They were significantly different, $P < .005$, student t-test two tailed.

Section Three Table Three

Mean TOH activity in Superior Cervical Ganglion

after ICSS in the region of locus coeruleus

| Mean TOH activity picomoles DOPA/hour/pair ganglion ± S.E. | Self-stimulated ( n = 6) | Un-stimulated (n = 7) |
|---|---|---|
| | 125 ± 19.2 | 159 ± 23.5<br>n.s. |

Groups were compared with the student t-test, unpaired.

results are stated as mean TOH activity, expressed in picomoles Dopa formed/hour/locus coeruleus, ± S.E.M.

In the self-stimulated group, TOH activity in the left locus was 495 ± 52.3. The activity in the right locus was 451.9 ± 49.1. This difference was not statistically significant, using the paired-t-test. In the unstimulated group the TOH activity in the left locus was 344.0, and in the right locus 344.1. This difference was again not significant. However, the TOH activity in the stimulated (left) locus coeruleus in the self-stimulating group was significantly higher than in the left locus coeruleus of the unstimulated group ($P < .05$, student-t-test, unpaired two-tailed). The right locus on the stimulated group had a greater activity than its unstimulated comparator, but this difference just failed to achieve significance at the .05 level.(Fig 1)

The mean TOH activity of both left and right locus coeruleus in the self-stimulated group was 473.6 ± 35.0 and in the unstimulated group was 344.1 ± 23.7 . This difference was highly significant, $P < .005$, student-t-test, two-tailed. (Fig 2)

The mean TOH activity per pair of superior cervical ganglion was 125 ± 19.2 in the self-stimulated group, and 159 ± 23.5 in the unstimulated group. This difference was not significant. (Table 3)

There was no histological confirmation of the electrode positions due to the design of the experiment.

Discussion

The results of this experiment have shown that there was an increase in tyrosine hydroxylase (TOH) activity in locus coeruleus after self-administered electrical stimulation in vivo through electrodes aimed at the area of locus coeruleus in the dorsal brain stem (Table One: Fig One). This reaction of a central noradrenergic system was similar to that seen in the peripheral sympathetic nervous system after electrical stimulation, both in vitro in the guinea-pig vas deferens (Morgenroth et al 1974) and in vivo in the superior cervical ganglion (Ben-Ari & Zigmond 1975).

It has previously been shown that both reserpine and cold stress cause increased TOH activity in the central nervous system (Thoenen 1970) in particular the locus coeruleus (Zigmond et al 1974). This activation was also parallel to the effects of reserpine and cold stress on TOH in the peripheral sympathetic system (Mueller et al 1969: Thoenen 1970). There thus seems to be considerable similarity between peripheral and central TOH in their reaction to such external stimuli.

The in vivo activation of TOH in central NA neurones was subsequently demonstrated in anaesthetized rats with acute electrical stimulation (Roth et al 1975). The TOH activity in hippocampus was measured after electrical stimulation of locus coeruleus. The dorsal noradrenergic pathway has a unilateral projection to hippocampus (Ungerstedt 1971) and hence the experiment measured TOH in the nerve terminals of the dorsal noradrenergic system. After electrical stimulation for 15 minutes, an increase in TOH activity of 300% was seen when compared to TOH activity in unstimulated contralateral hippocampus. In the present experiment, the comparable increase comparing left locus in the stimulated animals with left locus in

unstimulated controls was only 44%. Apart from large procedural differences, especially the effect of anaesthesia and the use of terminal areas as opposed to cell bodies, there were other possible explanations for this difference.

It has been found that the degree of TOH activation in central adrenergic neurones was stimulus dependent in an in vitro model which used field stimulated hippocampal slices (Bustos et al 1978). In the anaesthetised preparation, approximately 18,000 pulses were passed whilst in a comparable period in the self-stimulating group an average of about 3,000 pulses would have been passed. This would have helped to explain the difference if this in vitro model was an accurate predictor of in vivo occurrences.

There was evidence of a higher level of TOH activity in the unstimulated (right) locus coeruleus in the +ve ICSS group, indeed the two sides were not significantly different. Furthermore, the mean total TOH activity (left and right combined) in locus coeruleus was much enhanced in the stimulated group compared to the unstimulated group. This difference was significant ($P < .005$). In a similar experiment to the present one, when changes in the metabolism of noradrenaline as measured by HMPG concentrations were investigated, a rise in HMPG was observed on both ipsi- and contralateral sides of the brain to the stimulating electrode, in conscious self-stimulating rats. In anaesthetised animals a unilateral rise in HMPG was found on the stimulated side, after stimulation comparable to that received by the self-stimulating animals. It was suggested that the rise in the contralateral side could be a reflection of motor activity or the operant behaviour (Anlezark et al 1975). Interestingly unilateral increases in TOH activity have been reported after electrical stimulation, but in anaesthetised animals (Roth et al 1975). It thus

appeared that the effects of electrical stimulation of noradrenergic neurones was to cause an increase in NA metabolism as measured both by HMPG and TOH changes. In the freely self-stimulating rat however, both these measures were increased in the contralateral NA system also. It has been suggested previously that operant behaviour was associated with changes in NA metabolism (Lewy & Seiden 1972: Olds & Yuwiler 1972). However the results of a recent experiment have indicated that operant behaviour itself does not alter TOH activity in the LC. The TOH activity was the same whether the animals performed a bar-press response for a food reward, was automatically fed in the test chamber by the responses of another rat, or was simply subjected to the pre-trial starvation period. Moreover none of these were significantly different to the completely untreated controls (Arbuthnott 1976). It would seem unlikely that the operant responding was in itself capable of raising TOH activity in a general manner, and hence the contralateral effect might indeed have been a result of ICSS of LC. It must be pointed out that although there was an increase in TOH activity in the contralateral locus, this was not statistically significant compared with the unstimulated controls, whereas the stimulated side was significantly increased.

The possibility of some interaction between the locus coeruleus in the conscious animal was suggested (Anlezark et al 1975). Anatomical evidence for a connection between the two nuclei has been reported. A projection to the contralateral nucleus was first demonstrated in the cat (using the horseradish peroxidase (HRP) retrograde tracing technique) (Sakai et al 1977). Also using the HRP method, with small amounts injected by iontophoresis, some contralateral cells were detected in the rat (Cederbaum & Aghajanian 1978). When a larger injection of HRP

was used a large number of contralateral locus coeruleus cells were labelled. This labelling was not seen after HRP injections into brain areas adjoining locus coeruleus (Clavier 1979).

As the noradrenergic cells of locus coeruleus have been shown to alter their rate of firing both after clonidine, an α-adrenergic agonist (Svensson et al 1975) and after iontophoretically applied NA itself (Cederbaum & Aghajanian 1976), the electrophysiological evidence also supported the possibility of a NA input to LC although the nature of that input was not determined and indeed Aghajanian suggested the possibility of recurrent collateral fibres. The more recent anatomy however might suggest a NA link. It could be suggested that indirect anatomical and electrophysiological evidence has supported the contention that activity in one LC might influence the activity in the contralateral LC.

The present experiment was only designed to demonstrate changes in the activity of TOH, and did not attempt to delineate the mechanism of such changes. For instance the pteridine cofactor was present in excess in the assays, and changes in affinity for it were not measured. Other experiments have suggested possible mechanisms however. After electrical stimulation of the locus coeruleus in vivo, and of hippocampal slices in vitro, the increased activity of TOH was shown to be attributable to changes in the kinetic properties of the enzyme. The affinity of the enzyme for the substrate tyrosine was increased, and the affinity for the end-product inhibitor was decreased (Roth et al 1975: Bustos et al 1978). A similar pattern was seen in the DA nigro-striatal system (Murrin et al 1976) and in the peripheral sympathetic system, after electrical stimulation of the guinea pig vas deferens (Morgenroth et al 1974). It seems possible that similar

mechanisms were responsible for the increased activity seen in the present experiment.

There was no rise in the TOH activity in the superior cervical ganglion (SCG) in the stimulated group compared with the control group. This suggested that the rise in TOH in locus after self-stimulation was not a generalised rise due to stressful factors inherent in the behavioural procedures. TOH activity has been shown to increase in the peripheral and central nervous systems after cold stress (Thoenen 1970: Zigmond et al 1974), and after foot shock (Weiss et al 1975). Neither did it appear likely that the implantation of electrodes was itself responsible for the rise in TOH activity as the control group had undergone the same procedure although it must be pointed out that they probably consisted of a different population of electrode locations.

Evidence from similar work on changes in TOH activity in DA systems has indicated that damaging the DA neurones caused an increase in DA levels (Walters et al 1973) and that such an increase could be associated with increased TOH activity (Walters & Roth 1976). However this effect was transient, with a maximum time course of 48 hours. Furthermore it was shown that the insertion of electrodes in itself did not increase DA synthesis, but that this was directly related to electrical stimulation (Murrin & Roth 1976). This would seem to indicate that in the present experiment the insertion of electrodes alone would not have resulted in an elevation of TOH activity during the test period as this was at least one week after implantation. It seemed reasonable to compare these results with the NA system as electrical stimulation of this DA system resulted in a comparable increase in TOH activity (Murrin et al 1976).

The results indicated that activation of the dorsal noradrenergic system was associated with the electrical self-stimulation supported by electrodes in the dorsal brain stem. This was in complete agreement with previous studies which showed increased formation of HMPG, and hence suggested increased activity in NA neurones, after electrical self-stimulation or enforced stimulation of the locus coeruleus (Anlezark et al 1975: Korf et al 1973). However, it was impossible to determine the location of the stimulating electrodes in the present study and hence no absolute link between self-stimulation, the locus coeruleus and TOH activity could be proved. Other studies have shown that there was a very close anatomical correlation between sites in the dorsal brain stem which supported ICSS and the NA cell bodies in the locus coeruleus (Crow et al 1972: Ritter & Stein 1973). Although it might be inferred that the electrodes in the present study were stimulating the dorsal noradrenergic system, there was no proof of direct stimulation.

In the sympathetic ganglion, the increase in TOH activity produced by reserpine was prevented by sectioning of the preganglionic cholinergic nerve (Thoenen et al 1969) or by nicotinic ganglionic receptor blockers (Mueller et al 1970). This suggested that the release of acetyl choline from the pre-ganglionic nerve could stimulate TOH activity in the ganglion. This observation has been extended to the central nervous system. The centrally active cholinergic agonist, oxytremorine has been shown to increase TOH activity in the locus coeruleus after systemic administration, and this increase was blocked by the centrally active cholinergic antagonist atropine (Lewander et al 1975). The area of the locus coeruleus contains large amounts of enzymes which are involved in the metabolism of acetylcholine (Shute & Lewis 1967). With the aid of histochemical techniques which

visualise acetylcholinesterase, an enzyme which degrades acetylcholine (Stedman et al 1932) it has been shown that large amounts of acetylcholinesterase could be found in cells of the locus coeruleus with a similar morphology to those which contained catecholamines (Pallkovits & Jacobowitz 1974). It has been shown that the acetylcholinesterase disappeared after 6-OHDA treatment and hence these cells were in fact the LC noradrenergic cells (Lewis & Schon 1975). Interestingly this situation was also described in the cells of the superior cervical ganglion (Koelle 1955). A hypothesis could be suggested whereby activation of a cholinergic input to the locus coeruleus by electrical stimulation might have resulted in an activation of TOH in that nucleus. Although this idea must be highly speculative, it could be a valid alternative interpretation of the present data, and might be taken to illustrate other similar explanations for the experimental data under discussion.

Although it would have been preferable to have had other experimental control groups included in the present experiment other work has indicated that these might not have been essential. The inclusion of a completely untreated group would have demonstrated that implantation of electrodes into this brain area did not cause the rise in TOH activity. However, as previously discussed when comparable work on the DA system was taken into consideration, this would have been unlikely (Walters et al 1973; Murrin & Roth 1976). The stimulation of anaesthetised animals through electrodes which had previously supported ICSS might have been useful. The observation of a unilateral rise in TOH activity after electrical stimulation of a NA pathway has been made elsewhere (Roth et al 1975). If this and the present experiment were considered together, the combined results had a marked resemblance to that seen when NA metabolite concentration was measured after electrical

stimulation and ICSS from the LC (Anlezark et al 1975).

The possibility that the performance of the operant response itself was responsible for the increased TOH activity would seem unlikely. Neither the experimental situation nor bar-pressing for a food reward has caused any change in TOH activity in the LC (Arbuthnott 1976).

In conclusion, an increase in tyrosine hydroxylase activity in locus coeruleus was observed after electrical self-stimulation with electrodes in the dorsal brain stem. This provided indirect evidence that ICSS in the dorsal brain stem was associated with increased activity in the dorsal noradrenergic system. It also implied that electrical stimulation of a central noradrenergic system in vivo could produce activation of TOH as has been reported for the peripheral sympathetic nervous system. However, the lack of anatomical verification of electrode positions has prevented such direct conclusions from being made. Further experimental evidence has indicated that this was the correct deduction as electrical stimulation of NA neurones in the CNS has been shown to increase TOH activity both in vivo (Roth et al 1975) and in vitro (Bustos et al 1978).

Section 4

The effect of selective lesions to the tegmental dopaminergic system on pontine electrical self-stimulation.

Introduction

Electrical self-stimulation behaviour was found to be elicited by electrodes in the dorsal tegmentum (Olds & Olds 1963) in an area later found to be traversed by the dorsal noradrenergic bundle (Ungerstedt 1971a). Further investigation revealed that ICSS could be sustained from electrodes in the pons, in or around the locus coeruleus the nucleus containing the cell bodies A6 of the dorsal noradrenergic system (Dahlstrom and Fuxe 1964). It was suggested that self-stimulation behaviour elicited from this region was qualitatively different from that produced in lateral hypothalamus and the ventral tegmental area. ICSS from electrodes in locus coeruleus was characterised by low rates of lever pressing, little excitation (Crow et al 1972). The behaviour associated with ventral tegmental ICSS was very different, with generally very high rates of lever pressing, and much excitation, with increased rearing, sniffing and licking. There was a very close anatomical relationship between sites supporting this type of ICSS behaviour and the dopaminergic systems of the ventral tegmental area and hence this type of self-stimulation behaviour was associated with activation of a dopaminergic system (Crow 1972a) However, high rates of self-stimulation have been observed with dorsal pontine electrodes, comparable to tegmental stimulation in all aspects (Ellman et al 1975).

The sites supporting such ICSS were predominantly in the anterior locus coeruleus, or medial to the brachium conjunctivum in the dorsal midbrain (Ellman et al 1975).

