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THE ROLE OF CATECHOLAMINERGIC AND NON-CATECHOLAMINERGIC MECHANISMS IN SELF-STIMULATION OF FOREBRAIN SITES IN THE RAT

by
Ann Margaret Robertson

Department of Psychology

Submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy

Faculty of Graduate Studies
The University of Western Ontario
London, Ontario.
August, 1978

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ABSTRACT

There has been a good deal of interest recently in the role of central neurotransmitters in brain-stimulation reward. A series of experiments was done using neuropharmacological techniques to investigate the contribution of noradrenalin, dopamine, acetylcholine and serotonin neurons to self-stimulation of selected sites in the forebrain of the rat. Self-stimulation was observed with electrodes in the supracallosal bundle, which contains fibers of noradrenergic neurons projecting from the locus ceruleus to the cerebral cortex and hippocampus. Evidence that noradrenergic neurons were being stimulated came from histological verification of stimulation sites, from antidromic activation of locus ceruleus neurons, from the attenuation of self-stimulation of the supracallosal bundle by administration of colchicine (which temporarily disrupts neural transmission), and from the demonstration of differential effects of D- and L-amphetamine on self-stimulation of the supracallosal bundle. However, self-stimulation of the supracallosal bundle was suppressed by the administration of spiroperidol, a dopaminergic antagonist, so that the possibility of contribution of dopamine neurons could not be excluded. Self-stimulation was observed with electrodes in the subfornical organ, which receives cholinergic and serotonergic neurons, and response rates were reduced by the administration of atropine sulphate, a cholinergic antagonist, and by methysergide, a serotonergic antagonist. Evidence that these drug effects were not mediated through a non-specific disruption of operant behavior came from the observation that self-stimulation of the lateral hypothalamus was not altered by administration of atropine or methysergide. Self-stimulation was observed with electrodes in the nucleus accumbens,
and prefrontal cortex, both of which contain dopaminergic projections from the ventral tegmental area. Microinjections of spiroperidol into the area of stimulating electrodes in the nucleus accumbens reduced response rates for stimulation through those electrodes but did not alter response rates for stimulation through electrodes in the contralateral nucleus accumbens, evidence that dopamine neurons contribute to self-stimulation of the nucleus accumbens. However, the role of dopamine neurons in self-stimulation of the prefrontal cortex was less clear since microinjections of spiroperidol into the area of stimulating electrodes in the prefrontal cortex was effective only at the highest dose. Microinjections of spiroperidol into the prefrontal cortex did not alter self-stimulation of the nucleus accumbens and microinjections of spiroperidol into the nucleus accumbens did not change self-stimulation of the prefrontal cortex. In order to further investigate the role of dopamine in self-stimulation of the prefrontal cortex, spiroperidol was administered systemically for nine days. When spiroperidol was administered two hours before daily tests, self-stimulation was suppressed during the period of administration and facilitated after the period of administration. When spiroperidol was administered after the tests, and approximately 22 hours before the subsequent daily tests, self-stimulation was facilitated both during and after the period of administration. These facilitatory effects were specific to self-stimulation of the prefrontal cortex since self-stimulation of the nucleus accumbens, subfornical organ, supracallosal bundle, caudate-putamen, or ventral tegmental area was not facilitated by daily administration of spiroperidol. Chronic administration of D-amphetamine also caused a facilitation of self-stimu-
lation of the prefrontal cortex but a suppression of self-stimulation of the supra- callosal bundle. The results provide evidence that dopamine is involved in self-stimulation of the prefrontal cortex although its role may be modulatory rather than essential. Additionally, there are similarities between the effects of spiroperidol on self-stimulation of the prefrontal cortex and the therapeutic effects of neuroleptics in humans. Both may be mediated by changes in prefrontal dopaminergic activity. Overall, the data indicate that brain-stimulation reward is not exclusively dependent on one transmitter-specific system. Several catecholaminergic and non-catecholaminergic mechanisms may mediate or modulate self-stimulation.
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I would like to thank my advisory committee—Drs. Cain, Hirst, Innis, and Vanderwolf—for their suggestions and encouragement throughout the course of this work.

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INTRODUCTION

Electrical stimulation delivered through electrodes implanted into several regions of the vertebrate brain can act as a positive reinforcer in that animals will learn an operant response to gain access to the stimulation. This behavior is termed "self-stimulation" (SS) and was first reported by Olds and Milner (1954), who noted that rats receiving electrical stimulation of the septal area in an open field repeatedly returned to the place where they had last been stimulated. The experimenters subsequently discovered that rats would press a bar in a Skinner box to receive the stimulation. Olds (1958) later demonstrated that animals will learn other operants, such as traversing a maze, running down an alleyway, and crossing an electrified grid delivering painful electrical shocks to the paws, to gain access to the stimulation.

Since these pioneering studies, there has been a lively and continuing interest in the behavior of SS, its characteristics and neural substrates. Not only is SS an intriguing behavior in itself, but the study of SS has other implications. It is relevant to many issues and problems in the psychology of learning. Under the appropriate conditions, central and conventional reinforcers can be shown to be quite similar (Mogenson & Cloé, 1977). Therefore SS may provide the means of directly studying the neural substrates of reinforcement processes. Additionally, the study of SS has sparked theories regarding the etiology of mental disorders in man, based on the idea that central "reward systems" are somehow altered or disordered in such cases (example, Crow, 1973; Stein, 1971). Finally, the study of SS holds the promise of revealing basic and common properties of neuronal functioning in
relation to behavior since SS has been demonstrated in a wide variety of species, from fish (Boyd & Gardner, 1962) to man (Heath, 1963).

This thesis is concerned specifically with the neurochemical substrates of SS in several regions of the forebrain of the rat. Interest in identifying the neurotransmitters critical for maintaining central reinforcement dates back many years. Four putative neurotransmitters have received particular attention: acetylcholine (ACh), serotonin (5-hydroxytryptamine or 5-HT), dopamine (DA) and noradrenaline (norepinephrine or NE). The development of new techniques for visualizing and mapping the catecholamine (DA and NE) neurons in the rat central nervous system (see Appendix 1), particularly in the early part of this decade, focused the attention of many scientists on the possibility that either or both of these neurotransmitters might play a critical role in central reinforcement. Evidence which has been used to implicate NE and DA in SS is critically reviewed in separate sections below and the possible roles of 5-HT and ACh in the mediation of SS are also considered. As this review of the literature will make clear, it has proven difficult to assess the importance of these neurotransmitters in SS. In large part, this is due to some major methodological problems and problems of interpretation which have made the several theories of the neurochemical bases of SS quite controversial in recent years. These problems are discussed following the literature review. Finally, experiments designed to investigate the neurochemical substrates of SS in several selected brain sites in the rat are described.
The Role of Noradrenaline in Sulf-Stimulation

Anatomical and Neurochemical Evidence

The "noradrenergic hypothesis" of SS, which states that SS depends on the activation of NE neurons and the subsequent release of NE from terminals, was the first and for a long time the foremost theory which sought to explain SS in terms of stimulation and release of a specific neurotransmitter. Formulated originally by Stein (1962, 1964) and later by others (Poschel & Ninteman, 1963; Dresse, 1966a), it was based primarily on evidence from anatomical, neurochemical and pharmacological studies. Olds, Travis and Schwing (1960) and Olds and Olds (1963) identified the medial forebrain bundle as the main neuroanatomical substrate of SS. Since NE neurons were known to be contained within the medial forebrain bundle, it was proposed that stimulation of NE fibres in this bundle was necessary for SS (Stein, 1964). Later, it was shown that the locus ceruleus, the origin of a dorsal group of NE fibres innervating the cortex and hippocampus, also supports SS (Crow, Spear & Arbuthnott, 1972; Ritter & Stein, 1973), as do other areas containing NE perikarya in the hindbrain (Ritter & Stein, 1974). In fact, many if not all NE areas of the brain support SS (German & Bowden, 1974). However, as Fibiger (1978) noted, it is not surprising that NE neurons should be located in areas supporting SS, given their widespread distribution in the central nervous system. Therefore the anatomical data must be interpreted with care.

A number of neurochemical studies have demonstrated a relationship between noradrenergic activity and SS. There is an increased turnover of NE follow-
ing stimulation or lesions through electrodes in the midbrain and hindbrain which support SS (Dresse, 1966a; Stinus, Thierry, Blanc, Glowinski & Cardo, 1973; Arbuthnott, Fuxe & Ungerstedt, 1971). Stein and colleagues (Stein, 1968a, b; Stein & Wise, 1969) infused labelled NE through push-pull cannulae in the lateral hypothalamus, amygdala, or ventricles. Stimulation of points in the medial forebrain bundle that supported SS was accompanied by high radioactivity counts in the perfusate whereas stimulation of points not supporting SS was not.

It is important to note, however, that electrical stimulation through electrodes supporting SS might well be expected to affect NE activity if fibres were intermingled with other more critical fibres in the area. Additionally, since some degree of arousal can accompany the electrical stimulation in unanesthetized subjects, NE might be released as a result of a general arousal. Thus the data must be interpreted with caution.