It seemed possible that activation of a dopaminergic system in the mesencephalon could explain the sites supporting ICSS in the dorsal pons. There appeared to be an anatomical connection between the substantia nigra and the dorsal pons. After horseradish peroxidase was injected into the substantia nigra pars compacta, it was transported retrogradely to cells in the dorsal pons, near the superior cerebellar peduncle (Tulloch, Ph.D. thesis). Horseradish peroxidase has been shown to be taken up by nerve terminals and transported retrogradely to the cell body (Kristensson et al 1971; La Vail and La Vail 1972). Pharmacological evidence also existed to suggest a stimulant effect of noradrenaline on dopamine systems. Low doses of clonidine (0.1 mg/kg), which decreased NA utilization, also decreased DA synthesis and release, and yohimbine which increased NA utilization, also increased DA synthesis and release (Anden and Grabowska 1976). It has been suggested that ICSS from LC was produced by some kind of learning which gave access to DA reward mechanisms, possibly involving synaptic plasticity (Milner 1977).

In order to investigate whether the dopaminergic systems of the mesencephalon, especially the substantia nigra pars compacta, provided an essential functional link in ICSS from the dorsal pons, it was decided to investigate the effect of lesioning these DA systems on ICSS from this area. It has been shown that 6-hydroxydopamine (6-OHDA) can produce lesions of the brain catecholamine systems when administered intracerebrally (Ungerstedt 1968) or intraventricularly (Uretsky & Iversen 1969). It had been shown that 6-OHDA produced a selective degeneration of the sympathetic noradrenergic nerve terminals in the peripheral nervous system when given systemically (Tranzer and Thoenen 1968). Investigation of the biochemical consequences of brain administration of 6-OHDA showed that catecholamines were selectively

depleted, with little or no effect on indoleamine levels (Bloom et al 1969; Uretsky & Iversen 1970). The selective destruction of catecholaminergic neurones has been ascribed to the active uptake of 6-OHDA into such neurones, as drugs which inhibit the uptake of noradrenaline prevent the destruction of noradrenergic neurones (Breese & Traylor 1971). The formation of oxidative products of 6-OHDA that can undergo covalent binding with nucleophilic groups of some important constituents of the neuronal membrane might produce the neurotoxic action (Saner & Thoenen 1971). It was possible to cause increased destruction of dopaminergic neurones by giving a monoamine oxidase inhibitor prior to 6-OHDA (Breese & Traylor 1971) as 6-OHDA could be metabolized by this enzyme also (Jonsson et al 1972). A selective destruction of dopaminergic neurones was produced by giving a highly specific inhibitor of noradrenaline uptake, desimipramine (Lidbrink et al 1971) which prevents 6-OHDA induced destruction of noradrenergic neurones (Breese & Traylor 1971) prior to 6-OHDA treatment. The use of 6-OHDA combined with these pharmacological treatments seemed to provide a suitable method for making selective lesions to the dopaminergic systems in the mesencephalon without destroying noradrenergic systems.

It was decided to lesion the nigro-striatal DA system on the ipsilateral and contralateral side of the brain (relative to the stimulating electrode) in different groups of animals. By this experimental design, any effects of a dopaminergic lesion which were not specific to the neural system supporting self-stimulation might become apparent. It was to be supposed that if non-specific effects of a unilateral nigro-striatal DA lesion produced deficits in ICSS, then such a deficit would be equally severe in both an ipsilateral and a contralateral lesion. If however this DA system was an essential link

in the ICSS produced from the dorsal pons then only one or other of the lesions would produce a deficit, depending upon whether this putative pathway projected in a unilateral manner or whether it crossed over. The HRP studies indicated a unilateral link to the substantia nigra from the dorsal pons (Tulloch Ph.D thesis) and hence the ipsilateral lesion would be the one expected to produce a deficit in ICSS if this was a functional link.

The extent of the lesion to the DA systems was verified in two ways. It has been shown that rats with lesions to the nigro-striatal DA system produced asymmetric body postures and pronounced rotational behaviour when given drugs which release dopamine (Anden et al 1966a ). With 6-OHDA lesions to the nigro-striatal DA system, similar results were obtained, and pronounced rotational behaviour in a direction contralateral to the side of the lesion was produced by the dopamine agonist apomorphine (Ungerstedt 1971[b]). Hence rats which had had their nigro-striatal DA system lesioned could be identified by the ability of apomorphine to induce rotational behaviour. Further verification of these lesions was provided by biochemical analyses of the levels of DA and NA in various brain areas after the behavioural testing was completed. The biochemical analysis also showed the extent of damage to the different DA systems, and any unintentional damage to the NAergic systems. DA and NA were estimated by a modification of the radioenzymatic method (Coyle and Hendry 1973).

In order to minimise non-specific mechanical destruction of tissue by the injection cannula, an indwelling guide cannula was inserted along with the stimulating electrode. It was supposed that if a pathway necessary for ICSS was disrupted by this procedure then no ICSS would be seen with that animal.

It was considered, therefore, that the combination of an indwelling cannula, ipsi-and contralateral lesions, and the pharmacological protection of noradrenergic systems would provide reasonable evidence as to whether the mesencephalic DA systems were neural substrates for pontine self-stimulation, or if they were involved in some non-specific way.

Methods

(1) Self-stimulation procedures

Male Wistar rats, 180 - 200 gm in weight, were anaesthetised with halothane, and bipolar electrodes were implanted in the dorsal pons, using the methods described in section (1). The stereotaxic co-ordinates used were 1.4 mm posterior to the lambda suture, 1.0 mm lateral to the midline, and 6.4 mm below the skull surface at this point. During the same operation an indwelling cannula was implanted in posterior hypothalamus using the co-ordinates 4.1 mm posterior to the bregma suture, ± 1.1 mm lateral to the midline, and 8.3 mm below the skull surface at this point. The cannula was constructed from a disposable hypodermic needle, 26G and 10 mm in length (Gillete Surgical, Eng.), with a stainless steel stylet inserted to keep the cannula patent. All rats were allowed at least seven days to recover from surgery.

All animals were tested for ICSS as described in section (1), except for the stimulus parameters. These were 200 msec trains of monophasic square wave pulses, each 0.2 msec duration at 50 Hz. They were produced by a Neurolog stimulator, using an NL800 isolated constant current output stage (Digitimer Ltd.). The animals were tested at 50 μA, and the current was increased until interest was shown (i.e. the animal repeatedly returned to the place where stimulation had been given) or until an aversive response was elicited. Current levels used to produce reliable ICSS were typically 100 - 200 μA and routinely monitored on an oscilloscope, using the voltage drop across a 1 Kohm resistor. Rats were shaped to the lever until spontaneous ICSS was seen.

The rats were allowed to self-stimulate for 15 minutes each day for 5 days, by which time their response rates were stable. The current

used for each rat was adjusted so that it produced sub-maximal response rates. The response rates were recorded at the end of each 15 minute session.

Once the response rate was stable, the animals were subjected to the lesioning procedure, and typically allowed two days to recover from this. Thereafter, all rats were placed in the test chamber for 15 minutes a day, until 21-24 days post-lesioning when the experiment was terminated. Currents to the animal were maintained at pre-lesion levels and no retraining was attempted.

(2) Lesion procedures

All lesions to the nigro-striatal dopaminergic system were made through indwelling cannulae, which were placed either ipsi or contra-lateral to the stimulating electrode. After the training and stabilisation of response rates was completed, the rats were treated with 50 mg/kg pargyline and 25 mg/kg desmethylimipramine i.p. 30 minutes prior to being anaesthetized with 4% halothane, and anaesthesia maintained with appropriate levels of this agent. The stainless steel sylet was removed, and a 32G stainless steel injection cannula attached to a 10 µl glass syringe (Hamilton) was lowered down the indwelling cannula. The injection cannula projected approximately 1 mm below the outer cannula. 8 µg of 6-OHDA hydrobromide (AB Biotec, Sweden) dissolved in 4 µl of chilled isotonic saline containing 0.25 mg/ml ascorbic acid as an anti-oxidant was injected at a rate of 1 µl/minute. The cannula was left in position for two minutes after this, then slowly withdrawn. A plug of bonewax (Ethicon) was inserted into the top of the indwelling cannula. The animals were allowed to recover from the anaesthetic and returned to a clean cage.

The extent of the lesion in each animal was verified after the ICSS testing was completed. The rats were given apomorphine, 0.3 mg/kg intra-peritoneally and observed for rotation in a semi-spherical bowl. If a criteria of 150 turns contralateral to the side of the lesion was observed in 30 minutes, then that rat was assumed to have a substantial lesion of the nigro-striatal system (Ungerstedt 1971), and was included in the subsequent biochemical analysis. Those which failed to satisfy this criteria were retested with the same dose of apomorphine after an interval of three days. Rats which did not produce such rotation were deemed to have an unsuccessful lesion and were not included in further analysis. Eventually there were two groups of 8 rats with successful lesions, ipsi - and contralateral to the stimulating electrode respectively.

(3) Biochemical analysis

(a) Dissection procedure

All the rats which had satisfied the rotation criteria were sacrificed 30 days post-lesion by stunning and decapitation. The brains were removed, and placed in ice-cold isotonic saline. They were then dissected over ice. The brain was placed ventral side upward, and the olfactory tubercles pinched off with curved forceps. A coronal cut was made 2 mm anterior to the optic chiasma, followed by a similar cut through the optic chiasma. The resulting tissue slice was placed horizontally, and a vertical cut at $90^{o}$ to the previous cuts made through the anterior commissure. The ventral part of this slice was removed, and tissue lateral to the lateral olfactory tract was cut away. The remaining tissue comprised of left and right nucleus accumbens. The dorsal part of this slice included the striata, which were picked-out with curved forceps, as was the striatal

tissue caudal to the optic chiasma in the remainder of the brain. The cortices caudal to the level of the optic chiasma were removed. A coronal cut was made at the level of the mamillary bodies, and this slice was then cut horizontally through the anterior commissure. The cuboid thus produced was the hypothalamus. Immediately after dissection the tissue was frozen in aluminium foil on solid $CO_2$ before weighing and storage in liquid $N_2$. The remainder of the brain was snap frozen to be cut into thin sections in a cryostat for histological evidence of the electrode placement.

(b) Estimation of noradrenaline and dopamine in the same tissue sample by a radioenzymatic method modified from Coyle J.T. & Henry D. J. Neurochem 1973 Vol 21 pp 61 - 67.

The tissue was homogenised in 300 µl of 0.1N perchloric acid, then centrifuged at 10,000 G for 15 minutes. The standards contained 25 ng of DA or NA added to 300 µl of brain extract. After centrifugation, the supernatant was transferred to 15 ml glass-stoppered tubes and 100 µl of a reaction mixture containing:-

500 µg dithiothreitol
0.5 µmol $MgCl_2$
140 µmol of Tris buffer pH 9.6
25 µl COMT (catechol-O-methyl-transferase)
25 µl Tritium S-Adenosyl-L-(Methyl-$^3$H) Methionine (2.5 µCi)

This was incubated for 60 minutes at 37$^{o}$C, then the reaction was stopped by the addition of 500 µl of 0.5M Borate buffer pH 10. 50 µl of nonradioactive carrier was now added. This contained:-

7 µg Methoxytryramine
3 µg Normetanephrine
3 µg Metanephrine
1 mg EDTA (ethyl enediamine tetra-acetic acid)

The O-methylated products were extracted into 9 mls of water saturated ethyl acetate - methanol (10:1) by shaking for 30 seconds, and low

speed centrifugation. 8.5 mls of the organic phase was transferred into a tube containing 0.5 mls of 0.5M borate buffer pH 10. 8 mls of the organic phase was transferred to a glass tube containing 0.5 mls of 0.1N HCl, then shaken for 30 seconds and centrifuged at low speed. The organic phase was aspirated off, and 8 mls water saturated ethyl acetate added. The tube was shaken for 30 seconds, centrifuged, and the organic phase aspirated off. This was transferred to an ice bath, and 0.5 mls of 0.5M $PO_4$ buffer pH 7.5 added to each tube. In order to separate DA from NA, 50 µl of freshly prepared 3% Sodium Metaperiodate was added, and allowed to react for 3 minutes, until stopped by the addition of 50 µl 10% glycerol. 10 mls of toluene was added, the tubes shaken for 30 seconds and centrifuged as before.

For NA estimation 9 mls of the toluence phase were removed and 1 ml 1N NaOH added. The aqueous phase was kept for DA estimation. The tubes were shaken and centrifuged. The organic phase was removed by aspiration, and 0.1 ml glacial acetic acid was added to the NaOH phase. 10 mls toluene was added, the tubes then shaken and centrifuged. 9 mls of the toluene phase were removed and added to 0.4 mls Liquifluor for liquid scintillation counting. For the estimation of DA the aqueous phase (see above) was added to 5 mls toluene, then the tubes were shaken and centrifuged as before. The organic phase was aspirated off, and to the aqueous phase was added 0.5 mls 1M borate buffer and 6 mls of toluene - isoamyl alcohol mixture (3:2). The tubes were shaken and centrifuged, then 5 mls of the organic phase was added to 10 mls of liquid scintillant, for liquid scintillation counting.

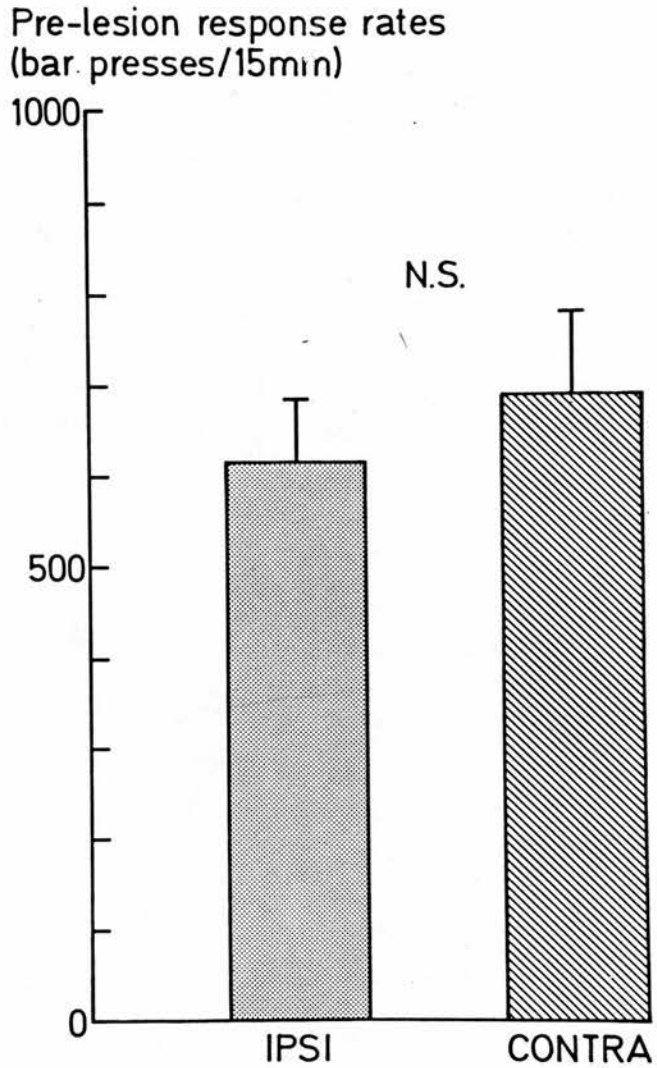
(c) Counting and estimation details

Samples were counted for 10 minutes on a Nuclear-Chicago, liquid scintillation system. C.P.M. (counts per minute) were converted to

D.P.M. (disintegrations per minute) using a channels ratio quench curve for the machine. Actual concentrations were calculated from the NA and DA standards in the assay. (ng/g wet weight tissue).

4. Histological procedures

The ventral mesencephalon and brain stem which remained after the dissection of the brain for biochemical analysis were stained using a modified Kluver Barrera method (see Section One, methods, this thesis), and examined in a light microscope. The location of the electrode tip was verified and the location determined by comparison with the atlas of Pellegrino & Cushman (1967). By examining the sections of the mesencephalon it was possible to look for the unilateral disappearance of cell bodies in the pars compacta of the substantia nigra.




Section Four Fig 1

Mean ICSS response rates before the 6-OHDA lesion. The two groups were not different significantly.

Section Four Table One (a)

The effect of a unilateral 6-OHDA lesion of the ascending DA systems on ICSS from the dorsal pons

| Days post-lesion | Mean difference from control, response rate/15 mins Ipsilateral group n = 8 | | Contralateral group n = 8 | |
|---|---|---|---|---|
| | Responses ± SE | % Control | Responses ± SE | % Control |
| 3 | -156 ± 73 n.s. | -25 | -248 ± 95 n.s. | -36 |
| 4 | -197 ± 83 n.s. | -32 | -306 ± 88 * | -44 |
| 6/7 | -111 ± 66 n.s. | -18 | -118 ± 92 n.s. | -17 |
| 13/14 | 20 ± 39 n.s. | 3 | - 41 ± 50 n.s. | - 6 |

* $p < .05$ Paired Student t-test

Table One (b)

| | | | | |
|---|---|---|---|---|
| Maximum recovery up to 21 days post-lesion | 83 ± 19 | +14 n.s. | 77 ± 36 | +11 n.s. |

Significantly different from pre-lesion control, paired student t-test

Section Four Table Two

The mean number of days to recovery of pre-lesion response rates (to within 15% of pre-lesion control rate)

| Mean number of days to recovery ± S.E.M. | Ipsilateral n = 8 | Contralateral n = 8 | |
|---|---|---|---|
| | 5.9 ± 1.2 | 5.6 ± 0.3 | n.s. |

Student t-test.

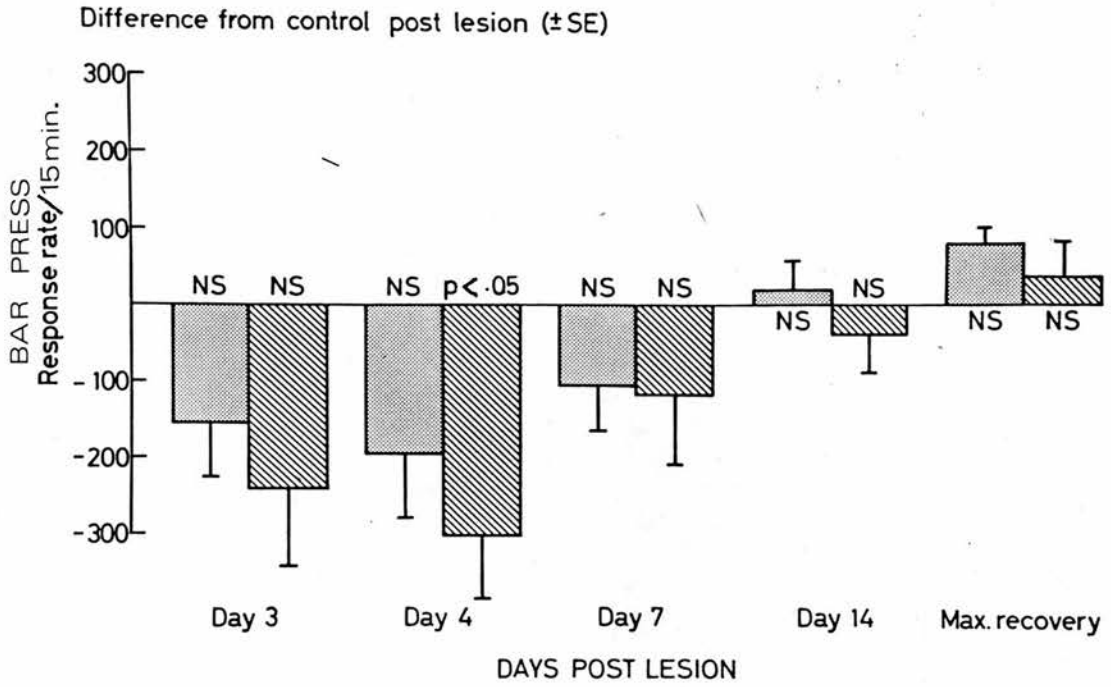
## Results

### Pre-lesion response rates

The baseline response rate of each rat was defined as the mean of the rates on the two days prior to lesioning. The group of 8 rats which had ipsilateral lesions, designated Ipsi, had an average response rate of 617 $\pm$ 66 (Standard Error S.E.) for each 15 minute test session. The groups of 8 rats which had contralateral lesions, designated Contra, had a response rate of 691 $\pm$ 91. These response rates did not differ significantly (Fig One).

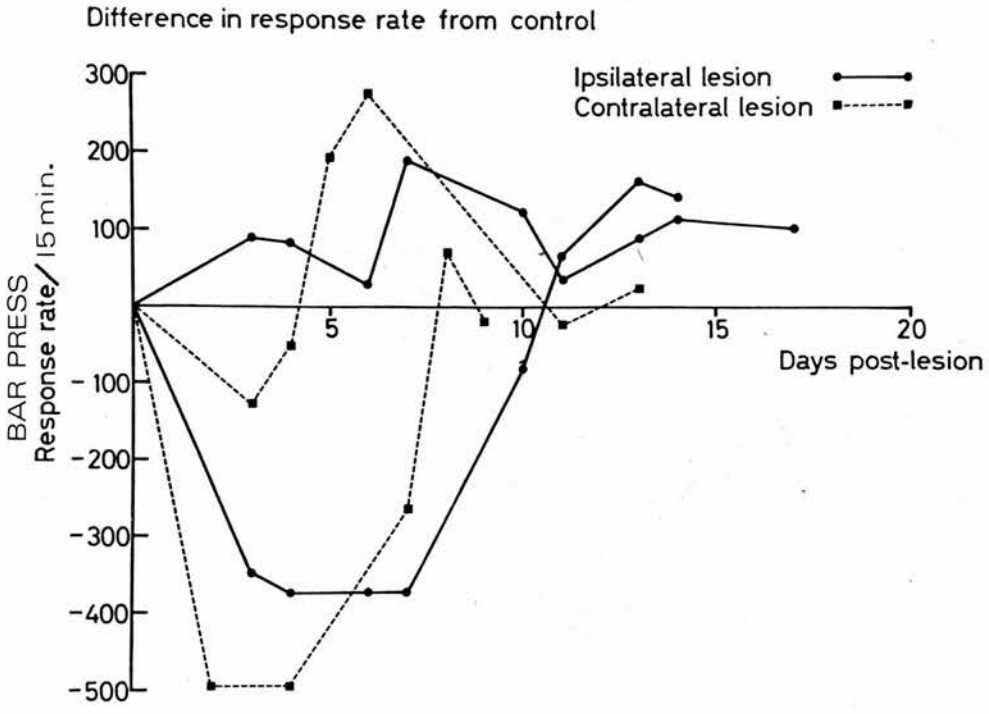
### Post lesion response rates

The effect of lesions to the dopaminergic systems in the ventral mesencephalon was to produce a transient decrement in lever pressing rates for ICSS from the dorsal pons (Table One (a), Figure Two). The decrement was of the order of 30 - 40% of pre-lesion control rates, and only significant on day 4 in the contralateral lesion group (student paired t-test). A complete recovery to pre-lesion levels was seen by 14 days post-lesion, with a considerable recovery to above 80% of control values by day 7 (Table One). There was no significant difference between the Ipsi and the Contra groups at any time. Although there was considerable variation within lesion groups in the time taken to full recovery, this variation was similar in both groups. Animals with considerable deficits in responding immediately after a lesion was made recovered with a similar time course in both groups (Fig 3). The maximum rate which animals recovered to within the 21 day post-lesion experimental period was highly comparable in both Ipsi and Contra groups, and in both cases was at least to pre-lesion response rates (Table One (b)). The mean number of days to recovery



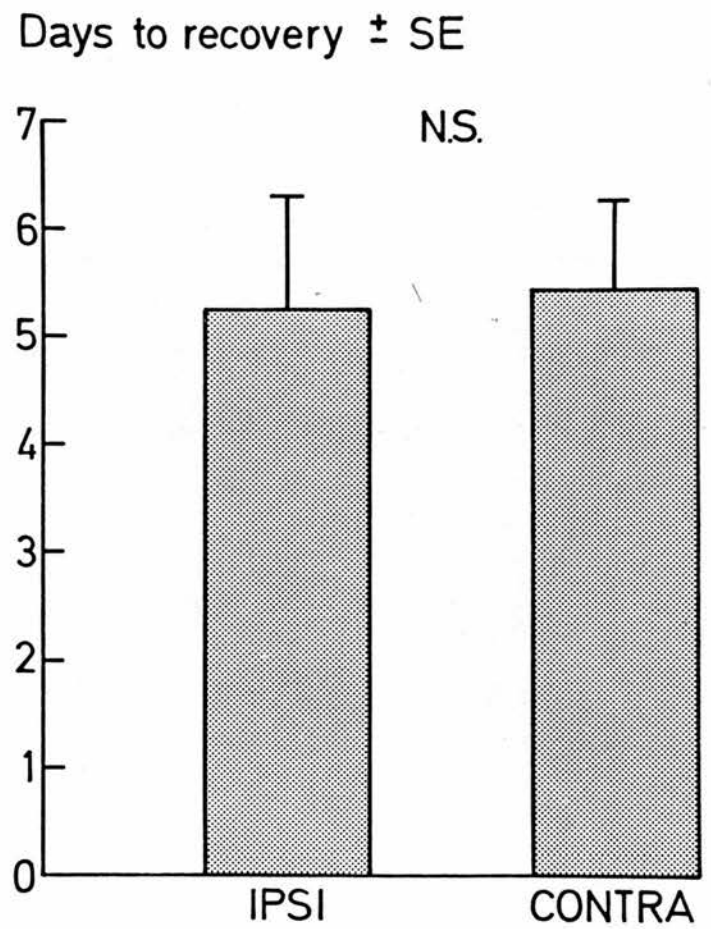

Section Four Fig 2

The mean difference in response rates from pre-lesion controls after the 6-OHDA lesion. The ipsilateral lesion group (dots) were never statistically different either from pre-lesion control or from the contralateral lesion group (hatched lines). The contralateral lesion group was statistically different from its pre-lesion control only on day 4 ($P < .05$, student paired t-test).




Section Four Fig 3

The differences in response rates from pre-lesion control for individual animals. Both after successful ipsi - and contra-lateral lesions, which severely depleted forebrain DA, deficits at 4 days post-lesion ranged from severe to virtually non-existant. All animals however had recovered by 10 days post-lesion.




Section Four Fig 4

Mean number of days to recovery in the ipsi - and contralateral lesion groups. Recovery was defined as recovering to within 15% of pre-lesion response rates. There was no difference between the two groups.

of response rates to within 15% of pre-lesion rates was 5.9 ± 1.2 days for the Ipsi groups and 5.6 ± 0.8 for the Contra group. There was no significant difference between groups (Student-t). (Table Two Fig 4).

In summary, no long lasting deficit in ICSS response rates was seen after the lesioning procedure. A temporary deficit was induced in some cases with variations between animals in the extent and duration of this deficit. However, these effects were common to both Ipsi and Contra groups, and the effect of the lesion was not significantly different between groups.

Biochemical results

The concentrations of DA and NA in the brain areas assayed were as shown in Table three. The results were expressed in absolute amounts of transmitter in ng/gm of wet weight of tissue. The lesion produced very marked depletions of DA in the corpus striatum ipsilateral to the 6-OHDA injection in both the Ipsi and Contra groups, of the order of 86 and 95% depletions respectively (% of lesioned to unlesioned side). A similar pattern of depletion of DA was seen in the nucleus accumbens in both Ipsi and Contra groups, with depletions of 77 and 91% respectively. These differences were statistically significant, $P < .001$, student t-test. Cortical dopamine was also reduced on the lesioned side, $P < .001$ in the Ipsi group and $P < .05$ in the Contra group. The concentration of dopamine in hypothalamus was the same in both Ipsi and Contra groups.

The concentrations of noradrenaline was markedly reduced in the corpus striatum in both Ipsi and Contra groups (Ipsi $P < .01$, Contra $P < .001$) to 37 and 16% respectively.