Pharmacological Evidence

A number of pharmacological data have also been used in support of a role for NE. Table 1 lists many of these studies and the main results. Generally, it has been observed that substances which release NE or mimic the action of NE at receptor sites facilitate SS and decrease the stimulation threshold for eliciting SS. Thus amphetamine, phenylethylamine (with an MAO inhibitor), iproniazid (an MAO inhibitor), and l-NE (when injected centrally) all increase SS rates. Conversely, drugs which deplete NE or block its action such as alpha-methyl-p-tyrosine, chlorpromazine, reserpine, and parachlorophenylalanine (PCPA) decrease SS rates.
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<td>Wise, Berger &amp; Stein (1973)</td>
<td>-phentolamine</td>
<td>-alpha-adrenergic block*</td>
<td>-suppression</td>
</tr>
<tr>
<td>Haggard (1976, a-c)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Harburg, Stephens &amp; Franklin (1976)</td>
<td>-propranolol</td>
<td>-blocks NE reuptake &amp; blocks NE receptors</td>
<td>-suppression</td>
</tr>
<tr>
<td>Risler &amp; Stein (1972)</td>
<td>Reserpine</td>
<td>-alpha-adrenergic block (depletes NE stores)</td>
<td>-suppression</td>
</tr>
<tr>
<td>Huglery, Fisher &amp; Kamen (1968)</td>
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<tr>
<td>Gibson, McGee &amp; McGee (1970)</td>
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<tr>
<td>Poschel &amp; Ninteman (1963)</td>
<td>-tetraethane</td>
<td>-depletes NE</td>
<td>-suppression</td>
</tr>
</tbody>
</table>

* Relatively specific to NE transmission
Since all of these agents affect the transmission of more than just NE, it is difficult to conclude that NE mediates SS. For example, chlorpromazine can also affect DA transmission. The data could therefore be interpreted to mean that DA is involved in SS. On the other hand, there are drugs which are relatively specific in their effects on NE transmission (see Table 1). Disulfiram, which inhibits NE synthesis by blocking the converting enzyme dopamine-beta-hydroxylase, inhibits SS and this inhibition can be reversed by intraventricular administration of NE (Wise & Stein, 1969). Phentolamine, an alpha-adrenergic blocking agent, will also suppress SS (although propranolol, which blocks beta-adrenergic receptors, does not) (Wise, Berger & Stein, 1973; Hasegawa, 1976a, c).

The main problem with interpreting these data is that the drugs may alter SS rates not because the positive reinforcement is altered but because the animal is sedated. For example, Roll (1970) observed that rats given disulfiram appeared asleep or sedated. If, however, a rat was picked up and placed back on the response lever, it resumed bar-pressing at normal rates, which should not have been the case if disulfiram had decreased reward. Similarly, Rolls, Kelly and Shaw (1974) used measures of locomotor activity and rearing to assess the sedative effects of phentolamine and disulfiram and found that the suppression of response rates for electrical stimulation could be accounted for by the sedation observed. On the other hand, Liebman and Butcher (1973) observed that doubling the electrical current reinstated normal response rates in rats after disulfiram treatment. Since the animals could bar press at normal rates, they suggested that non-specific behavioral deficits could
not account for the inhibition. It is possible, however, that doubling the current
increased general arousal to a point where the sedative effects of disulfiram were
counteracted.

There are two dopamine-beta-hydroxylase inhibitors, FLA-63, and
U-14, 624, which do not exert marked sedative effects (Corrodi, Fuxe, Hamberger
& Ljungdahl, 1970). Hasegawa (1976a) and Hasegawa and Sakai (1976) have report-
ed that both these compounds decrease SS of the substantia nigra, lateral hypoth-
alamus, and dorsal noradrenergic bundle. In apparent contradiction to these studies
are reports by Breese and Cooper (1975) who found no change in SS of the locus
ceruleus after U-14, 624 treatment, and Stinus, Thierry and Cardo (1976) who admin-
istered reserpine to rats 24 hours before administering FLA-63 or U-14, 624 and found
no change in SS of the lateral hypothalamus, despite massive reductions in NE levels
in the brain. However, Franklin and Herberg (1975) reported that, with reserpine
pretreatment three to five days before administration of FLA-63, SS of the lateral
hypothalamus was suppressed and that this suppression could be reversed by intra-
ventricular administration of NE. They concluded that NE in intraneuronal, reser-
pine-sensitive reserve pools is required for SS to occur. As Fibiger (1978) observed,
however, adequate behavioral controls were not included in this study. Even though
the rats displayed no overt signs of behavioral debilitation, more subtle deficits may
have been present and caused the changes in SS rates.

Other evidence from pharmacological studies clearly does not support
a role for NE in SS. For example, Gibson, McGeer and McGeer (1970) measured
NE levels after administration of DL-5-bromotryptophan, a tyrosine hydroxylase inhibitor, and correlated changes in NE levels with changes in SS. They found that, although sizeable changes in NE occurred, there were only small changes in SS rates. Therefore, even if specific NE drugs are used, it is difficult to conclude that NE is involved in SS, particularly if stringent behavioral controls are not used.

One such behavioral control is to show that responding from electrodes in one site is affected by a drug while responding from electrodes in another site is not. Olds and colleagues in several studies demonstrated that animals with posterior hypothalamic electrodes are more sensitive to the rate-decreasing effects of chlorpromazine or iproniazid (an MAO inhibitor) than are animals with electrodes in the anterior hypothalamus (Olds & Travis, 1960; Olds, 1958; Olds & Olds, 1958). This could be interpreted to mean that NE is more important in SS of the posterior hypothalamus than the anterior hypothalamus. This further suggests that NE is not the only neurotransmitter involved in SS. Another study by Stark, Turk, Redman and Henderson (1969) reported apparently contradictory results in that rats with anterior hypothalamic placements were more sensitive to chlorpromazine than were animals with posterior hypothalamic electrodes even though both groups had the same baseline rates of responding before drug treatment. This discrepancy might be accounted for by procedural differences; at any rate it is difficult to conclude what role NE might play from these data, particularly since both chlorpromazine and iproniazid affect DA as well as NE activity.
Evidence from Lesion Experiments

Evidence from studies using techniques to selectively destroy areas of the central nervous system has been used in support of the NE hypothesis of SS. Stein, Belluzzi, Ritter and Wise (1974) abolished SS of the DA-containing substantia nigra by knife cuts made caudal to the SS electrodes which destroyed ascending NE neurons. The knife cuts would probably, however, destroy other neurons in the area therefore it is difficult to conclude that destruction of NE neurons was responsible for the changes in SS. The experimenters also reported that injections of the neurotoxin 6-OHDA into the dorsal and ventral NE pathways suppressed SS of the substantia nigra on the treated side. It is possible, however, that 6-OHDA spread also to DA pathways to suppress responding since there was a drop in striatal DA levels. Again, it is unclear from these results that NE plays a critical role in SS of the substantia nigra.

Not only is the supporting evidence inconclusive, but there is evidence which is contrary to the NE hypothesis of SS. Several studies have demonstrated that lesions of NE cell bodies or their projections fail to disrupt SS. Clavier, Fibiger and Phillips (1976) observed no changes in SS of the midbrain tegmentum after 6-OHDA lesions of the locus ceruleus or ventral noradrenergic bundle. Similarly, Corbett, Skelton and Wise (1977) observed no changes in locus ceruleus SS after electrolytic lesions of the dorsal noradrenergic bundle. Placing electrodes further rostral in the dorsal noradrenergic bundle, Clavier and Routtenberg (1976) failed to disrupt SS after locus ceruleus or ventral noradrenergic bundle lesions even though telencephalic
NE was decreased by more than 80%. Finally, it has been observed that 6-OHDA lesions of the dorsal noradrenergic bundle projecting to the hippocampus and olfactory bulb does not disrupt SS of these sites (Van Der Kooy, Phillips & Fibiger, 1977; Phillips, Van Der Kooy & Fibiger, 1977).

If SS of structures such as the locus ceruleus, which is caudal to the DA-containing neurons does not depend on activation of NE neurons, then other mechanisms must be sought. Rolls and Cooper (1974) suggested that SS of the locus ceruleus depends on descending, not ascending, projections from the prefrontal cortex, since prefrontal cortex neurons were antidromically activated by stimulation of rewarding locus ceruleus or pontine tegmental sites. More precise data concerning the critical neural substrates of SS of the locus ceruleus are provided by Koob, Fray and Iverson (1978), who found they could disrupt SS of the locus ceruleus by 6-OHDA injections into the ipsilateral but not the contralateral ventral tegmental area, an area containing major groups of DA cell bodies. These data suggest that the ventral tegmental area may be important in SS of the locus ceruleus and, by inference, that NE neurons of the locus ceruleus itself may not be important.

Summary and Conclusions

Anatomical, neurochemical and pharmacological data have been used to implicate NE neurons in SS. The anatomical and neurochemical data are inferential, however. The pharmacological data are largely inconclusive since it is unclear whether some of the drugs used were altering SS rates due to effects on
NE or due to effects on other neurotransmitters. Additionally, drugs affecting NE transmission might decrease SS rates due to sedation or increase SS rates due to arousal. Lesion data have been used to support a role for NE, but there are several studies which have failed to disrupt SS in spite of severe depletions of brain NE, which inevitably places doubt on the necessity of NE for SS. Thus the role of NE in SS is unclear. NE has not been demonstrated to be the sole or necessary mediator of SS. It may, however, be sufficient to support SS at least in some parts of the brain. This possibility deserves further attention in view of the experiments by Olds and colleagues (Olds, 1958; Olds & Olds, 1958; Olds & Travis, 1960) which do report site-specific drug effects on responding. Additionally, the generally negative findings of the lesion studies should not be construed as evidence that NE does not play a role in SS. SS of sites other than those tested might be mediated by NE.
The Role of Dopamine in Self-Stimulation

Evidence from Anatomical and Lesion Experiments

Dopamine, another catecholamine whose distribution within neurons has been extensively mapped in the central nervous system, has received much attention lately. Crow (1972b) proposed that DA mediates SS after observing that SS can be obtained from A9 and A10 DA cell bodies in the mesencephalon (Routtenberg & Malsbury, 1969; Crow, 1972a). This proposal is supported by anatomical studies showing that SS in other DA-containing areas will support SS (cf. review by German & Bowden, 1974). However, SS is observed when electrodes are in areas where there is limited or no dopaminergic innervation such as the hippocampus (Van Der Kooy, Fibiger & Phillips, 1977) and the dorsal tegmental area (Crow, Spear & Arbuthnott, 1972). Therefore direct stimulation of DA neurons is not necessary for SS to occur although this does not preclude transynaptic activation of DA neurons.

If DA is involved in SS, then destruction of DA neurons should decrease or abolish SS. One popular technique for destroying DA neurons is by injection of 6-OHDA. In one of the first studies using this technique, Breese, Howard and Leahy (1971) found that 6-OHDA injections into the ventricles of rats self-stimulating through lateral hypothalamic electrodes caused sharp drops in responding. Intraventricular injections of 6-OHDA might also alter NE transmission thus it is difficult to conclude which transmitter was critical from this study.

Even when destruction of tissue is limited to dopaminergic neurons, the possibility that changes in SS rates are secondary to a general disruption of
operant behavior must be controlled for. 6-OHDA given in combination with desimi-
pramine (which prevents uptake of 6-OHDA into NE neurons) causes a selective de-
struction of DA neurons. Using this method, Cooper, Cott and Breese (1974) demon-
strated a suppression of SS of the lateral hypothalamus after intracisternal injections
of 6-OHDA. SS rates did, however, recover fully. When alpha-methyltyrosine was
given along with the 6-OHDA treatment to completely deplete DA in the brain, SS
rates dropped significantly. 6-OHDA treatments which depleted NE levels but not
DA levels had no effects on SS. The authors therefore suggested that DA is impor-
tant in maintaining SS of the lateral hypothalamus. Since, however, the severe
depletion of DA associated with this treatment might have disrupted operant abilities,
It is difficult to conclude that the deficit is specific to SS behavior.

Other studies using 6-OHDA to deplete DA have controlled for non-
specific effects by injecting 6-OHDA unilaterally and testing SS ipsilateral and con-
tralateral to the site of injection. For example, Phillips, Carter and Fibiger (1976)
examined the role of nigrostriatal DA neurons in SS of the caudate-putamen. They
injected 6-OHDA into the substantia nigra unilaterally and examined SS of the
caudate-putamen both ipsilateral and contralateral to the site of injection. SS of
both ipsilateral and contralateral sites dropped initially, however SS of the contra-
lateral caudate-putamen returned to nearly normal rates over the three week test
period whereas SS of the ipsilateral caudate-putamen remained at very low levels.
Thus, although the 6-OHDA lesions may have produced an initial general disruption
of bar-pressing, the results provide evidence that DA neurons from the substantia
nigra innervating the caudate-putamen are normally involved in SS of this site. Using a similar paradigm, Clavier and Fibiger (1977) examined the role of nigrostriatal DA neurons in SS of the substantia nigra. They observed that 6-OHDA lesions of the nigrostriatal bundle both ipsilateral and contralateral to the SS electrodes resulted in only temporary decrements of SS, suggesting that transient motor deficits produced the initial suppression of SS.

More recently, Phillips and Fibiger (1978) examined the role of mesocorticolimbic DA neurons in SS of the ventral tegmental area, nucleus accumbens and prefrontal cortex. They made 6-OHDA lesions of these DA neurons in the region of the far lateral hypothalamus and subsequently examined responding from the origin of these fibres in the ventral tegmental area and from termination points in the nucleus accumbens, medial prefrontal cortex, and sulcal prefrontal cortex. They observed that SS of the ventral tegmental area was disrupted whereas SS of the other three sites was not altered or suffered only temporary disruption. The attenuation of SS of the VTA could not be accounted for by general disruption of operant behavior since SS of the anterior sites was still elicited. Thus they concluded that DA systems are critical for the maintenance of SS of the ventral tegmental area.

Using a similar paradigm, Koob, Fray and Iverson (1978) recently tested the hypothesis that intact DA systems are required for the maintenance of lateral hypothalamic and locus ceruleus SS. They observed, following unilateral 6-OHDA injections into the substantia nigra and ventral tegmental area which produced significant decreases in DA in telencephalic areas, that SS of the ipsilateral
lateral hypothalamus or locus ceruleus was severely and permanently attenuated. On the other hand, contralateral SS was only transiently affected, although the results were quite variable. Interestingly, sham injections produced disruptions of responding accompanied by decreases of both DA and NE, suggesting that non-specific damage to neurons in the area by the injection itself might disrupt activity in critical neural systems in that area. Additionally, since NE levels were depleted on the injection side in several of the groups tested, it is difficult to determine just how critical DA neurons are to SS of the lateral hypothalamus or locus ceruleus. The results do suggest, however, that some neurons in the area of the ventral tegmental area or substantia nigra might be critical.

Experiments by Huston and Ornstein (1975) and Ornstein and Huston (1975) provide conflicting evidence with respect to the importance of the substantia nigra DA neurons in SS of the lateral hypothalamus. They found that 6-OHDA injections into the substantia nigra or knife cuts of the nigrostriatal bundle disrupted responding from lateral hypothalamic electrodes both contralateral and ipsilateral to the lesion site. It seems likely, therefore, that the bar-pressing ability was impaired. In order to more accurately assess this possibility, the authors used simpler operants such as head-turning or a curling-up response. They found that rats could still perform these simpler responses to receive brain stimulation, implying that the lesions had affected primarily the ability to perform complex operant responses.

One possible explanation for the discrepancy between these results and those of Koob, Fray and Iverson (1978) (who concluded that DA systems are important in SS
of the lateral hypothalamus) is that, in the latter case, mesolimbic and mesocortical DA pathways were also destroyed. These pathways may be critical in SS of the lateral hypothalamus. Perhaps more important, however, is the observation of other experimenters that SS of the lateral hypothalamus may depend on different substrates, depending on the precise location of the electrode (Anderson, Leith & Barrett, 1978; Herberg, Stephens & Franklin, 1976; Eichler, Antelman & Fisher, 1976).

Pharmacological Evidence

Much of the pharmacological evidence which has been used in support of the NE hypothesis can also be used to support a role for DA in SS. Referring back to Table 1, the effects of drugs such as alpha-methyl-m-tyrosine, tetrabenazine, alpha-methyl-p-tyrosine, and chlorpromazine could be interpreted as support for either the NE or the DA hypothesis of SS. There are, however, many drugs which act fairly selectively on DA neurons and which alter SS rates. An example of a group of drugs which do so are the neuroleptics, including such agents as haloperidol, spiroperidol, and pimozide. Experiments showing that such drugs inhibit SS were first reported by Dresse (1966b). Dresse, however, attributed the effects to changes in activity of NE neurons. Since the demonstration by Janssen (1970) and others (see review by Iverson, 1975) that these drugs act primarily to block DA receptors, this evidence might now be regarded as implicating DA in brain-stimulation reward.

Subsequent studies confirmed that neuroleptics suppress SS (Wauquier & Niemegeers, 1972; Rolls, Rolls, Kelly, Shaw, Wood and Dale, 1974; Phillips,
Brooke & Fibiger, 1975; Liebman & Butcher, 1973). There is disagreement however, regarding the specificity of action of these drugs on SS. As was the case in examining the effects of NE drugs on SS and the effects of lesions of DA neurons on SS, it has been proposed that neuroleptics might disrupt responding because of non-specific impairments in operant behavior. For example, neuroleptics disrupt shock-avoidance responding (Niemsegeers, Verbruggen & Janssen, 1969). In support of this "motor deficit" interpretation, Rolls, Rolls, Kelly, Shaw, Wood and Dale (1974) showed that doses of spiroperidol which suppressed SS responding also disrupted bar-pressing for food and water. It might be argued that bar-pressing for food or water is not strictly comparable to bar-pressing for SS since the rates of responding are likely to be different. When rates of responding for food or for SS are equalized by use of a variable interval schedule of reinforcement, however, there is still an equal attenuation of responding for electrical stimulation and responding for food (Fibiger, Carter, & Phillips, 1976). Wise, Spindler, De Wit and Gerber (1978), have recently suggested that DA may mediate the rewarding value of food to a food-deprived animal, much the same as DA has been proposed to mediate SS. Thus, it might be expected that neuroleptics would suppress responding for food and water. Additionally, the presence of a non-specific deficit in responding might "mask" any specific effect on positive reinforcement from brain-stimulation so that such an effect cannot be measured.

In another approach to the problem, Mora, Sanguinetti, Rolls and Shaw (1975) hypothesized that a simpler operant might be more immune to any gen-
eral disruptive effects of neuroleptics and therefore any decrement observed would likely be due to disruption of brain-stimulation reward. They selected a licking response rather than bar-pressing response for brain stimulation and found that licking was as severely attenuated as bar-pressing. As Fibiger and Phillips (1978) observed, however, base line rates of responding, which might have affected the results if they were different, were not reported. Thus the data do not provide unequivocal evidence about the role of DA.