Section Four Table Three

The effect of a unilateral 6-OHDA lesion to the ascending DA systems on the concentration of noradrenaline and dopamine in various brain areas

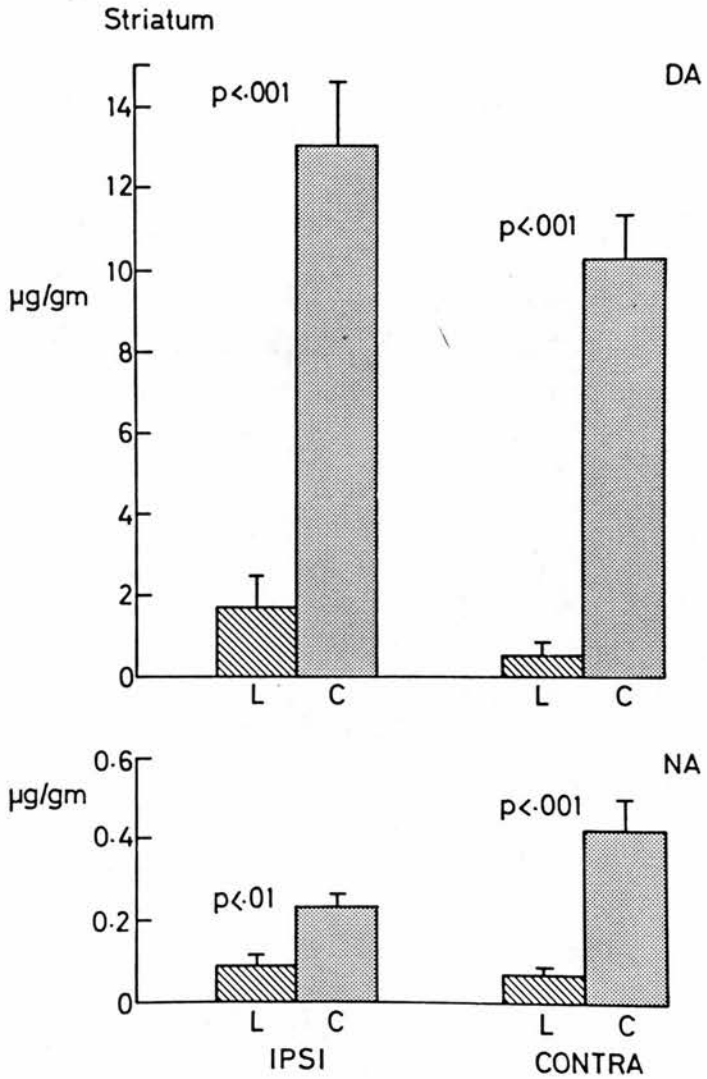
| Dopamine | Corpus Striatum Left | Corpus Striatum Right | Nucleus Accumbens Left | Nucleus Accumbens Right | Parietal cortex Left | Parietal cortex Right | Hypothalamus |
|---|---|---|---|---|---|---|---|
| Ipsilateral lesion n = 7 | 1756 *** ± 687 | 13012 ± 1557 | 1723 *** ± 460 | 7441 ± 703 | 39 *** ± 6 | 125 ± 14 | 970 ± 127 |
| Contralateral lesion n = 7 | 10227 ± 1033 | 521 *** ±300 | 6945 ± 1105 | 624 *** ± 103 | 141 ± 37 | 16 * ± 6 | 1013 ± 126 |
| **Noradrenaline** | | | | | | | |
| Ipsilateral lesion n = 7 | 85 ** ± 25 | 227 ± 32 | 1911 n.s. ± 222 | 1984 ± 309 | 297 n.s. ±38 | 367 ± 55 | 6115 ± 923 |
| Contralateral lesion n = 7 | 419 ± 78 | 64 *** ±18 | 2242 ±414 | 3048 ± 790 | 434 ±64 | 291 n.s. ±29 | 6416 ± 753 |

Results expressed as mean concentration in nanograms/gram ± S.E.M.

Significantly different from unlesioned side, student t-test

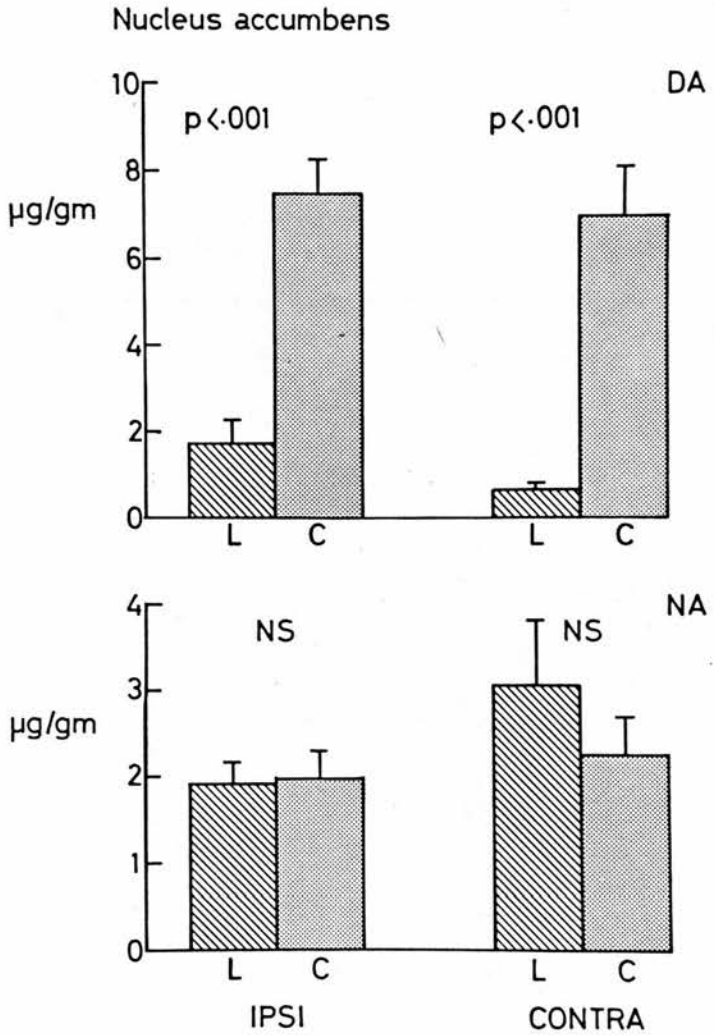
Electrode on left side. Lesion relative to this.

*** P < .001
** P < .01
* P < .05



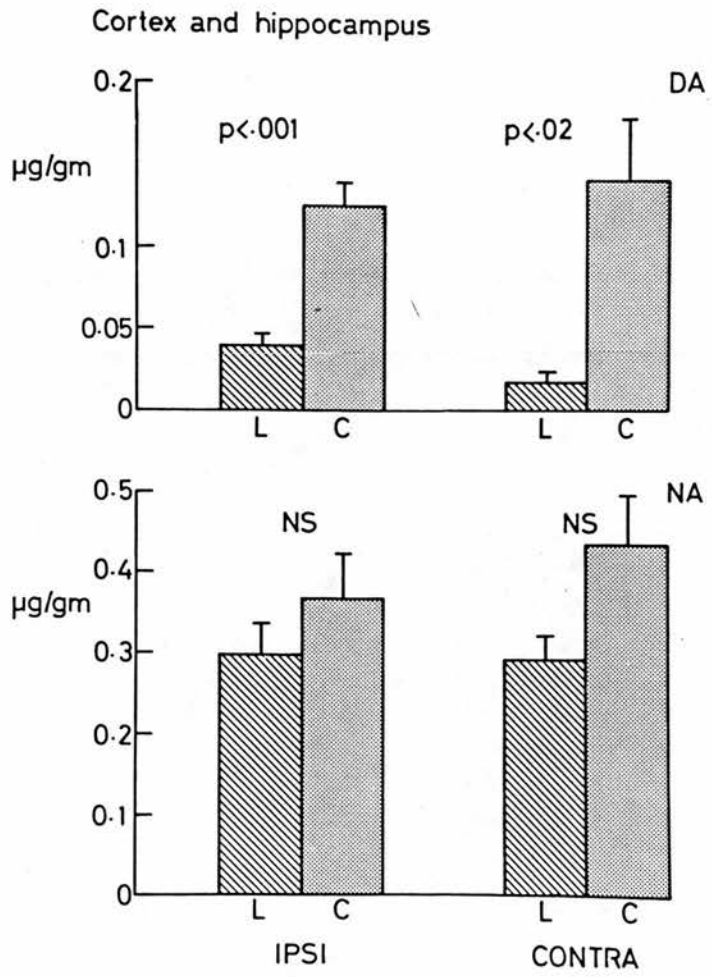

Section Four Fig 5

The mean (+ S.E.M.) concentrations of NA and DA in the striatum after a unilateral 6-OHDA lesion of the ascending DA pathways. The lesioned side (L) was compared to the unlesioned control side (c) of the same animals. Lesions were made in posterior hypothalamus either Ipsi or Contra lateral to the stimulating electrode.



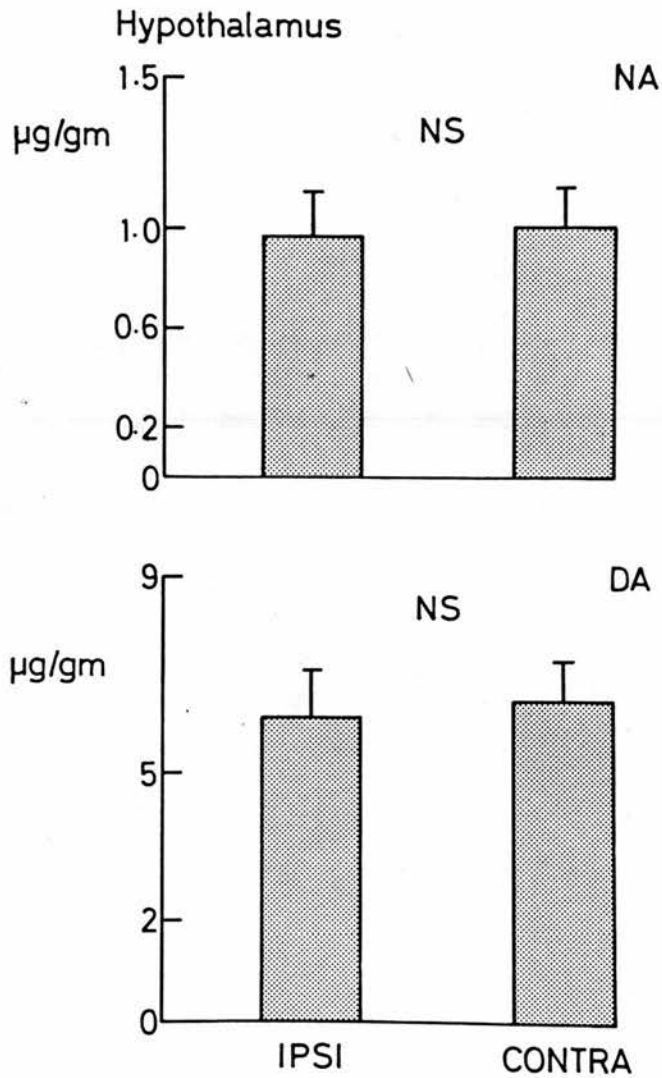

Section Four Fig 6

This shows the mean concentrations of NA and DA in the nucleus accumbens after the unilateral 6-OHDA lesion; the lesioned side (L) was compared to the unlesioned control side (C) of the same animals. Lesions were made in posterior hypothalamus either Ipsi or Contra-lateral to the stimulating electrode.




Section Four Fig 7

This shows the mean concentrations of NA and DA in cortex and hippocampus together, after the unilateral 6-OHDA lesion; the lesioned side (L) was compared to the unlesioned control side (C) of the same animals. Lesions were made in posterior hypothalamus either Ipsi or Contra-lateral to the stimulating electrode.




Section Four Fig 8

This shows the mean concentrations of NA and DA in hypothalamus after the unilateral 6-OHDA lesion; the lesioned side (L) was compared to the unlesioned control side (C) of the same animals. Lesions were made in posterior hypothalamus either Ipsi - or Contra-lateral to the stimulating electrode.

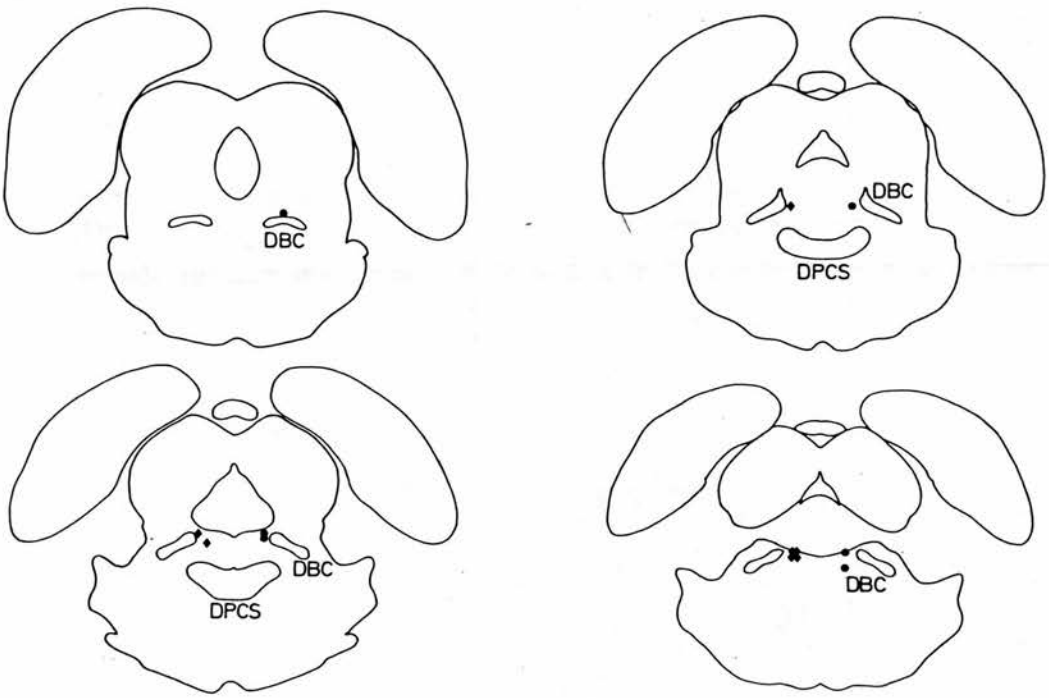
In no other brain areas studied was there any significant difference between lesioned and unlesioned sides in either of the treatment groups. Although there were slight reductions in the concentrations of NA in parietal cortex (19% in the Ipsi group, 33% in the Contra) these were not significant. The concentration of NA was the same in the hypothalamus in both Ipsi and Contra groups. The injection of 6-OHDA into the ventral tegmental area did not produce a general depletion of noradrenaline throughout the forebrain and cortical areas.

Correlation of biochemical assay results with behavioural effects of the lesion

In order to assess the effect of lowering DA concentration in the corpus striatum on the response rate for ICSS, the correlation between the concentration of DA which remained in corpus striatum after the lesion and the difference from control response rates on day 3/4 was calculated. The correlation coefficient between these two measures was 0.46 in the Ipsi group and 0.18 in the Contra group. This indicates that these two measures were not highly correlated.