Another method of distinguishing the effects of neuroleptics on SS from effects on general operant behavior has been used by Atrens, Ungerstedt and Ljungberg (1976). They tested animals for SS in a "rate-free" shuttle box (where the animal must move to one side of the box to initiate stimulation and leave it to escape stimulation), reasoning that, if a drug were affecting behavior non-specifically, both the latency to initiate and the latency to escape stimulation should change in the same direction. If only one changed, or if the two latencies changed in opposite directions, it would suggest a specific effect on brain-stimulation reward. They tested the effects of the neuroleptics clozapine and haloperidol on SS of the hypothalamus and found that, whereas haloperidol increased both latencies, clozapine in low doses increased the latency of initiation but caused no change in escape latency. Thus, they concluded that haloperidol affected general operant performance and clozapine affected reward. Although both these drugs would presumably act on the same DA neurons, clozapine might have lessened any extrapyramidal side effects due to its anticholinergic action (Iverson, 1975), a property which appears to count-
eract the extrapyramidal side effects associated with these antidopaminergic drugs. It is possible, however, that escape behavior is less sensitive to any general disruption than is approach behavior.

A different approach has been to examine the cumulative response curves after drug administration. If a drug produces the same effects as turning off the current, then one should observe an initially high rate of responding followed by a much lower rate. A non-specific effect on operant behavior would be more likely to produce a steady rate of responding throughout the test session. Fouriezos and Wise (1976) reported that pimozide treatment tended to produce a cumulative response curve similar to extinction curves seen in normal animals, evidence that pimozide produced the same effects as turning off the current. However in a similar experiment, Fibiger and Phillips (1978) reported that cumulative response curves under extinction conditions are different when the animal is administered haloperidol than when it is not. If haloperidol acts to decrease brain-stimulation reward, the response curves should have been the same or very similar.

Therefore any conclusions regarding the role of DA in brain-stimulation reward based upon the effects of DA receptor blocking agents must be regarded as tentative. There is considerable evidence indicating that these drugs do have non-specific effects even in very low doses. The majority of experiments attempting to modify or circumvent these non-specific effects have not been conclusive. In this regard, a recent experiment by Franklin (unpublished) is worthy of note. He used the runway method of Edmonds and Gallistel (1977) to distinguish reward from
performance decrements after the administration of pimozide. Under non-drug conditions, a reward-summation curve can be generated which relates running speed in the alley to the number of pulses received at the end of the alley. The location of the sharp rise in running speed on the curve generated, as the number of pulses increases, is altered by pimozide treatment. The optimal running speed, however, is not changed, indicating that the rats probably did not have a performance or motor deficit and that pimozide may have altered the rewarding aspects of stimulation in the runway.

Drugs which stimulate DA receptors such as apomorphine (Ernst, 1967) have also been used to implicate DA neurons in brain-stimulation reward. Apomorphine has been reported to decrease SS at some sites, particularly in the ventral mesencephalon and prefrontal cortex (Liebman & Butcher, 1973; Herberg, Stephens & Franklin, 1976; St. Laurent, Leclerc, Mitchell & Miliaressis, 1973; Mora, Phillips, Koolhas & Rolls, 1976). This may seem paradoxical since administration of DA receptor blockers also suppresses SS at these sites. Recent evidence suggests, however, that apomorphine acts, in low doses, preferentially on autoreceptors located on DA neurons, resulting in inhibition of activity of post-synaptic DA receptors (Carlsson, 1978). Apomorphine can also facilitate SS (Mora, Phillips, Koolhas & Rolls, 1976; Cazalo & Cardo, 1977; Kadzielawa, 1974; Herberg, Stephens & Franklin, 1976). Wauquier and Niemegeers (1973) found mixed facilitatory and inhibitory effects and noted that, although the effect of apomorphine depends on the dose, the stimulus parameters, and the time of measurement, even when these factors are held constant
both facilitation and inhibition of SS were found. Broekkamp and Van Rossum (1974) also reported mixed effects which could not be related to the site of stimulation. They suggested that apomorphine reinforces whatever response is occurring predominantly at the time of administration of action of the drug. If a rat is pressing at high rates, bar-pressing will be likely to be reinforced; if the rat responds at low rates, other behaviors might be reinforced, producing suppression of SS rates. This proposal might provide an explanation of the mixed effects of apomorphine on SS since apomorphine itself acts as a positive reinforcer in that animals will perform a response to obtain injections of the drug (Baxter, Gluckman, Stein & Scerni, 1974; Baxter, Gluckman & Scerni, 1976).

Summary and Conclusions

It is difficult to interpret the effects of DA drugs on SS, due to problems of non-specificity. Evidence for a role for DA is made stronger by lesion studies. Unilateral 6-OHDA lesions of the SN and ventral tegmental area produce a relatively permanent suppression of SS of the ipsilateral caudate-putamen (Phillips, Carter and Fibiger, 1976a) and locus ceruleus or lateral hypothalamus (Koob, Fray and Iverson, 1978), but only transient effects on SS of the contralateral sites. This is good evidence that DA, or neurons in that area, contribute to SS of these structures. Additionally, the finding that rats self-administer apomorphine suggest that DA may play a role in SS.

These studies also suggest that DA is not the unitary substrate of SS. If this is true, then the finding that neuroleptics, when administered peripherally,
suppress SS from all brain regions tested further emphasizes the possibility that such
effects may be non-specific. Obviously very stringent controls are required in
pharmacological studies.
The Role of Acetylcholine in Self-Stimulation

A role for ACh in SS is suggested primarily by pharmacological studies. There is ample evidence that administration of cholinergic compounds affect SS rates. Stark and Boyd (1963) first demonstrated that physostigmine, which retards the metabolism of ACh by inhibiting AChE, suppresses SS in dogs. This was subsequently confirmed in the rat (Jung and Boyd, 1966; Newman, 1972; Pradhan & Kamat, 1972; Malick & Goldberg, 1970; Domino & Olds, 1968; Olds and Domino, 1969a, b). The effects of physostigmine on SS are, moreover, immediately preceded by a decrease in AChE activity and increased ACh levels (Domino & Olds, 1968; Olds & Domino, 1969b). However, since physostigmine might also disrupt food-reinforced behavior, the effects on SS might be due to a general disruption of operant activity, as suggested by Newman (1972). Olds and Domino (1969b) attempted to control for this possibility by examining both escape behavior from aversive stimulation of midbrain loci and SS behavior through LH electrodes. They found that escape behavior was much less sensitive to physostigmine than was SS behavior. Escape behavior might be more resistant to a general disruption; however sodium pentobarbital in low doses suppressed both behaviors to the same degree—evidence that physostigmine might have had specific effects on SS.

Arecoline and pilocarpine, both cholinomimetics, also suppress SS (Newman, 1972; Olds and Domino, 1969a, b; Pradhan and Kamat, 1972). The effects of these drugs and of physostigmine on SS can be blocked by administration of muscarinic cholinergic blocking agents such as atropine (Stark & Boyd, 1963; Jung & Boyd,
1966) and scopolamine (Olds & Domino, 1969a, b; Newman, 1972; Pradhan & Kamat, 1972, 1973). These data suggest that activation of muscarinic receptors might inhibit SS. If so, then administration of muscarinic blockers should facilitate SS. Atropine, however, has been reported to have mixed effects, including suppression (Newman, 1972), acceleration (Pradhan & Kamat, 1972, 1973) and no change at all (Newman, 1972; Stark & Boyd, 1963). Scopolamine has been reported to increase SS (Olds & Domino, 1969a; Pradhan & Kamat, 1972). However since it also increases escape behavior from aversive stimulation of midbrain loci (Olds & Domino, 1969b), the effects might be explained by general arousal.

Largely on the basis of this apparent lack of uniform effects of atropine and scopolamine, it has been proposed that muscarinic cholinergic activation can modulate activity in neurons mediating SS but is not essential for SS (Newman, 1972; Olds and Domino, 1969a, b). It has also been proposed that activation of nicotinic cholinergic neurons facilitates SS (Pradhan, 1975), since the administration of nicotinic compounds facilitates SS and mecamylamine, a nicotinic receptor blocker, blocks this facilitation (Newman, 1972; Pradhan & Bowling, 1971; Pradhan & Kamat, 1972). Olds and Domino (1969a, b) reported, however, transient suppression of SS following nicotine.
The Role of Serotonin in Self-Stimulation

As was the case in examining the role of ACh in SS, the evidence suggesting that 5-HT is involved in SS comprises primarily of pharmacological data. There are several studies using drugs to implicate 5-HT in SS which are particularly interesting because of the site-specific effects of the drugs. One drug which has been used frequently to investigate 5-HT mechanisms is p-chlorophenylalanine (PCPA), which was originally reported to selectively deplete 5-HT levels with little or no effect on NE levels 24 hours or more after drug administration (Koe & Weisssman, 1966). When SS is tested anywhere from 24 hours to three days after injection of PCPA, facilitatory effects are usually observed with electrodes in the lateral hypothalamus (Van Der Kooy, Fibiger & Phillips, 1977; Phillips, Carter & Fibiger, 1976b; Poschel & Ninteman, 1971) or in the ventral tegmental area or the substantia nigra (Poschel & Ninteman, 1971; Miliareissis, 1977). These are regions through which ascending 5-HT axons project (Dahlstrom, Haggendal & Atak, 1973). 5-6-dihydroxytryptamine, which is taken up by 5-HT neurons and destroys them, facilitates SS when electrodes are in the medial forebrain bundle (through which 5-HT axons project), but not when electrodes are outside the medial forebrain bundle (Poschel, Ninteman, McLean, & Potoczak, 1974). 5-HTP, the precursor of 5-HT, causes small decreases in SS when electrodes are in the medial forebrain bundle (Poschel & Ninteman, 1973). Similarly, p-chloroamphetamine, which releases 5-HT, has been reported to suppress SS of the medial forebrain bundle. Contrary to these reports, however, Gibson, McGeer and McGeer, (1970) reported that PCPA and
DL-6-fluorotryptophan (another tryptophan hydroxylase inhibitor) increased the threshold for SS of the majority of animals self-stimulating through electrodes in the medial forebrain bundle-posterior hypothalamic region.

When electrodes are in termination areas of 5-HT neurons such as the hippocampus (Van Der Kooy, Fibiger & Phillips, 1977) or caudate-putamen, PCPA suppresses SS four days after administration, at a time when 5-HT levels in the brain are only 20% of normal levels (Van Der Kooy, Fibiger & Phillips, 1977). When the effects of PCPA are examined with SS electrodes in the nuclei of origin of these fibres, the dorsal and medial raphe nuclei, variable effects are reported. Margules (1969) reported no effects on dorsal raphe SS 24 hours after PCPA. However, Simon, LeMoal, & Cardo (1976) have reported facilitation of responding. On the other hand, SS of the medial raphe reportedly decreases 24 hours after PCPA administration (Miliaressis, 1977).

One of the major problems with the interpretation of the effects of these drugs is that the results might also be accounted for by changes in NE transmission. PCPA, although it was first reported by Koe and Weissman (1966) to have negligible effects on NE neurons after 24 hours, has since been reported to have marked effects on NE levels in many if not all areas of the rat brain up to five days after administration (Miller, Cox, Snodgrass, & Maickel, 1970). Similarly, 5-6-dihydroxytryptamine, which was used by Poschel, Ninteman, McLean and Potoczak (1974) to implicate 5-HT in SS of the medial forebrain bundle, was reported by the authors to cause small but significant depletions of brain NE during the time SS was
being tested. p-chloroamphetamine has also been reported to affect NE levels
(Miller, Cox, Snodgrass & Maickel, 1970).

There is support for the suggestion that changes in SS rates might be
unrelated to changes in 5-HT levels after administration of PCPA and similar comp-
ounds. Stark, Fuller, Hartley, Schaffer and Turk (1970) and Stark and Fuller (1972)
found that changes in 5-HT levels in the brains of dogs and rats were poorly corre-
lated with changes in SS rates after administration of PCPA or metabolites of PCPA.

Atrens, Ungerstedt and Ljungberg (1977) administered a compound,
LU10-171, which blocks 5-HT uptake and apparently is selective with respect to 5-
HT activity, to rats self-stimulating through lateral hypothalamic electrodes. The
authors reported an apparent decrement of the rewarding value of SS since, in a two-
way shuttle box, rats took longer to initiate the stimulation. Since the latency to
escape was not changed, the authors concluded that LU10-171 was not exerting any
non-specific effects on behavior. It is possible, however, that escape behavior was
less sensitive to the disruptive effects of this drug.

Even if PCPA and related compounds do have effects on NE activity
as well as 5-HT activity, it is still difficult to explain the results in terms of non-
specific effects since the changes in SS rates appear to be site-dependent. Differ-
ent methodologies and different baseline rates of SS might account for some of these
site-specific effects. The experiment by Phillips, Carter and Fibiger (1976b) comp-
ared SS from the lateral hypothalamus and caudate-putamen after PCPA adminis-
tration and found facilitation of lateral hypothalamic SS and suppression of caudate-
putamen SS although both groups responded at identical baseline rates before drug
treatment. Similarly, Miliaressis (1977) used two bars simultaneously in his testing
procedure so that a rat could respond for medial raphe and for ventral tegmental sti-
mulation during the same test session. Although rates were similar on both bars before
drug treatment, PCPA administration caused a slight enhancement of ventral teg-
mental SS and a large suppression of medial raphe SS rates.

On the basis of these types of experiments, it must be concluded that
PCPA did selectively alter the rewarding characteristics of stimulation of the ventral
tegmental area, lateral hypothalamus, caudate-putamen and medial raphe nucleus.
In view of this, it seems particularly relevant to determine through which action or
actions PCPA produced these effects. If it was at least partly through changes in
5-HT activity, the evidence would favour a role for 5-HT in SS.
Summary, Conclusions and Rationale

The possible roles of each of four putative neurotransmitters in the central nervous system in the mediation of SS were considered separately in this review, although the experimental techniques used to implicate these neurotransmitters are relatively similar. These include the initial anatomical evidence implicating DA and NE in SS, based on the overlap of catecholamine pathways with sites positive for brain-stimulation reward; evidence from lesion studies wherein the effects of selective destruction of catecholamine-containing areas on SS of various brain loci were assessed; and pharmacological evidence from examination of the responses of self-stimulating animals to the administration of drugs. This final technique has most frequently been used to implicate a given neurotransmitter in SS. Consideration of these pharmacological data as a whole, however, points to certain common problems of interpretation:

(1) Anatomical Specificity—In areas of the brain such as the medial forebrain bundle, electrical stimulation is likely to excite both dopaminergic and noradrenergic neurons. Many of the studies done have used electrode placements in these areas, making it difficult to relate changes in response rates to specific alterations in only one of these neurotransmitters. One solution is to place electrodes further rostral in the forebrain area, where the problem of overlap of NE and DA neurons is considerably lessened.

(2) Pharmacological Specificity—Unless drugs with very specific actions are used, it is difficult to relate the change in activity of a particular neuro-
transmitter to the behavioral action of the drug. For example, drugs such as amphetamine, chlorpromazine, alpha-methyl-p-tyrosine, and MAO inhibitors affect both DA and NE transmission. These are the drugs, however, which have often been used to implicate either NE or DA. The use of drugs with known and relatively specific properties may offer more information insofar as changes in SS rates may be attributed to alterations in the activity of a specific neurotransmitter.

(3) Behavioral specificity—Perhaps the most obvious and most difficult problem in using drugs to implicate a neurotransmitter in SS is that many drugs can alter the animal’s capacity to respond. SS is an operant behavior and, as such, depends on the ability of the animal to respond optimally or maximally at all times. If a drug which affects this ability is administered, changes in response rates do not necessarily mean that the positively-reinforcing value of the electrical stimulation has been altered in any way. Thus very stringent controls must be used when assessing drug effects on SS. It is necessary to have a direct method of assessing any "nonspecific" effects. Examination of the response rates from one part of the brain relative to another offers one such method. If a drug alters responding when electrodes are in one part of the brain but not another, then non-specific deficits seem unlikely.
Description Of Experiments

The experiments described in this thesis were designed to evaluate the roles of DA, NE, 5-HT and ACh in several regions of the forebrain of the rat. The experiments were designed so that the three problems of specificity (anatomical, pharmacological, and behavioral) were controlled for or assessed directly.

When these experiments were begun, DA and NE were considered two major "candidates" for the role of mediator of SS. In order to maintain anatomical or biochemical specificity, it was initially decided to select sites in the brain where primarily dopaminergic or primarily noradrenergic neurons could be stimulated. The nucleus accumbens (NAS) and caudate-putamen (CPU) were selected as areas with high levels of DA but minimal levels of NE. Selection of a noradrenergic site was more difficult since, although NE neurons are widely spread throughout the brain, they are intermingled with DA neurons in most areas which had been shown to support SS. There was, however, an area which had not been systematically explored—the supracallosal bundle (SCB), a fibre bundle containing NE neurons from the dorsal pontine tegmentum. Therefore, as described in Paper 1 (Brain Stimulation Reward Associated With Stimulation of the Supracallosal Bundle), the SCB was tested for SS. SS was elicited from rostral portions of the bundle and experiments were conducted to see if SS of this site was mediated by NE fibres. Since this proved to be a difficult task, pharmacological methods were supplemented by electrophysiological and lesions techniques. In fact, it was not possible to exclude involvement of other neurotransmitters in SS of this area. It was concluded that, although NE
was implicated in SS of the SCB, DA might also be involved. This possibility became particularly apparent because, at the time these studies were conducted, reports of dopaminergic innervation of the prefrontal cortex and cingulate cortex, areas rostral and dorsal to the stimulation sites in the SCB, were published. The prefrontal cortex (PFC) was thereafter included in studies of SS.

It was also of interest to find a site which could support SS but which did not contain DA or NE neurons. The subfornical organ (SFO) was selected for investigation on this basis. SS was demonstrated there (Paper III: Subfornical Organ: A Site of Brain Self-Stimulation). Since, at that time, there was no indication that this area contained NE or DA but there was evidence of cholinergic and serotonergic innervation, experiments involving injections of drugs which specifically blocked ACh or 5-HT receptors were carried out, in order to see if 5-HT or ACh were involved in SS of this site (Paper IIII: Self-Stimulation of the Subfornical Organ and Lateral Hypothalamus: Differential Effects of Atropine and Methysergide). By use of the technique of comparison of SS from the SFO and the lateral hypothalamus to assess any non-specific effects of the drugs, it was concluded that 5-HT and ACh might play a role in SS of this site. These observations also implied that DA and/or NE were not "exclusive" mediators of SS in the central nervous system.

In the experiments thus far completed, the role of DA had been investigated in SS of the SCB, SFO and the dopaminergic areas selected, by injecting spiroperidol, a drug which blocks DA receptors. It became apparent both from this work and other published studies that this drug might suppress SS rates by changing
the animal's ability to perform the operant response. Therefore in order to investigate the role of DA, an alternative method of administering spiroperidol was used, involving direct administration of spiroperidol into areas of the brain containing DA receptors (Paper IV: Evidence for a Role for Dopamine in Self-Stimulation Of The Nucleus Accumbens in the Rat). Using this method, evidence was found for the involvement of DA in SS of the NAS.