Electrode placements

The electrodes were found to lie in the dorsal pontine area, ventral to the 4th ventricle. A number were in or close to the anterior locus coeruleus, and the rest were located in or near the projection of the dorsal tegmental bundle as defined by Lindvall & Bjorklund (1974). These were medial to the brachium conjunctivum, dorsal of the superior cerebellar peduncle. They were mostly on the margins of the periventricular gray matter. One electrode was at the level




Section Four Fig 9

This shows the electrode positions in the pons. All electrodes were situated on the same side of the brain but for ease of illustration the ipsilateral lesion group (diamonds) are shown on the opposite side from the contralateral lesion group (filled circles). (DBC - dorsal brachium conjunctivum: DPCS - decussations of the superior cerebellar peduncle).

of the dorsal raphe nucleus, on the dorsal edge of the brachium conjunctivum, in or near to the dorsal tegmental bundle as it has been represented at this brain level(Fig 9).

DISCUSSION

The results of this experiment indicated that unilateral injections of 6-OHDA into the dopaminergic systems in the ventral tegmentum, which produced marked destruction of these systems, had no lasting effect on ICSS from electrodes in the dorsal pons. This was the case whether the 6-OHDA injection was ipsi - or contra - lateral to the stimulating electrode.

There were transient deficits in response rates after the lesion, but these were seen in both Ipsi and Contra groups. Some animals had severe deficits, and had recovery periods of 10 days, while others had little or no deficit after 3 days. However, this variation was common to both treatment groups. The time course of recovery was also similar to both treatment groups, with full recovery by 14 days post-lesion, and recovery to within 15% of control values by a mean time of approximately 6 days in both groups. Response rates post-lesion recovered to a maximum only slightly above pre-lesion control values (+14% in Ipsi group, +11% in Contra) but this was not significantly different. There was no evidence for any facilitation of ICSS by this lesion.

There remains the fact that large initial deficits in responding were produced by the 6-OHDA injection in some animals. Unilateral lesions of the nigro-striatal pathway have been reported to produce impairment in the contralateral limbs (Marshall et al 1974) and to produce deficits in sensorimotor integration (Frigesyi et al 1971, Marshall et al 1974). A lever-pressing task would be susceptible to disruption with such impairments. Further evidence for the possibility that destruction of nigro-striatal DA system produced motor system

impairment came from an experiment which tested swimming ability. It was found that there was a high correlation between the number of DA cells remaining, DA concentrations in striatum and motor performance (Ranje & Ungerstedt 1977). As some animals did not suffer large deficits in response rates after a successful DA lesion the possibility of non-specific damage to some other pathway might be the cause of these deficits. An efferent pathway from the substantia nigra pons reticulata projects rostro-dorsally through the injection area and runs lateral to the mamillo-thalamic tract into the thalamus (Beckstead et al 1979; Faull and Carman 1968). It has been suggested that this pathway modulates posture (Olianas et al 1978) or acts as part of the motor output of the extra-pyramidal system (Arbuthnott et al 1977). It would seem reasonable to suggest that damage to such a pathway could result in motor deficits. Electrolytic lesions of the mamillothalamic tract produced deficits in ICSS from the posterior lateral hypothalamus (Boyd and Gardner 1967). This study did not differentiate between impaired performance and impaired reward function. It would seem possible that the deficits seen in the present study could arise from non-specific damage to such a system, or damage to the nigro-striatal DA system.

The results of this present study firmly indicated that the nigro-striatal DA system was not essential for ICSS. Previous studies involving lesions to the nigro-striatal DA system have suggested an essential role for DA in ICSS from sites rostral to the ventral tegmental area. ICSS may be elicited from the caudate nucleus, especially the medial regions (Phillips et al 1976), and this nucleus has been shown to be the major terminal area for the DA neurones A8, of the substantia nigra (Ungerstedt 1971). Lesions of these neurones

by injections of 6-OHDA produced large deficits in ICSS which slowly reversed if the lesion was contralateral but which were still only at free operant levels at the end of the 21 day test after an ipsilateral lesion (Phillips et al 1976). This experiment provided very strong evidence that the nigro-striatal DA system mediated ICSS in at least the caudate nucleus. This finding was extended by others to suggest that the nigro-striatal DA system was an essential link in ICSS from an area in the dorsal pons, in and around locus coeruleus. Lesions to the nigro-striatal system with 6-OHDA severely attenuated ICSS from this area, and the deficit was long lasting (at least 20 days) in the case of the Ipsi lesion but very short lasting (3 days) after a contralateral lesion (Koob et al 1978). This latter experiment suggested a functional role in the reward process for the nigro-striatal system owing to the unilateral nature of the ICSS deficit. However, the results of the present study directly contradict this latter experiment. The effect of lesions to the nigro-striatal system on ICSS from the SN itself was in accord with the present study. The nigro-striatal system was lesioned at the level of the hypothalamus with 6-OHDA, and the effect on ICSS from electrodes in the SN pars compacta (the DA cell group A8) examined. A deficit was produced, but it disappeared by 8 - 10 days post-lesion in both Ipsi and Contra groups (Clavier & Fibiger 1977).

This indicated that the nigro-striatal pathway plays an important role in ICSS but a non essential one in the reward process. The equivalent effect on both Ipsi and Contra groups in this and the present experiment supports the motor deficit hypothesis.

The experiment of Koob et al (1978) has results which appear contradictory to the present study. There were differences in procedure however, as well as possible differences in interpretation. Firstly, the exact location of the stimulating electrodes varied slightly as the majority of sites in the present study were anterior to the locus coeruleus whereas with Koob et al they were situated in or adjacent to this nucleus. This, or the different type of stimuli used, might have explained the difference in pre-lesion response rates, which were almost three times as high in the present study. Secondly, an indwelling guide cannula was used in the present study and hence mechanical damage at the time of the lesion was minimised. It might be supposed that non-specific damage to any neural pathway essential for self-stimulation caused by the insertion of the guide cannula would have prevented the elicitation of ICSS from that particular subject. Thirdly, the concentration of ascorbic acid used in the 6-OHDA injection vehicle was only 0.25 mg/ml in the present study, but 1 mg/ml was used by Koob et al. There was evidence for some non-specific damage to the NSB, either by the vehicle or the cannula in the study of Koob et al as the SHAM injected controls had considerable depletions of striatal DA. The ability of a saline ascorbate vehicle as used by Koob et al to induce a functional change, namely rotation in response to 5-MethoxyDMT, has been documented (Waddington & Crow 1979). This change appeared to be unrelated to changes in forebrain monamine levels, and hence could have been due to damage to a non-monaminergic pathway. This effect was not seen with a saline - ascorbate vehicle similar to the present study (0.25 mg/ml ascorbic acid) and hence it might be inferred that this vehicle causes less non-specific damage. Unfortunately, no sham-injection group was included in the present study so no direct evidence was available to prove this.

Although no actual numbers were published in the Koob et al study of post-lesion response rates, examination of Fig 5, Koob et al (1978), indicated a possible alternative interpretation of their results. There appeared to be a recovery to approximately 80% of pre-lesion rates on day 14 post-lesion, and it seemed unlikely that there was any statistical significance between the Ipsi and Control groups at that time. Furthermore, there was a marked trend for response rates to rise from days 8-14 towards full recovery of pre-lesion rates. Although this rise does not continue it should be noted that the Control group show a marked decline in response rates from days 14-20 for no apparent reason. Finally, there was no significant difference between Ipsi and Control groups for the main groups effect using the Neuman-Keuls test. It must be suggested therefore that the evidence for a critical functional role for the nigro-striatal system in this experiment was perhaps not as strong as the writers suggested.

There remained the possibility that the nigro-striatal system was indeed critical for ICSS but that functional recovery took place over a period of time either by regeneration of neurones of the formation of new synaptic connections (neuronal plasticity), or alternatively due to supersensitivity of the post-synaptic receptors following the degeneration of the pre-synaptic neurones.

The neurones of the central nervous system were believed to have a limited regenerative capacity, and regeneration after transection was considered abortive i.e. no functional connections were made (Ramon Y Cajal 1928). Furthermore adult mammalian neurones could not produce new neurones by differentiation and mitosis (Addison 1911). The study of the transected spinal cord has indicated that some limited functional capacity was restored by regeneration (Windle 1956) and it has been suggested that this relies on the integrity of the cell body

to sprout and develop new processes (Clemente 1964). The ability to visualise the monoamine neurones (Fuxe 1965) has made it possible to investigate the consequences of lesioning. It has been shown that mesencephalic CA axons regenerated 7 - 19 days after an electrolytic lesion (Katzman et al 1971). Regeneration of spinal CA neurones was observed after intraspinal 6-OHDA but this was considered abortive (Wiklune & Bjorklund 1975). After neuronal degeneration caused by 5, 6-dihydroxytryptamine (5, 6-DHT) regeneration of serotoninergic neurones has been seen, although with altered patterns of innervation. This regeneration commenced 10 - 17 days after the lesion and was considerable after three months (Bjorklund et al 1973). There this seemed to be the potential for regeneration in central CA neurones but whether it was functionally relevant was uncertain.

Of possibly greater significance has been the work which has investigated axonal sprouting, and plasticity i.e. the formation of new synaptic pathways which take over the same function. After partial destruction of the optic tract undamaged axons have been found to emit sprouts which invaded adjacent denervated regions (Goodman & Horel 1966). The ability of NA & 5-HT neurones to form axonal sprouts in the rat spinal cord has been shown (Bjorklund et al 1971). Subsequently the importance of axonal sprouting after lesions has been demonstrated. The destruction of the hippocampal input to the septum has been shown to cause a reinnervation of the septal nuclei by NA neurones as shown by a long lasting increase in the number of NA terminals using fluorescence histochemistry. These NA axons invaded areas which normally had a much lesser NA innervation (Moore et al 1971). After silimar lesions the synaptic ultrastructure has been investigated and showed that, after initial synaptic destruction, new synapses appeared over a month (Raisman & Field 1973). These

experiments would suggest that a functional reinnervation of these septal cells by NA neurones occurred after dennervation of another septal input. Thus although true regeneration was not seen reinnervation was possible, although whether it was of functional significance was not determined.

The importance of these discoveries has been emphasised by another study. After lesion of one 5-HT input to the hippocampus it was found that reinnervation took place after 28 days by collateral sprouting of another intact 5-HT hippocampal input. This was accompanied by functional recovery as demonstrated by the disappearance of an asymmetrical behavioural response (Azmitia et al 1978). It would thus appear that collateral reinnervation at least by neurones containing the same neurotransmitter could produce functional restoration. Of possible significance was the finding that injection of nerve growth factor enhanced recovery of feeding after a lateral hypothalamic lesion (Berger et al 1973).

Interesting and important as these indications of functional recovery and neuronal plasticity after lesions were, their immediate relevance to the present experiment must be doubtful. In all these experiments reinnervation was found to occur on a time-scale of 2 weeks - 3 months, and, in the one measurement of functional recovery this occurred 28 - 42 days after lesioning. In the present experiment functional recovery was observed with a mean time of 6 days and hence could not be explained on this basis. A mechanism with a much faster response to lesion damage would be needed. The phenomenon of supersensitivity might be such a mechanism.

The appearance of denervation supersensitivity after the destruction of catecholamine neurones has been much documented. It was initially described as paradoxical contraction when an exagerated response to

adrenaline was seen after lesions of sympathetic nerves (Anderson 1904). Such results led to the formulation of Cannon's law of denervation, Cannon (1939).

> "When in a series of efferent neurones a unit is destroyed, an increased irritability to chemical agents develops in the isolated structure or structures"

Dennervation supersensitivity has been extensively described in the peripheral sympathetic nervous system, when an exaggerated response by the post-synaptic effector cells to exogenous sympathomimetic amines has been observed (Trendelenburg 1963, 1966). The ability of systemic 6-OHDA to mimic this effect has been demonstrated (Haeusler et al 1969; Trendelenburg & Wagner 1971). This observation has been extended to the CNS. An enhanced behavioural responsiveness to the DA agonist apomorphine has been seen after destruction of the nigro-striatal system with 6-OHDA (Ungerstedt 1971b). Alternative evidence for a post-synaptic supersensitivity was enhanced changes in striatal acetylcholine levels after similar treatment (Grewaal et al 1974).

The time course for the development of supersensitivity has been determined in various experiments. In the periphery denervation supersensitivity has been shown to develop rapidly within hours but this probably reflected the disappearance of pre-synaptic uptake mechanisms. Post-synaptic supersensitivity developed over the period of 2 - 28 days after lesioning (Langer et al 1967). Similar results have been obtained in the CNS. Enhanced locomotor activity after intra-ventricular L-DOPA was demonstrated three days after 6-OHDA (Schoenfield & Uretsky 1973) although this might have in part been due to pre-synaptic supersensitivity. The development of the rotational response to apomorphine which reflected post-synaptic supersensitivity was apparent by three days after 6-OHDA treatment but was not fully developed

until 6 days after the lesion (Ungerstedt 1971b). The development of increased locomotor activity after apomorphine was potentiated by 6-OHDA lesions in the nucleus accumbens and this increase in sensitivity was apparent two days after the lesion though it increased to a maximum at about 14 days (Kelly et al 1975). It would appear that super-sensitivity mechanisms had a similarily rapid development in the CNS.

The development of supersensitivity has appeared to possess functional significance. The recovery of food intake after unilateral nigrostriatal lesions was also found to occur in the period 7 - 11 days after 6-OHDA lesion (Baez et al 1977). After pre-treatment with α-MPT, which could cause supersensitivity, recovery of function after LH lesions was enhanced (Glick et al 1972). It would appear that denervation supersensitivity could facilitate recovery of function. The time course of this phenomenon was similar to that observed for the recovery of ICSS in the present experiment.

It must be considered possible therefore that development of supersensitivity after the 6-OHDA lesion was able to produce functional recovery. It could be suggested that initial recovery mechanims were provided by supersensitivity, and this was followed by a period of reinnervation. Interestingly the normal response to amphetamine has been shown to reappear in a variety of studies around one month after a lesion to the DA systems (Kelly et al 1975 ; Ungerstedt 1974), which might suggest reinervation. Whatever the later events, super-sensitivity might explain the recovery of function after the DA lesion, whether the recovery of motor function or an ICSS reward system. U seful as this explanation might be, there was an indication that the response rates on the first day of testing after lesioning (day 3 or 4) were not correlated with the extent of striatal DA destruction. As previously postulated non-specific damage to another

motor system might have caused the deficits observed in some animals. DA destruction might also have contributed to such a deficit, or perhaps damage to both resulted in a more profound deficit. Unfortunately systematic evaluation of non-specific damage was impossible as the dissection of hypothalamus usually cut through the injection site. It must be noted that even with a non-specific lesion supersensitivity could have developed in another system and hence recovery.