In a final series of experiments, spiroperidol was administered chronically to self-stimulating rats (Paper V: Facilitation of Self-Stimulation of the Prefrontal Cortex in Rats Following Chronic Administration of Spiroperidol or Amphetamine). This method offered the advantage of being more comparable to the clinical use of drugs such as spiroperidol, which are administered chronically to control symptoms of schizophrenia. One interesting result of this study was that SS of the PFC was differentially sensitive to administration of spiroperidol over long periods of time, compared to SS of other sites. The study was then expanded to include administration of amphetamine, a drug which liberates DA and NE from presynaptic terminals. SS of the PFC was also differentially sensitive to the effects of this drug. Although the biochemical basis of this facilitation remains unclear, the finding was relevant because DA was implicated in SS of this site and because the differential sensitivity of SS of the PFC compared to other areas suggested that the PFC might mediate some of the long-term effects of neuroleptics in humans.
PAPER 1

Brain-Stimulation Reward Associated With Stimulation of the Supracallosal Bundle

Published in *Experimental Neurology*, 1978, 58, 81-94.
Self-stimulation was observed with electrodes in the supracallosal bundle. Histological verification of the locus of the stimulating electrodes, the antidromic activation of locus ceruleus neurons, the attenuation of self-stimulation of the supracallosal bundle by administering colchicine to the locus ceruleus, and the differential effects of D- and L-amphetamine suggest that during self-stimulation of the bundle there is activation of noradrenergic fibers projecting from the locus ceruleus. The electrodes in the supracallosal bundle are also in close proximity to the cingulate gyrus, a region of dopamine innervation, but electrodes there yielded a low percentage of sites positive for self-stimulation. The results suggest that self-stimulation of the supracallosal bundle is associated with the activation of noradrenergic neurons but involvement of dopaminergic neurons cannot be ruled out.
INTRODUCTION

The locus ceruleus is the site of noradrenergic neurons which project to the hippocampus and cerebral cortex as well as to the cerebellum and spinal cord (Loizou, 1969; Swanson, 1976; Tohyama, Maeda, and Shimizu, 1974; Ungerstedt, 1971). The major rostral projection of the locus ceruleus is via the dorsal noradrenergic bundle ascending in the midbrain (Huang & Maas, 1976; Lindvall, Bjorklund, Nobin, & y. nevi, 1974; Shimizu, Ohnishi, Tohyama, & Maeda, 1974) passing ventrally through the lateral hypothalamus. These fibers project through the septum and form the supracallosal bundle (SCB) on their way to the cerebral cortex and the hippocampus (Olson & Fuxe, 1972; Pickel, Segal, & Bloo v, 1974).

It was shown in acute electrophysiological recording experiments that electrical stimulation of the SCB antidromically activates neurons in the locus ceruleus and subceruleus (Faiers, & Mogenson, 1976; Takigawa, & Mogenson, 1977). The present study was undertaken to see whether or not rats would self-stimulate the SCB (Routtenberg, 1971). This possibility is of interest in relation to a number of recent investigations which implicate catecholaminergic neurons in brain-stimulation reward [reviewed in (Mogenson, and Phillips, 1976; Wauquier, & Rolls 1976)] and specifically in relation to controversial results concerning the role of the locus ceruleus and of the dorsal noradrenergic bundle in brain-stimulation reward (Amaral, & Routtenberg, 1975; Clavier, Fibiger, & Phillips, 1976; Clavier, & Routtenberg, 1976; Crow, 1972: Crow, Spear, & Arbuthnott, 1972; Phillips, Van Der Kooy, & Fibiger 1977; Stein, Belluzzi, & Wise, 1976). Accordingly additional experiments were carried out in attempting to see whether or not self-stimulation
of the SCB is associated with the activation of noradrenergic fibers from locus ceruleus neurons. A preliminary report of these experiments has already appeared.

(Takigawa, Robertson, & Mogenson, 1976).
METHODS

Subjects and Electrode Implantation

Male Albino rats, weighing 250 to 300 g at the time of surgery, were used. They were housed in individual wire mesh cages with food and water provided ad libitum.

In preparation for implantation of electrodes into the SCB, rats were anesthetized with sodium pentobarbital (Nembutal, 40 to 50 mg/kg, i.p.). Under stereotaxic control, bipolar electrodes (Plastic Products Ltd.), made of stainless-steel wire 127 μm in diameter and insulated except for the cross-sectional area at the tip, were lowered into the SCB, into sites dorsal or rostral to it, or into the medial prefrontal cortex. Most SCB electrodes were positioned 2.0 to 2.6 mm anterior to bregma, 0.8 to 1.0 mm lateral to the midline, and 3.5 mm ventral to the dorsal surface of the skull, with the incisor bar set 5.0 mm above the interaural line. Dental acrylic was used to attach the electrodes to the jeweler's screws placed in the skull. Two or three electrodes were placed chronically in each rat.

Testing for Self-Stimulation

After a 1-week recovery period, rats were tested for self-stimulation. Testing took place in a Plexiglas box (28 x 16 x 30 cm.) with a lever at one end which could be depressed with a force of approximately 16 g to deliver a 0.20-s train of electrical stimulation. The stimulation provided by a Grass S44 stimulator consisted of monophasic rectangular pulses of 0.20-ms duration presented at 80 Hz through a Grass stimulus isolation unit. Current was monitored on an oscilloscope and was varied systematically between 50 and 600 μA. Each rat was tested for a
minimum of four daily 60-min. sessions, preceded if necessary by a shaping procedure of as much as 100 min. to establish the lever-pressing response. After establishment of the response, rats were tested with current levels constant, for 15 min./day at approximately the same time each day.

Antidromic Activation of Locus Ceruleus Neurons

After self-stimulation tests, rats were anesthetized with urethane (160 mg./100 g. ip) in preparation for extracellular recording from neurons in the locus ceruleus and subceruleus according to the procedures described previously (Takigawa, & Mogenson, 1977). Rectal temperature was monitored throughout the recording session with a Yellow Springs telethermometer and was maintained between 36 & 38°C. A strip of bone over the cerebellum was removed, the dura deflected, and the exposed cortical surface covered with mineral oil. Stainless-steel microelectrodes etched to a tip diameter of 2 to 4 μm were lowered into the area of the locus ceruleus. When a neuron was identified, single monophasic square-wave pulses (0.2 ms in duration and 0.2 to 1.2 mA in intensity) were delivered through the ipsilateral SCB electrode from a Grass S44 stimulator. Antidromically activated units were identified using the criteria of constant latency of action potentials initiated by the stimulation and collision with spontaneous action potentials (Faires, & Mogenson, 1976).

Colchicine Injections

Electrodes were placed bilaterally into the SCB and cannulae bilaterally into the locus ceruleus. The guide cannulae, constructed from 23-gauge
hypodermic needles, were lowered so that the tips were 1.0 mm above the locus ceruleus. The injection cannulae, made from 30-gauge hypodermic needles, extended 1 mm from the guide cannulae.

Self-stimulation of the SCB was established bilaterally. After stabilization of response rates, 2 μg/0.2 μl colchicine or a control injection of sterile saline was infused during 3 min. into one locus ceruleus cannula using a Hamilton microsyringe. Colchicine, by disassembling microtubule protein, temporarily disrupts axoplasmic transport and the release of neurotransmitter from axon terminals and has been used to provide a reversible functional block (Møgensen, & Avrit, 1977). Self-stimulation of the two SCB electrodes, ipsilateral and contralateral to the site of colchicine administration, was determined during the next 7 days.

Drug Administration

Animals were prepared each with electrodes in two of the following sites: the SCB, the medial prefrontal cortex rostral to the SCB, the caudate putamen, and the nucleus accumbens. Animals which self-stimulated were tested 10 min. daily at current levels (between 100 and 400 μA) adjusted so as to generate approximately equal response rates from the three electrode loci.

Prior to administration of a drug, animals were injected with the appropriate vehicle control. A minimum of 3 days elapsed between successive drug administrations in those rats which received more than one injection. Drug solutions were prepared immediately prior to administration. D- or L-amphetamine HCl, dissolved in isotonic saline, was administered in a dosage of 1.0 mg/kg 30 min. prior to
test session to rats with SCB, medial prefrontal cortex, and nucleus accumbens electrodes. Spiropelidol, dissolved in tartaric acid (0.01 M), pH adjusted to 6.5 to 6.8 with 1 N NaOH, was administered (0.01, 0.05, and 0.10 mg/kg.) 2 h before the test session to rats with electrodes in SCB, caudate-putamen, and nucleus accumbens.

Data Analysis and Histology

All response rates following an experimental procedure were expressed as percentages of baseline response rates prior to the experimental manipulation. These baseline response rates were averaged from two or three daily test sessions immediately prior to the lesion or drug. After completion of testing, animals were killed by an overdose of sodium pentobarbital, and their brains were removed and sectioned with a freezing microtome. The sections were stained with thionine and examined under a microscope to determine the site of the electrode tips.
RESULTS

Self-Stimulation of the Supracallosal Bundle

Tests of self-stimulation were made of 82 electrodes in the SCB and in surrounding regions of the forebrain (Fig. 1). Self-stimulation occurred for 22 of 26 electrodes confirmed histologically to terminate in the SCB (Table 1). During self-stimulation these animals were hyperactive, with increased sniffing, rearing, and locomotion. When stimulation electrodes were more posterior in the bundle (Fig. 1, section 8920) clonic movements of the forelimbs and head were occasionally observed. Such movements also occurred in response to applied stimulation in some animals that did not self-stimulate, and were possibly the result of current spread to motor cortex.

Only 10 of 35 electrode sites in the cingulate cortex dorsal to the SCB were positive for self-stimulation. Self-stimulation did not occur for electrodes immediately dorsolateral to the bundle or for electrodes that penetrated into the corpus callosum (Fig. 1, Table 1). The rate of self-stimulation and the current

<table>
<thead>
<tr>
<th>Stimulation Site</th>
<th>Self-Stimulation (N)</th>
<th>Rate (15 min) (\pm SE)</th>
<th>Current ((\mu A)) (\pm SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td>Supracallosal bundle</td>
<td>22</td>
<td>4</td>
<td>517 (\pm) 52</td>
</tr>
<tr>
<td>Dorsal Cingulate</td>
<td>10</td>
<td>25</td>
<td>570 (\pm) 120</td>
</tr>
<tr>
<td>Neocortex</td>
<td>0</td>
<td>3</td>
<td>-</td>
</tr>
<tr>
<td>Frontal cortex</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dopamine region</td>
<td>4</td>
<td>4</td>
<td>362 (\pm) 123</td>
</tr>
<tr>
<td>Nondopamine</td>
<td>0</td>
<td>7</td>
<td>-</td>
</tr>
<tr>
<td>Corpus callosum</td>
<td>0</td>
<td>3</td>
<td>-</td>
</tr>
</tbody>
</table>
Fig. 1. Coronal sections showing electrode sites in the supracallosal bundle and surrounding region (Konig and Klippel (1963)). Sites positive for self-stimulation are shown by filled triangles (rate > 50 responses/15 min.) and negative sites by open triangles. Numbers at the lower right-hand corner of each section indicate the distance in micrometers anterior to the interaural plane. Abbreviations: FM1 - forceps minor, GCC-genu of corpus callosum, RCC-radiation of corpus callosum, CP-caudate-putamen, TCC-trunk of corpus callosum, SL-lateral septal nucleus.
intensities for the electrodes in the SCB were similar to those for the cingulate cortex and prefrontal cortex.

Antidromic Activation of Neurons in the Locus Ceruleus from Electrical Stimulation of Sites in the Supracallosal Bundle

In order to provide further evidence that the SCB was being stimulated attempts were made to antidromically activate locus ceruleus neurons by stimulation of 19 SCB sites (16 positive for self-stimulation and 3 negative) and of 4 negative cingulate cortex sites in 16 rats anesthetized with urethane. For 7 neurons confirmed histologically to be in the locus ceruleus or subceruleus, stimulation of the SCB via chronic electrodes positive for self-stimulation produced antidromic responses. Antidromic responses recorded from a locus ceruleus neuron (A) and from a subceruleus neuron (B) are shown in Fig. 2, and stimulating and recording sites are shown in Fig. 3. Five of the antidromically activated neurons were in the locus ceruleus and two were 0.3 to 0.7 mm ventral to it in the region of the subceruleus. The latency of the antidromic action potentials in the locus ceruleus was 42.3 ± 3.7 ms.

Stimulation of electrode sites negative for self-stimulation did not elicit antidromic responses in neurons of the locus ceruleus or subceruleus.

Effect of Colchicine Administration to the Locus Ceruleus on Self-Stimulation of the Supracallosal Bundle

The effect of disrupting locus ceruleus neurons on self-stimulation of the SCB was investigated by infusing colchicine (2 μg/0.2 μl) unilaterally into it in rats self-stimulating through bilateral SCB electrodes. As shown in Fig. 4 rats whose
Fig. 2. Examples of antidromically activated neurons in the locus ceruleus (A) and subceruleus (B) in response to single pulses delivered to the supracallosal bundle. Arrows indicate application of the stimulus pulse. The top panels show the latency of the activated units from five superimposed sweeps with current intensity 1.2 times threshold. The bottom panel in each case shows collision of an antidromically activated response with a spontaneous action potential, indicated by a solid triangle.
Fig. 3. Coronal sections of three rat brains stained with Thionine. A and B show sites (indicated by arrows) in the supracallosal bundle positive for self-stimulation and corresponding sites (to the right) of antidromically activated neurons in the locus ceruleus (A) and subceruleus (B). C is an example of a site negative for self-stimulation (indicated by arrow) in which no antidromically activated units in the locus ceruleus could be identified. Horizontal bars represent 5 mm.
Fig. 4. The effect of a unilateral infusion of colchicine (2 μg/0.2 μl) into cannulae in the locus ceruleus on self-stimulation of bilateral supracallosal bundle sites. Solid lines in each case show self-stimulation from the ipsilateral electrode and dashed lines from the contralateral electrode. The upper right-hand panel shows the effect of control saline injections on self-stimulation of ipsilateral (closed circles) and contralateral (open circles) electrodes in five rats. Note that rat 13 exhibited ataxia for 2 days following colchicine administration which might have been due to spread of colchicine up the cannulae shafts into the cerebellum.
cannulae and electrodes were histologically verified as being in the locus ceruleus and SCB, respectively, displayed a suppression of self-stimulation through the electrodes ipsilateral to the injection site. Control injections of isotonic saline had no effect. The decrease in self-stimulation after colchicine injections appeared maximal at 3 to 4 days postinjection, consistent with the time course of suppression of neuronal transmission following colchicine administration (Perisic, & Cuenod, 1972). Some rats also displayed a decrease in responding from the contralateral electrode but this was of smaller magnitude.

Effects of Spiroperidol on Self-Stimulation of the Supracallosal Bundle

Spiroperidol, a specific dopaminergic antagonist, was administered to rats self-stimulating the SCB, caudate-putamen, or nucleus accumbens. Doses of 0.01 and 0.05 mg/kg produced little change. After an injection of 0.10 mg/kg spiroperidol there was a significant suppression ($P < 0.005$) of self-stimulation of the SCB as well as for electrodes in the caudate-putamen and nucleus accumbens (Fig. 5).

Effects of D- and L-Amphetamine on Self-Stimulation of the Supracallosal Bundle

D- and L-amphetamine were previously shown to have differential effects on self-stimulation according to whether the electrode was in a predominantly dopaminergic or a predominantly noradrenergic area of the brain (Phillips, Brooke & Fibiger, 1975; Phillips, & Fibiger, 1973). Therefore the effects of these isomers were tested for self-stimulation of SCB electrodes and compared to self-stimulation of electrodes in dopamine-containing regions, the
Fig. 5. The effect of spiroperidol on self-stimulation of the supracauallosal bundle (SCB), nucleus accumbens (NAS), and caudate-putamen (CPU). The number of rats per group is indicated at the bottom of each column and standard errors are represented by vertical bars.
medial prefrontal cortex and nucleus accumbens. Each animal was tested with D- and L-amphetamine (1 mg/kg in each case). A response differential, based on the difference between response rates for the D- and the L-isomers, was calculated. As shown in Table 2, there was a small, nonsignificant D-L differential for animals with nucleus accumbens electrodes. Animals with SCB or medial prefrontal electrodes showed a significantly high response differential (P < 0.05); response rates after D-amphetamine were considerably elevated compared to response rates after L-amphetamine.

### TABLE 2
Effects of D- and L-Amphetamine on Self-Stimulation

<table>
<thead>
<tr>
<th>Electrode site</th>
<th>Rat No.</th>
<th>D-Am- Amphetamine</th>
<th>L-Am- Amphetamine</th>
<th>D - L</th>
<th>Mean difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Supracallosal bundle</td>
<td>38</td>
<td>281</td>
<td>116</td>
<td>165</td>
<td>83</td>
</tr>
<tr>
<td></td>
<td>42</td>
<td>142</td>
<td>102</td>
<td>40</td>
<td></td>
</tr>
<tr>
<td></td>
<td>46</td>
<td>123</td>
<td>78</td>
<td>45</td>
<td></td>
</tr>
<tr>
<td>PFC</td>
<td>30</td>
<td>203</td>
<td>102</td>
<td>101</td>
<td>73</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>166</td>
<td>103</td>
<td>63</td>
<td></td>
</tr>
<tr>
<td></td>
<td>46</td>
<td>149</td>
<td>95</td>
<td>54</td>
<td></td>
</tr>
<tr>
<td>NAS</td>
<td>41</td>
<td>106</td>
<td>88</td>
<td>18</td>
<td>-4</td>
</tr>
<tr>
<td></td>
<td>43</td>
<td>76</td>
<td>100</td>
<td>-24</td>
<td></td>
</tr>
<tr>
<td></td>
<td>27</td>
<td>99</td>
<td>103</td>
<td>-4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>45</td>
<td>90</td>
<td>96</td>
<td>-6</td>
<td></td>
</tr>
</tbody>
</table>

*a* Self-stimulation rates are expressed as a percentage of preinjection rates.
DISCUSSION

The major finding of the present study is that self-stimulation was obtained with electrodes histologically verified as being in the SCB. Electrodes dorsal and lateral to it were mainly negative (see Fig. 1, sections 8920 and 9410). Electrodes that penetrated into the corpus callosum were also not associated with self-stimulation. The SCB contains fibers that project from noradrenergic neurons in the locus ceruleus (Pickel, Segal, & Bloom 1974; Ungerstedt, 1971) and, in view of the recent controversy about the role of catecholaminergic neurons in brain-stimulation reward (Mogenson, & Phillips, 1976; Wauquier, & Rolls (Eds.) 1976), evidence will first be considered which suggests that fibers of locus ceruleus neurons were activated by stimulating SCB self-stimulation sites.