There has been some indication that lesions to the nigro-striatal system have resulted in motor or performance deficits rather than an effect on reward systems. Unilateral 6-OHDA lesions have resulted in an equal disruption of LH (Ornstein & Huston 1975) and SN ICSS (Clavier & Fibiger 1977) whether the lesion was ipsi- or contralateral to the stimulating electrode. More importantly bilateral 6-OHDA lesions of the SN abolished ICSS with a lever press response but not when a simple motor response was used (Ornstein & Huston 1975). These experiments would support a more generalised deficit after such lesions. Even in studies which have demonstrated a long lasting deficit after unilateral DA lesions, the contralateral lesion has produced a short-lasting deficit (Phillips et al 1976: Phillips & Fibiger 1978: Clavier & Gerfen 1979). These results confirmed that unilateral DA lesions produced a temporary impairment of a complex motor response, and they showed that DA systems might have a direct role in reward insome areas as well as motor performance. It must be suggested tentatively that in the present study the motor performance effect has been demonstrated but not the effect on reward.

The experimental results suggested that an independent pontine pathway supported ICSS. The obvious candidate for such a pathway must be the dorsal tegmental noradrenergic bundle originating from the locus coeruleus (Anden et al 1966B Ungerstedt 1971a.

The stimulating electrodes were all in or near this pathway, and it was not destroyed by the 6-OHDA procedure. The slight decrease in NA concentrations seen in cortical tissue, a major projection area of DTB (Ungerstedt 1971a) could have been due to mechanical destruction of DTB fibres by the stimulating electrode. This decrease was not significant in either lesion group. It could be argued that this experiment provided supportive evidence for an independent functional role in ICSS for the dorsal NA system, but not conclusive evidence for this role. The role of this NA system in ICSS has remained controversial. Various studies have demonstrated ICSS from electrodes within or close to the locus coeruleus (Crow et al 1972; Ritter & Stein 1973; Ellman et al 1975; this thesis), although other groups have not been able to produce ICSS from this area at all (Amaral and Routtenberg 1975; Simon et al 1975). It seemed that training procedures could account for this difference, as multiple training sessions were often needed to produce ICSS from this region. The results of lesioning the locus coeruleus have however not in general been supportive of a vital role for this NA system in ICSS. Electrolytic lesions of the locus coeruleus have failed to attenuate ICSS from the MFB in lateral hypothalamus, and in fact increases in response rate have been reported in these experiments (Koob et al 1976; Corbett et al 1977). Similar lesions, both unilateral and bilateral also failed to permanently attenuate ICSS from the dorsal brain stem in the area of the DTB although a transient deficit was produced (Clavier and Routtenberg 1976). More direct evidence against LC involvement in brain stem ICSS was provided by experiments which had electrodes in the LC, and lesions were made to the DTB. In one experiment, 6-OHDA was injected into the DTB bundle, and large depletions of cortical NA were achieved (by 96%), but without any attenuation of

LC ICSS (Clavier et al 1976). In another electrolytic lesions were made to the DTB, both uni and bilateral, but again ICSS from LC was unaffected (Corbett et al 1977). These experiments indicated that the ICSS produced from the dorsal brain stem did not rely on the integrity of the LC.

There does exist evidence for some link between the dorsal brain stem and lateral hypothalamus in the mediation of ICSS. Brain stem self-stimulation was attenuated by MFB electrolytic lesions, with the attenuation proportional to the degree of damage to MFB (Clavier & Routtenberg 1976). In a completely different type of experiment, simultaneous stimulation of the dorsal brain stem and hypothalamic sites at subthreshold intensities interacted to produce suprathreshold response rates (Ellman et al 1975). These experiments have indicated that a common neural substrate might underly self-stimulation in both these areas, although they gave no indication of the putative pathway. Anatomical studies have suggested that there were descending pathways from LH which at least passed through these dorsal pontine areas. An autoradiographic study has shown that efferent pathways from LH projected to the rostral LC through the ventral aspects of the Central gray (Saper et al 1979). Conversely the use of retrograde tracing techniques utilising horseradish peroxidase have also demonstrated such a projection. Both iontophoretic application of small amounts of HRP (Cedarbaum & Aghajanian 1978) and larger intracerebral injections of HRP (Clavier 1979) have shown that the axons of LH cells project to the area of the LC. The possibility that activation of such a pathway could have supported ICSS in the dorsal brain stem must remain open to speculation.

Other pathways have also been suggested to support ICSS in the dorsal pontine area. One interesting possibility was that the afferent

sensory pathway containing gustatory information supported ICSS. This pathway from the nucleus of the solitary tract has a relay centre in the area of the brachium conjunctivum, the so-called pontine taste area (Norgren & Leonard 1973). This area was close to the LC, and its rostral projections ran close to those of LC (Norgren 1978). The possibility that this taste pathway supported ICSS must be considered. The brachium conjunctivum has been claimed to support ICSS (Routtenberg & Malsbury 1969), but this was not supported by a later study with electrodes actually within the superior cerebellar peduncle (brachium conjunctivum) (Crow et al 1972). The possibility that the mesencephalic nucleus of the trigeminal nerve was the possible mediator of ICSS in this region was considered, although the motor trigeminal nucleus did not itself support ICSS (Crow et al 1972). Subsequently ICSS has been obtained from the motor trigeminal nucleus (Motor V) area (Van der Kooy & Phillips 1977), and from the lateral aspects of locus coeruleus and the mesencephalic nucleus of the trigeminal nerve (Van der Kooy 1979). In the latter experiment 6-OHDA injections failed to disrupt this ICSS, and hence it appeared that the LC was not essential for ICSS from this area. Another extensive mapping study has indicated that ICSS in the area of LC was most reliably elicited in lateral but not in medial aspects of this nucleus (Corbett and Wise 1979). It would seem reasonable to suggest that either the trigeminal system or the pontine taste system, or both could be the mediators of ICSS in the dorsal pons.

In summary, the present study has indicated that ICSS from the dorsal brain stem, in the region of the DTB, does not rely on the integrity of the nigro-striatal DA system. Therefore, there was no evidence for the involvement of those neurones in the dorsal tegmentum which projected to the substantia nigra in such ICSS. However, it

seemed possible that this DA system played a functional role in the bar pressing response used to elicit ICSS. The suggestion that ICSS from LC was evoked by some type of learning process whereby LC stimulation gains access to DA reward mechanisms (Milner 1977) seemed to be refuted. The wider question of whether the LC itself was the neural substrate for dorsal brain stem ICSS remained unresolved by this study. However, caution must be used, after consideration of the afore mentioned lesion studies, in ascribing the non-DA ICSS system in brain stem to the NA system originating in locus coeruleus.

General Discussion

Since the discovery of electrical self-stimulation by Olds and Milner (1954), extensive research has been undertaken to discover the neural systems which supported this behaviour. Initial mapping studies indicated that discrete brain areas were involved and that the focus of positive sites was the medial forebrain bundle (Olds 1956: Olds et al 1960). Pharmacological investigations suggested that catecholamines were directly involved in ICSS. Drugs which increased the availability of catecholamines at the synapse potentiated ICSS (Stein 1962: Poschel and Ninteman 1963) and conversely the drug reserpine which decreased catecholamine brain levels decreased ICSS (Olds et al 1956).

The anatomical basis for this relationship was uncovered when the medial forebrain bundle was shown to contain a large catecholaminergic component by fluorescence histochemistry (Anden et al 1966b.

The cell bodies of origin of these CA pathways were located in the ventral mesencephalon and the brain stem (Dahlstrom et al 1964). The ventral mesencephalon supported ICSS (Dresse 1966), in the area of the A10 CA cells (Dahlstrom & Fuxe 1964). However this was considered to be a noradrenergic cell group but was subsequently shown to be dopaminergic (Anden et al 1966b). The dorsal brain stem also supported ICSS, in the area of the superior cerebellar peduncle (Routtenberg & Malsbury 1969), close to the fibres of the dorsal noradrenergic system (Ungerstedt 1971a. These results suggested it was indeed possible that the CA systems supported ICSS. A systematic evaluation of this hypothesis was conducted by Crow and co-workers. They investigated the ventral mesencephalon and the dorsal brain stem in the area of locus coeruleus. There was a close correlation between

the sites supporting ICSS and the DA cell groups A9 and A10 in the ventral mesencephalon and the sites in the brain stem and the NA cell group A6 in locus coeruleus (Crow 1971, 1972a; Crow et al 1972). It thus seemed that both NA and DA systems supported ICSS. Crow (1972b) proposed a hypothesis on their functions based on the behavioural consequences of electrical stimulation in these areas and on possible sensory inputs to these cell groups. Briefly, self-stimulation of the DA cells produced increased activity, sniffing and licking and rearing and high rates of responding. There was indirect anatomical evidence that there was in fact a link between the olfactory systems and those DA cells (Millhouse 1969). It was thus suggested that these dopaminergic systems mediated incentive motivation which was phylogenetically related to olfaction. Self-stimulation of the noradrenergic cells in locus coeruleus produced a different type of behaviour. There was little excitation with gnawing the predominant associated behaviour, and low response rates were seen. Anatomically the locus coeruleus lies in the visceral afferent column of the brain stem (Russell 1955), which contains the gustatory input although this is believed to terminate in the nucleus of the tractus solitarius. It was therefore suggested that this NA system might mediate reward or reinforcement, which was phylogentically related to gustation. In order for this dual catecholamine hypothesis to be sustainable it has been necessary to verify that ICSS from these areas was mediated by these CA systems. A substantial body of experimental evidence on this problem has now accumulated, and the justification for this theory may be reassessed.

There has been some dispute as to whether the locus coeruleus supported ICSS at all. The initial demonstration that ICSS could be elicited with electrodes in or around locus coeruleus (Crow et al 1972) could not be replicated in other laboratories (Amaral and Routtenberg 1975; Simon et al 1975). The studies used different behavioural procedures, omitting the shaping to the bar press response with multiple training sessions. If these procedures were used ICSS could be obtained from this area (Ritter and Stein 1973; Ellman et al 1975). There seemed little doubt that ICSS could be obtained from this area, and there was a qualitative difference from that obtained elsewhere.

Once a function has been ascribed to a brain area or neural system the classical approach to confirm this relationship has been to lesion it and then observe the changes in behaviour so produced. When electrolytic lesions were made through electrodes in the superior cerebellar peduncle which had supported ICSS there was a subsequent build up of catecholamines as demonstrated by histofluorescence. This indicated that catecholamine pathways had been close to the stimulating electrode (Clavier and Routtenburg 1974), most likely the dorsal tegmental system from locus coeruleus. The neurotoxin 6-OHDA (see section 4) was injected bilaterally to locus coeruleus, with subsequent considerable depletion of cortical NA, and hence presumably marked destruction of the dorsal tegmental pathway. There was no lasting attenuation of ICSS from these brain stem sites in the region of the superior cerebellar peduncle (Clavier and Routtenberg 1976). This experiment did not resolve the ability of LC to support ICSS as the lesions were not confirmed in all animals, and were not complete. Further more the stimulation sites were in dorsal brain stem, not LC itself. Subsequent experiments have helped resolve these problems.

ICSS was established from sites in or around LC, and then 6-OHDA was injected into the mesencephalic projection of the dorsal NA system. Although virtually complete depletion of cortical and hippocampal NA was achieved, there was no significant or lasting effect on LC ICSS (Clavier et al 1976). A very similar experiment was performed using electrolytic lesions of the dorsal noradrenergic bundle, after ICSS in LC had been established. Neither unilateral nor bilateral lesions attenuated LC ICSS (Corbett et al 1977). These experiments have shown that the telencephalic projections of the LC were not essential for ICSS from this nucleus, and question the role of the LC in dorsal brain stem ICSS.

Virtually all the mapping studies linking dorsal brain stem ICSS to CA systems have been assumptive i.e. the sites supporting ICSS were believed to lie in or near CA neurones whose location had been determined previously in other anatomical studies. There was thus considerable room for interpretation or even error. A study has now been made of ICSS sites in the dorsal brain stem and the electrode site's relationship to the CA systems determined by histofluorescence in the same animal. In addition a movable electrode was used and hence the electrode position relative to the CA systems was variable. There was no correlation observed between the density of CA innervation and ICSS response rates, and sites within the CA cells of locus coeruleus proved negative. This was true in the dorsal aspects of the nucleus when little damage had been done by the electrode. In spite of using methods including behavioural shaping and repeated testing the locus coeruleus nucleus failed to support ICSS although it was elicited from adjacent structures, especially the mesencephalic nucleus of the trigeminal nerve (Corbett and Wise 1979).

It could now be said that the dorsal noradrenergic system originating in locus coeruleus seems unlikely to be as essential component of ICSS from the dorsal brain stem. The convergence of the results from the divergent approaches of lesion and mapping studies was compelling. However it was shown that areas adjacent to locus coeruleus (both lateral and anterior), supported ICSS and it must be a possibility that electrodes in LC could stimulate these areas also which would explain the many reports of ICSS being obtained from this area of the brain stem. Indirect supportive evidence for the non-essential role of locus coeruleus in ICSS has been the number of studies showing no attenuation of medial forebrain bundle self-stimulation after locus coeruleus lesions (Farber et al 1976) and in fact increased response rates in some cases (Koob et al 1976: Corbett et al 1977). Conversely much of the supportive evidence for the central role of the dorsal noradrenergic system in ICSS has been only correlative. The release of NA in terminal areas of this system, associated with LC ICSS has been measured by electrophysiological (Segal and Bloom 1976) or by biochemical (Anlezark et al 1975) methods. An effect on NA metabolism in the cell bodies of LC has also been noticed (section 3, this thesis). However, these studies have only shown that these NA neurones lay within the field of stimulation from electrode sites which supported ICSS, and not that the NA neurones were essential. In view of the negative results from more direct tests of the dorsal NA hypothesis the value of these results must now be doubtful, as must the hypothesis itself.

The initial demonstration of self-stimulation from sites in the ventral mesencephalon, dorsal to the interpeduncular nucleus (Dresse 1966) has since been replicated and positive sites shown to extend into the adjacent area of the substantia nigra, mainly in the pars compacta (Crow 1972a. These mesencephalic areas contained the dopaminergic cell groups A9, in the substantia nigra pars compacta (SNC), and the A10 cell group dorsal to the interpeduncular nucleus. The A9 cells projected to the striatum to form the nigro-striatal-DA system and the A10 cells to limbic forebrain areas to form the meso-limbic DA system (Ungerstedt 1971a. This close anatomical relationship has been further demonstrated by the existence of ICSS in the terminal areas of both systems. The striatum has been shown to support ICSS (Phillips et al 1976) and areas in limbic forebrain shown to support ICSS include the olfactory bulb (Phillips & Mo genson 1969), frontal cortex (Routtenburg and Sloan 1972) and the nucleus accumbens (Phillips et al 1975). The associative evidence that dopaminergic systems supported ICSS was therefore strong. Further evidence was obtained by lesion experiments and by the use of pharmacological techniques.

Lesion experiments which directly investigated the role of dopamine were possible using the neurotoxin 6-OHDA which selectively destroys CA neurones (Ungerstedt 1971b. This selectivity could be increased by pre-treatment with desmethylimipramine which protected NA neurones from destruction presumably by blocking 6-OHDA uptake into these neurones (Roberts et al 1975). Although it has been shown that bilateral 6-OHDA lesions of the nigro-striatal bundles (NSB) produced generalised deficits in instrumental responding (Fibiger et al 1974) it has proved possible to control for this in

the ICSS experiments by observing the effect of lesions to the ipsi- and contralateral DA pathways (relative to the stimulating electrode).

Using these methods it was found that ICSS in the striatum was dependent on an intact DA innervation. 6-OHDA lesions of the nigro-striatal system produced a long lasting and severe attenuation of ICSS from the striatum when placed ipsilateral to the electrode but only a transient attenuation when contra-lateral (Phillips et al 1976). This seemed to indicate a vital role for this DA pathway in ICSS. However, in a subsequent experiment when the stimulating electrodes were placed in the SNC, and 6-OHDA lesions of the NSB were made both ipsi-and contralateral lesions produced only a short-lasting deficit in ICSS (Clavier & Fibiger 1977). It thus appeared that the DA cells of the substantia nigra were not essential for ICSS from that area. Previously it had been shown that ICSS from SN was seriously attenuated by bilateral electrolytic lesions of sulcal pre frontal cortex. This was interpreted as evidence for a descending pathway from frontal cortex which supported ICSS in SN (Clavier and Corcoran 1976).

Although it seemed that SN ICSS could be mediated by a non-DA system, possibly arising in sulcal prefrontal cortex, ICSS from pre-sulcal frontal cortex has been shown to depend on an intact DA system. Ipsilateral 6-OHDA lesions which greatly reduced DA in both the nigro-striatal and mesolimbic systems severely attenuated ICSS from sulcal frontal cortex (Clavier and Gerfen 1979). The results from these experiments suggested that ICSS in DA terminal regions was dependent on intact DA systems, while in the ventral mesencephalon other neural systems could support ICSS. This distinction has proved unfounded however.

Unilateral 6-OHDA lesions to the ascending DA pathways severely attenuated ICSS from the ventral tegmentum, in the A10 DA cells but had only a slight transient effect on nucleus accumbens ICSS in the same animals. A separate group of rats subjected to the same lesioning procedure also showed a pronounced attenuation of A10 ICSS and on medial frontal cortex ICSS but the latter site showed a progressive though incomplete recovery (Phillips & Fibiger 1978).

The results of these experiments must be considered paradoxical. Lesions of the nigro-striatal DA system virtually abolish striatal ICSS (i.e. terminal area) but have no such effect on nigral ICSS (i.e. cell bodies). Conversely lesions of the mesolimbic DA system have virtually no effect on nucleus accumbens ICSS (terminal) but severely attenuate ventral tegmentum (A10) ICSS (cell bodies).

The effects of 6-OHDA lesions on ICSS from frontal cortex, believed to have a mesolimbic DA innervation (Lindvall and Bjorklund 1974) were also equivocal. ICSS from sulcal prefrontal cortex was abolished after DA lesions, whilst medial prefrontal cortex ICSS was only moderately reduced. However, recent anatomical studies have indicated that the A9 DA cells also project to frontal cortex, with medial areas receiving their DA input from A10 and sulcal areas from A9 (Fallon and Moore 1978: Emson and Koob 1978) thus it appeared that in the terminal areas of the A9 system DA was essential for ICSS whereas in the terminal areas of A10 cells other neurones also supported ICSS. Likewise in the mesencephalon, in the area dorsal to the interpeduncular nucleus DA appears to have an essential role whilst in substantia nigra other neurones can support ICSS.

These interpretations must be treated with caution. In most of these experiments both mesolimbic and nigrostriatal pathways were destroyed unilaterally, hence functional discrimination was not really possible. A further factor which complicated these results was that recovery of ICSS behaviour was sometimes gradual e.g. in the medial prefrontal cortex. The possibility of recovery arising from adaptive changes in the damaged DA system, for example the development of supersensitivity must be considered (see section IV). In spite of these reservations the results which showed a profound decrement in ICSS after DA system lesions when ICSS at another site was intact or the contralateral control not similarly affected have shown a vital role for DA at some sites. Other sites which were previously assumed to be mediated by DA systems, such as the SNC, would seem to have other ICSS-supporting systems in close proximity. This has not excluded the SNC from a role in ICSS, indeed the results in its terminal regions suggested the opposite. It emphasised the need for caution in drawing conclusions from lesion experiments as they only indicated the role of a neural system at a particular stimulation site. ICSS from other points in the same system can have simultaneously stimulated other neural systems which also supported ICSS.

It has been suggested that the ventral NA system was responsible for ICSS in the region of SN (Belluzzi et al 1975). These experiments were distinctly crude with either large mechanical lesions or 6-OHDA lesions at concentrations which would produce non-specific damage. Additionally ICSS has not been obtained from the cell bodies of the ventral NA system (Anlezark et al 1974). This raised the interesting possibility of a non-CA system supporting ICSS in the ventral tegmentum.

In conclusion, experiments which have specifically lesioned the DA systems have indicated that at some points in these systems DA appeared to have an essential role in ICSS. At other points in the same neural system the destruction of DA neurones had either a non-specific or negligible effect on ICSS and the existence of non-DA neurones which supported ICSS was suggested. There remained the possibility that the mesencephalic DA systems supported ICSS, but that the effects of their destruction were masked by the close proximity of these other systems supporting ICSS.

The role of DA in ICSS has also been investigated by pharmacological means. The ability of amphetamine to potentiate ICSS (Stein 1964) was believed to indicate a facilitation of NA transmission. Subsequent analysis of the mode of action of amphetamine has shown that it increases release of both NA and DA, inhibits their reuptake and inhibits one of the CA degrading enzymes, monoamine oxidase (Carlsson 1970). Recent experiments with 6-OHDA lesions of the DA systems have indicated that these systems must be intact for this facilitation to occur. When whole brain DA was selectively depleted after intraventricular 6-OHDA the effect of d-amphetamine on ICSS from lateral hypothalamus was significantly reduced (Cooper et al 1974). More directly, ipsilateral 6-OHDA lesions of the DA systems abolished amphetamine facilitation of SN ICSS without significantly affecting the ICSS itself. A contralateral lesion had no such effect (Clavier and Fibiger 1977). A similar finding was obtained with ICSS from the nucleus accumbens and medial prefrontal cortex after ipsilateral 6-OHDA to the DA pathways (Phillips and Fibiger 1978). Conversely, unilateral destruction of the dorsal NA system did not affect the ability of d-amphetamine to potentiate ICSS (Clavier et al 1976). It thus appeared that the potentiation of ICSS by amphetamine crucially involved the DA systems, but not the dorsal NA system. The nature of

that involvement was not determined however, whether it was a direct effect on the "reward" system or whether it facilitated the performance of the operant behaviour. The latter must be a consideration as by the use of selective DA lesions it has been shown that the dopaminergic systems in the nucleus accumbens were essential for the facilitation of locomotor activity by amphetamine (Kelly et al 1975). The difficulty of separating reward from performance deficits has also influenced the interpretation of another pharmacological approach, the use of dopaminergic antagonists.

The first indication of the effectiveness of DA antagonists in blocking ICSS was when chlorpromazine was found to severely attenuate self-stimulation from lateral hypothalamus (Olds et al 1956). Chlorpromazine has been shown to block both DA and NA receptors however (Anden et al 1970). The use of more selective DA antagonists has produced similar effects on average rates of bar-press responses for ICSS. Haloperidol (Wauquier and Niemegeers1972: Phillips et al 1975) spiroperidol (Wauquier and Niemegeers 1972) and pimozide (Liebman and Butcher 1974: Fibiger et al 1976) have all been shown to decrease ICSS in a dose-related manner. The interpretation of these results as indicative of a direct effect in a reinforcement system has been questioned. It has been suggested that these drugs cause an impairment of complex motor acts such as bar-pressing (Fibiger et al 1976) or more generally the performance of learned motor responses (Fibiger et al 1975). Operant behaviours for natural reinforcers such as food and water have also been attenuated by similar low doses of neuroleptics (Fibiger et al 1976: Rolls et al 1974). This could be taken as evidence for the performance deficit theory only if the assumption that DA systems did not mediate natural reward also was correct. It was necessary however to prove that reward deficits could be dissociated from performance deficits for this line of experimentation to demonstrate an essential role for DA systems

in reinforcement.

An indication that this might be possible came from an experiment which showed that increased stimulation current would overcome neuroleptic blockade of ICSS (Liebman and Butcher 1973). This suggested that increased reward (whether by involvement of another adjacent system or not) could reinstate ICSS, and hence a generalised motor deficit was improbable. The use of operant tasks less dependent on complex motor performance than bar-pressing have also been used to dissociate reward from performance.

With a rate-free measure of ICSS in a shuttle box, pimozide was shown to significantly reduce the amount of stimulation received (Liebman & Butcher 1974), and hence presumably was reducing the rewarding properties of the stimulation. In another study, which confirmed the non-specific effects of pimozide on a variety of operant behaviours at higher doses, low doses of pimozide markedly reduced ICSS from sites in the far-lateral hypothalamus when a runway response was used. These stimulation sites were in the region of the ascending DA pathways (Ungerstedt 1971a) but ICSS from a more medial hypothalamic site was unaffected. The bar-press response was attenuated at both sites (White et al 1978). This was evidence for the direct involvement of DA in ICSS when DA pathways were being stimulated. In another experiment using a runway as part of the operant response, a measure known as the reward summation function which relates maximum running speed to the duration of the electrical stimulation (Edmonds & Gallistel 1974) was used. Pimozide caused a marked change on this function in the direction equivalent to reduced reward without so affecting the maximum running speed (Franklin 1978). This again indicated a selective affect on reward rather than performance at low doses of the neuroleptic.

An alternative approach which also investigated a direct measure of the rewarding strength of electrical stimuli has been investigated. The experimental design was such that reinforcement thresholds were determined by the animal itself as the current was automatically stepped down until a second operant task was performed which reset the current to its original level. In this way an overall decrease in responding reflected a general performance deficit whereas a change in reinforcement threshold could occur independantly (Stein and Ray 1960). In the present experiment pimozide at low doses produced a dose-related increase in reinforcement thresholds, without a general disruption of response rates (Zarevics and Setler 1979). This effect was similar to that seen when the quantity of electrical charge per stimulus was decreased, and hence suggested that pimozide had effectively reduced the rewarding properties of the stimulus.

Other experiments have used intra-cerebral microinjections of the DA antagonists in an attempt to dissociate reward from performance. Injections of spiroperidol into the nucleus accumbens almost completely abolished ICSS from LH but produced little motor impairment (this was comprehensively rated separately) while injections into the striatum caused motor impairment with little effect on ICSS (Mora et al 1975). In accord with this finding injections of haloperidol into either ipsi or contralateral striatum disrupted ICSS from the ventral tegmentum (Broekkamp and Van Rossum1975), which was presumably a non-specific effect. In a similar experiment microinjections of spiroperidol made close to the stimulating electrodesignificantly attenuated self-stimulation from the nucleus accumbens, with injections to the contra-lateral accumbens having no effect. The experiment was repeated in medial prefrontal cortex with less marked effects, and only at the highest dose used was any attenuation observed (Robertson and

Mogenson 1978). This again suggested that DA systems played an important role in ICSS in some brain areas, and that this was not simply related to performance of the task.

The use of simple response rate measures has been questioned without the presence of elaborate behavioural controls. The analysis of variations in response-rates throughout an ICSS period after drug treatment has usually been neglected, and only average response rates used. Experiments conducted with the self-administration of stimulant drugs (which function as reinforcers in this situation) have indicated that DA antagonists alter response patterns in particular ways. Low doses of pimozide were found to have the same effect as decreasing the amount of amphetamine delivered, namely to increase response rates, i.e. it reduced the affective reward value of a certain dose of stimulant. Higher doses of pimozide caused an unusual response pattern of initially accelerated responding followed by a gradual cessation. This effect was typically that seen during the extinction of responding caused by substitution of saline for the stimulant drug (Yokel and Wise 1975). These results had two main implications. Firstly that dopamine systems mediated the rewarding effect of these drug stimuli and secondly that low doses of pimozide did not impair the lever press response, as enhanced responding was produced.

When this analysis was performed in the response pattern seen with ICSS from LH after pimozide an equivalent effect was seen. Higher doses of pimozide caused an initial period of normal responding, a gradual deceleration and then cessation i.e. an extinction type pattern and low doses caused normal early responding followed by a low rate of responding (Fouriezos and Wise 1976). This finding was replicated in a later study which also investigated the extinction - like effect in more detail and compared it with the extinction produced

by current reduction. It has been found that after extinction trials if the animal was subsequently tested in the non-reward situation a brief period of responding would ensue. A high dose of pimozide which caused the extinction pattern of responding was shown to cause this pattern of short response periods after inter trial intervals and thus mimiced the extinction effect in this also. Finally the effect on a runway task for ICSS was used and again high doses of pimozide mirrored the extinction effect, with increased latencies and a reduction in running speed as the trial progressed although the first test runs were normal (Fouriezos et al 1978).

These experiments have suggested that DA antagonists specifically block neural systems critical for the rewarding property of electrical stimulation, and that performance deficits were not a sufficient explanation for the observed decrements in ICSS. It has been reported that these drugs also attenuate operant responding for natural reinforcers including food (Fibiger et al 1976) and water (Rolls et al 1974); which was interpreted as an effect on performance. This might not be the case as analysis of the effect of pimozide has shown that the extinction effect was also seen with food or saccharin rewarded animals (Wise et al 1978). This has suggested that DA systems might mediate positive reward, but further work would be necessary to substantiate this interesting possibility.

If the use of DA antagonists to define the role of DA in ICSS was to be valid, it was necessary to eliminate other possible actions of these drugs. It has been shown that many of the neuroleptics possess a pronounced antagonism at NA receptors (Anden et al 1970). This was especially so with haloperidol, although pimozide had a much more specific action on DA. It seemed unlikely that pimozides action on ICSS was a result of α-NA blockade as at doses which caused no significant

attenuation of the α-NA flexor reflex test (Zarevics et al 1977) it caused complete extinction of ICSS (Fouriezos et al 1978). Furthermore, α-NA blockade itself with phenoxybenzamine was ineffective in blocking ICSS at doses which blocked the flexor reflex (Zarevics et al 1977). The deficits seen in ICSS after high doses of phenoxybenzamine did not resemble the extinction-effect but caused erratic responding in the lever-press test, and caused reduced running speeds and increased latencies in the runway test (Fouriezos et al 1978). Finally pimozide given chronically did not cause an enhanced responsiveness to clonidine, an α-NA agonist whilst haloperidol did (Ettenberg and Milner 1977). It therefore seems that the results with pimozide reflected its DA antagonist activity, although results with other neuroleptics, especially haloperidol must be treated with some caution.

The latter experiment with chronic pimozide further emphasised the importance of DA in ICSS. It caused an enhanced sterotypy with amphetamine after pimozide was withdrawn, which indicated an increased DA receptor sensitivity as had previously been reported (Thornburg and Moore 1974). The effect on ICSS was both to increase response rates and to lower thresholds (Ettenberg and Milner 1977). As NA sensitivity had not changed, this was interpreted as evidence for a crucial role for DA systems in ICSS.

In conclusion, pharmacological experiments with DA antagonists have indicated that the DA systems have a functional role in the rewarding aspects of ICSS. Although at high doses non-specific effects on performance were demonstrated at low doses the effect on reward and performance could be separated with appropriate experimental design. The evidence for a reward function was particularly compelling as a wide variety of experiments had reached this conclusion. These had included measures such as the reward summation function, of reward thresholds and in particular the stimulating animals own control

of them, and of behavioural effects, the extinction-like effect which was shown both with lever pressing and runway tasks. All these measures were able to demonstrate performance and reward deficits and found that DA receptor blockade reduced the rewarding effect of ICSS.

When these results were considered in conjunction with those from the selective DA lesion experiments the evidence for a critical role for DA in ICSS seemed convincing. It was possible to severely disrupt ICSS with selective DA lesions and to reduce the rewarding effects of ICSS with selective DA blockade. However there remained certain problems. In most cases DA receptor blockade experiments did not discriminate between different DA systems whilst DA antagonists have varying affinities for DA receptors in different areas (Laduron et al 1978). One study which did discriminate between DA systems, with intra-cerebral spiroperidol has in fact raised further problems. It showed that the DA system in the nucleus accumbens was essential for ICSS from that area (Robertson and Mogenson 1978). After destruction of the DA pathway however there was no effect on ICSS from this area (Phillips and Fibiger 1978). It seemed unlikely that inadequate lesions were responsible for this absence of effect as ICSS from the ventral tegmentum was severely reduced. The possibility that spiroperidol had blocked other receptors must be considered as at high doses it has been shown to block NA (Anden et al 1970) and 5-HT receptors (Leyson et al 1978). However, low doses of spiroperidol which should have been specific for DA were used. The two results must be considered contradictory at present, and need resolving. Another area which supported ICSS which was presumed to be mediated by dopamine, was the medial prefrontal cortex. It has been shown to contain a high concentration of DA terminals (Berger et al 1976) and to have an increased release

of DA after ICSS in this area (Mora and Myers 1977). However, neither local injections of moderate doses of spiroperidol (Robertson and Mogenson 1978) nor DA lesions (Phillips and Fibiger 1978) have abolished ICSS from this area. It must therefore be concluded that although DA systems mediated the rewarding properties of ICSS at some sites in the brain, there existed other areas where this was not the case. However, the initial hypothesis that DA could mediate ICSS at certain areas now must be considered justified.

There have been other approaches to the problem of the role of CA's in ICSS. It has been shown that ICSS was associated with changes in the activity of CA neurones. There was some evidence that ICSS from the ventral tegmentum area caused a decreased intensity of fluorescence in NA - terminals (Dresse 1966). A similar result has been obtained after imposed stimulation in anaesthetised rats in this area (Arbuthnott et al 1970) and also after stimulation in conscious rats through electrodes which has previously supported ICSS (Arbuthnott et al 1971). Even in the latter experiment stimulation had to be imposed as the self-stimulation was impaired by the use of a tyrosine-hydroxylase inhibitor to measure turnover rates. This experiment however illustrated the problems of interpretation inherent in such work. The changes in NA metabolism were indicative of a role for the ventral NA system in ICSS from this area but it was subsequently demonstrated that this system did not support ICSS (Anlezark et al 1974: Clavier and Routtenberg 1974). Furthermore changes in DA metabolism have been shown after ICSS from this region (Section One) which contains the mesencephalic DA cell groups. However, lesion studies have indicated that non-DA systems might also support ICSS in the ventral mesencephalon and hence these results might not be so convincing either. If CA pathways were in close proximity to those

supporting ICSS then it would be possible for them to be stimulated fortuitously. The same argument could be applied to the studies which have shown changes in NA metabolism after ICSS in the region of locus coeruleus, in the dorsal NA system. These have shown both increased metabolism of NA in terminal areas (Anlezark et al 1975) and increased TOH activity in locus coeruleus itself (Section Three). In view of the subsequent lesion and anatomical studies which have consistently shown that the dorsal NA system was not the sole substrate of ICSS from this region such evidence must be regarded as more coincidental than essential. However, it must be pointed out that the two studies which have shown increased release of CA's as measured by metabolite concentrations after ICSS (Anlezark et al 1975: Section One, this thesis) have shown that electrical self-stimulation can cause release of these transmitters. The release of CA could therefore have contributed to the ICSS behaviour. Studies of amine metabolism have not produced definitive evidence for the CA hypothesis of ICSS.

One problem that the studies of CA release by electrical stimulation in anaesthetised animals seemed to pose was that maximal release of CA's was obtained by stimulation frequencies much lower than those commonly used in ICSS experiments (typically 50 - 100Hz). Indeed it has been shown to be very difficult to obtain ICSS with frequencies below 40Hz (Wauquier et al 1972). Maximal stimulation of HMPG, a NA metabolite was obtained from 2 - 20Hz (Walter and Eccleston 1973; Korf et al 1973). Similarly, maximal stimulation of HVA, a DA metabolite was obtained at 25 Hz (Korf et al 1976). One possible reason for this difference might be that in these experiments stimulation lasted for long continual periods of up to 45 minutes. In the ICSS stimulus trains of up to 0.5 seconds were typically used, with at least short intervals between trains and hence less stimulation would be delivered in a given

time. Even allowing for this, electrophysiological studies have indicated that the CA cells firing rates were also very low, typically 2Hz for NA cells of LC (Graham and Aghajanian 1971) and 2 - 10 Hz for DA cells (Bunney et al 1973). It might be felt that stimulation rates of 50 - 100 Hz would produce supra-maximal stimulation of such cells, and raises the possibility that cells with a higher firing rate might be responsible for supporting ICSS.

One very interesting experiment which seemed to support the involvement of the dorsal NA system in ICSS was one in which it was shown that this system was activated by rewarding stimulation in the region of locus coeruleus (Segal and Bloom 1976). They showed that the hippocampal pyramidal cells which responded both to iontophoretic NA and LC stimulation in an inhibitory manner, were inhibited by LC self-stimulation, but not by stimulation of non-rewarding sites in this area. Activation of the dorsal NA system appeared to be necessary for ICSS. An analysis of hippocampal self-stimulation has cast doubt on this simple relationship (Wise 1978). Briefly, hippocampal self-stimulation was found most reliably in the CA3 region of hippocampus (Ursin et al 1966), where the pyramidal cells were found. The pyramidal cells had been shown to be innervated by the dorsal NA system and to have an inhibitory NA input (Segal and Bloom 1976). It thus appeared that both direct stimulation and indirect inhibition of pyramidal cells in the hippocampus was rewarding, which made a simple role for NA as the mediator of reward in this area impossible. The possibility that hippocampal self-stimulation was mediated by other cells which were also stimulated must be considered a possibility.

Although there might be grounds for doubting the role of the dorsal NA system in ICSS, the problem has remained that the dorsal brain stem

supported ICSS. There has been some evidence that this ICSS did not involve the mesencephalic DA systems (Section 4) and hence at least two rewarding systems existed in the brain. Other possible explanations for brain stem ICSS have been suggested.

Although Crow suggested that the locus coeruleus was situated in a position to receive gustatory input from the visceral afferent column, no such input has been demonstrated (German and Fetz 1976). However a central gustatory pathway has been identified in close proximity to the locus coeruleus. The nucleus of the solitary tract which receives a gustatory input was shown to project to the parabrachial nuclei. These cells also responded to gustatory stimuli and were considered to form a pontine taste area around the brachium conjunctivum. These cells themselves projected to the gustatory nuclei of the thalamus, and to lateral hypothalamus (Norgren and Leonard 1973). This pathway might have some relevance to ICSS, especially if the theory that afferent stimulation had primary rewarding properties (Pfaffman 1960) was correct. It has been shown that ICSS could be easily obtained from areas around the brachium conjunctivum (Routtenberg and Malsbury 1969) and indeed one of the sites reported in the original report of locus coeruleus ICSS (Crow et al 1972) was situated lateral to the mesencephalic trigeminal nucleus in this very area. Further support has come from the demonstration of ICSS in the medulla oblongata, in or near the nucleus of the solitary tract itself (Carter and Phillips 1975). From the description of the rostral projection of the pontine taste area dorso-medial through the pons it must be considered possible that the area anterior to locus coeruleus which has been shown to support strong and easily obtained ICSS, (see Section 4; Wise (1979)) contained these fibres. Although the projections from the nucleus of the solitary tract were concentrated around the brachium conjunctivum, they were

also found in the mesencephalic trigeminal nucleus (Norgren 1978). It thus seems possible that there were very few sites in the locus coeruleus, which would not perhaps have stimulated these neurones also. They would certainly have been stimulated in the study which claimed to demonstrate that the mesencephalic trigeminal nucleus supported ICSS (Van der Kooy 1979). In view of these observations this central gustatory pathway must be considered a possible mediator of ICSS in pontine areas. However more conclusive evidence must be obtained. The major difficulty in this must be in the organisation of these pathways, this being both diffuse at the pontine level and multi-synaptic. The identification of the neurotransmitters in these pathways must also be realised.

The trigeminal system itself has convincing claims to be a mediator of ICSS. Although it was considered as a possible mediator of ICSS in the original mapping study of LC, this possibility was discounted as ICSS could not be elicited from another part of this system, the motor trigeminal nucleus (Motor V) (Crow et al 1972). Subsequent work has shown that ICSS can be reliably elicited within and adjacent to MOT V. Stimulation-induced oral behaviour was associated with these sites, and post-stimulation jaw movements (Van der Kooy and Phillips 1978, 1979). The discrepancy between this and the previous study would be difficult to explain especially as very similar behavioural methods were used including extensive shaping.

The area in and around MES V, which has monosynaptic connections with MOT V (Mehler 1963) has been shown to support ICSS also (Van der Kooy 1979). An extensive mapping study which demonstrated the relationship of the catecholamine systems to the electrode site has also indicated a much better correlation of ICSS with MES V than with LC (Corbett and Wise 1979). Both of these studies have indicated that

ICSS was more difficult to obtain from the medial aspects of LC. The original study of Crow et al did obtain ICSS from these regions, but interestingly those sites required fairly high currents and at one of them jaw movements were seen which could indicate current spread to MES V.

Further evidence for the independent nature of trigeminal ICSS was also obtained. It has been shown that the sub-coeruleus NA neurones projected to the area of MOT V (Olson and Fuxe 1972) and hence their involvement in the ICSS was a possibility. There was no significant effect of bilateral lesions of the LC on ICSS from MOT V, either with electrolytic (Van der Kooy and Phillips 1979) or with 6-OHDA lesions (Van der Kooy 1979). As well as indicating that activation of the LC was not responsible for MOT V ICSS these also illustrated the existence within the brain stem of a non-DTB system supporting ICSS.

The ICSS obtained from the area of the nucleus of the solitary tract (NST) (Carter and Phillips 1975) has been suggested to involve the motor trigeminal nucleus (Van der Kooy 1979). The NST has a projection which passes through the area of MOT V but whether terminals or fibres of passage was not determined (Norgren 1978). It must be suggested that the obverse might equally be true, that the fibres from NST might be mediating ICSS. The existence of some sites around MOT V which elicited jaw movements without supporting ICSS (Van der Kooy and Phillips 1979) might support this assumption. In addition the area around MES V forms the pontine taste area (Norgren and Leonard 1973), and hence these gustatory pathways might be supporting ICSS there also. It must be pointed out however that a close anatomical relationship between electrode sites supporting ICSS and MES V (Corbett and Wise 1979: Van der Kooy 1979) and MOT V (Van der

Kooy and Phillips 1977, 1979) has been demonstrated. This has not been accomplished with the pontine gustatory pathways.

In conclusion, the results from these more recent experiments have indicated that the dorsal NA system from locus coeruleus was not essential for ICSS from the dorsal brain stem. The evidence for the involvement of the mesencephalic DA systems has indicated that at some sites in the brain this neurotransmitter mediated ICSS. However the problem remained complex as ICSS sites with a large DA innervation did not necessarily depend on DA for the maintenance of ICSS. The major problem would seem to be to determine the nature of non-DA ICSS, which appeared to be obtainable in the present studies from the dorsal brain stem and possibly from medial posterior hypothalamus. Interestingly lesions produced at a site in the dorsal brain stem similar to the present study which supported ICSS caused degeneration in the area of medial posterior hypothalamus (Clavier and Routtenburg 1976). This possible connection might be an interesting subject for study.

The hypothesis that ICSS was mediated by these NA and DA pathways could not be refuted by the present series of experiments which could all be interpreted as supportive. The results of these other experiments have shown however that other neural systems have to be integrated into a comprehensive explanation of ICSS. Interestingly, if the pontine gustatory pathway did support ICSS, the original hypothesis relating gustatory and olfactory modalities to ICSS might still be relevant.

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