In acute electrophysiological recording experiments it was demonstrated that electrical stimulation of the SCB antidromically activates neurons in the locus ceruleus and subceruleus, suggesting that fibers from these neurons are activated by stimulation of the SCB (Faiers, & Mogenson, 1976; Takigawa, & Mogenson, 1977). Although it is difficult to record from locus ceruleus neurons, it was possible in the present study to record from seven neurons for a sufficient time to demonstrate that they were activated antidromically by stimulation of SCB sites previously shown to be associated with self-stimulation (see Fig. 2). No locus ceruleus neurons were antidromically activated from stimulation of a small number of sites outside the SCB. The latencies of the antidromic responses of locus ceruleus neurons were similar to those reported previously (Faiers & Mogenson, 1976; Takigawa & Mogenson, 1977), consistent with earlier evidence that small-diameter, slow-conducting fibers are
being stimulated. When colchicine, a reversible blocker of neural transmission, was administered to the locus ceruleus, self-stimulation of the ipsilateral SCB was markedly reduced for 3 or 4 days (See Fig. 4). Finally, D-amphetamine had a greater facilitating effect on self-stimulation of the SCB than L-amphetamine. This differential effect of D- and L-amphetamine is consistent with a previous study (Phillips, Brooke, & Fibiger, 1975) in which the dorsal noradrenergic bundle at the level of the midbrain was stimulated, presumably activating noradrenergic fibres of locus ceruleus neurons, and has been considered evidence that noradrenergic pathways are being stimulated (Herberg, Stephens, & Franklin, 1976; Phillips, Brooke, & Fibiger, 1975; Phillips & Fibiger, 1973; Stephens, Franklin & Herberg, 1976). In that earlier study differential effects of the two isomers on self-stimulation of the nucleus accumbens were also not observed. The differential facilitating effects of D- or L-amphetamine on self-stimulation of the medial prefrontal cortex is surprising and suggests the possibility that noradrenergic projections to this region (Kiévít, & Kuypers, 1975) may contribute to the rewarding effects. However, the results of the experiments using the isomers of amphetamine must be interpreted with caution (Phillips, Van Der Kooy, & Fibiger, 1977, and see Appendix 2).

Activation of fibers from locus ceruleus and from subceruleus neurons does not exclude the involvement of other transmitter-specific fibers in self-stimulation of the SCB and, indeed, does not necessarily mean that noradrenergic fibers have a crucial role in self-stimulation of bundle sites. Activation of fibers from locus ceruleus could be merely a concomitant of self-stimulation and not essential for self-
stimulation. Attenuation of self-stimulation of the SCB by spiroperidol (see Fig. 5) suggests that dopaminergic neurons may be involved (Mora, Rolls, & Shaw, 1976). The cingulate cortex received dopaminergic innervation (Simon, Le Moal, Galey, & Cardo, 1976; Thierry, Tassin, Blanc, & Glowinski, 1976) and some dopaminergic fibers projecting to the cingulate may pass through the SCB (Lindvall & Bjorklund, 1974). However, as shown in Table 1, 25 of 35 electrodes in the cingulate region were negative for self-stimulation. The medial prefrontal cortex (Leonard, 1969) also has dense dopaminergic innervation (Berger, Thierry, Tassin & Moyne, 1976); four of eight electrodes in the dopamine-containing prefrontal cortex were negative for self-stimulation in this study although in subsequent experiments we have shown that electrodes placed in the prefrontal region shown in section 11050, Fig. 1, are typically positive. This positive region is, however, more than 1 mm anterior to the most rostral SCB electrodes sites shown. If current spread to the dopamine-containing region of the prefrontal cortex were responsible for SCB self-stimulation, then the cingulate cortex immediately dorsal to the SCB should have been a better site for eliciting self-stimulation.

By comparison, 22 of 26 electrodes in the SCB were positive for self-stimulation. These electrodes, shown in Fig. 1, were in the region reported by Pickel et al. (Pickel, Segal, & Bloom, 1974), in an autoradiographic study, to be the site of the efferent fiber pathway from the locus ceruleus to cerebral cortex and hippocampus. Taken together with the results of the colchicine experiments and the differential effects of D- and L-amphetamine considered above, these findings point to
the importance of fibers of locus ceruleus neurons in self-stimulation of the SCB. However, the attenuation of self-stimulation of the SCB by spiroperidol implicates dopamine neurons [although nonspecific behavioral effects, e. g. motor (Fibiger, Carter, & Phillips, 1976; Rolls, Rolls, Kelly, Shaw, Wood & Dale, 1974, and see Appendix 3), associated with neuroleptic drugs cannot be excluded]. The possibility that self-stimulation of the SCB is associated with activation of both noradrenergic and dopaminergic neurons should also be considered in view of recent reports by Herberg and co-workers (Herberg, Stephens & Franklin, 1976; Stephens, Franklin, & Herberg, 1976). The hypothesis of coactivation of dopaminergic and noradrenergic neurons in brain-stimulation reward can account for the results of the present study, including the attenuation of self-stimulation of the SCB, nucleus accumbens, and caudate-putamen by spiroperidol.

In summary, self-stimulation was observed with electrodes in the SCB and evidence was presented which suggests that this is associated with activation of fibers of locus ceruleus neurons. These findings are consistent with previous reports of self-stimulation of the locus ceruleus (Crow, 1972; Ritter, & Stein, 1973) and dorsal noradrenergic bundle (Phillips, Brooke, & Fibiger, 1975) which have implicated the A6 locus ceruleus neurons in brain-stimulation reward. However, it was not possible to exclude definitively the involvement of dopaminergic neurons in self-stimulation of the SCB, even though a variety of techniques was used to supplement histological verification of the sites of the stimulating electrodes. Clearly, as shown in a number of previous studies, it is very difficult to dissociate noradrenergic and dopaminergic systems in brain-stimulation reward.
Subcomical Organ: A Site Of Brain Self-Stimulation

Published in Brain Research, 1976, 114, 511-516
Recently there has been considerable interest in the subfornical organ (SFO) as a possible locus of action for the dipsogenic effect of angiotensin II (Felix, & Akert, 1974; Johnson, & Epstein, 1975; Simpson, & Routtenberg, 1973; Simpson, & Routtenberg, 1975). Since an anatomical overlap of neural sites for elicited drinking and brain self-stimulation has been demonstrated previously (Mogenson, & Morgan, 1967; Mogenson & Stevenson, 1966) it was decided to determine whether self-stimulation could also be obtained from the SFO.

Male albino rats weighing 250-300 g at the time of surgery were used in the study. The animals were individually housed in wire mesh cages in a temperature-controlled room with lights on from 7:00 a.m. to 9:00 p.m. and with tap water and Purina rat chow available ad libitum. In preparation for stereotaxic surgery, rats were anesthetized with ketamine hydrochloride (Ketaset, 60 mg/kg body weight, i.p., Rogar/STB, London, Ont.) followed by sodium pentobarbital (Nembutal, 30-50 mg/kg i.p.) and then bipolar electrodes (Plastic Products Company, Roanoke, Va.) 127 μm in diameter and insulated except for the cross-sectional area at the tips were implanted in various brain areas. Forty-seven were aimed at the subfornical organ, 25 were implanted bilaterally in areas surrounding the SFO and 6 were implanted in the lateral hypothalamus (LH).

Following a 1 week recovery period the animals were tested for self-stimulation according to a standard procedure. Testing took place in a plexiglass box (27.8 cm. X 16.3 cm. X 30.6 cm.) with a lever at one end which could be depressed with a force of approximately 16 g to deliver a 0.20 sec. train of electrical stimu-
lation. The stimulating waveform provided by a Grass S44 stimulator consisted of monophasic rectangular pulses of 0.20 m sec. duration presented at 80 Hz through a Grass stimulus isolation unit. Current was monitored on an oscilloscope.

During testing the current was varied systematically between 200 and 600 μA. Each rat was tested for a minimum of four daily 60 min sessions, preceded if necessary by a shaping procedure of up to 100 min to establish the lever pressing response.

Of the 47 electrodes aimed at the SF0, 24 were confirmed histologically to terminate in this structure. Moderately high rates of self-stimulation were observed for 22 of these 24 electrodes (see Table 1) and no priming was required at the beginning of a test session. Although precise localization of the electrode tips within

<table>
<thead>
<tr>
<th>Area of electrode implantation</th>
<th>Positive sites (n)</th>
<th>Negative sites (n)</th>
<th>Mean current (μA)</th>
<th>Mean rate of SS (responses/15 min)</th>
<th>Associated Behavior</th>
</tr>
</thead>
<tbody>
<tr>
<td>SF0</td>
<td>22</td>
<td>2</td>
<td>480</td>
<td>246</td>
<td>None</td>
</tr>
<tr>
<td>Fornical comissure</td>
<td>3</td>
<td>11</td>
<td>600</td>
<td>170</td>
<td>None</td>
</tr>
<tr>
<td>Dorsal fornix</td>
<td>8</td>
<td>2</td>
<td>420</td>
<td>260</td>
<td>Motor***</td>
</tr>
<tr>
<td>Lateral hypothalamus</td>
<td>6</td>
<td>0</td>
<td>283</td>
<td>993</td>
<td>Hyperactive**</td>
</tr>
<tr>
<td>Thalamic nuclei</td>
<td>4</td>
<td>0</td>
<td>600</td>
<td>538</td>
<td>None</td>
</tr>
</tbody>
</table>

*Pulse duration, 0.2 m sec; frequency, 80 Hz; train duration 0.2 sec.
**All rats were 'hyperactive' (licking, chewing, increased locomotion, jumping, increased sniffing, etc.); one showed contraversive head turning and one showed contra-versive body turning.
***Out of 9 placements, 7 showed strong, non-lateralized backward jerking with each stimulation, at a variety of current levels. Two remaining placements (in a rat which self-stimulated) elicited shuddering. The jerking occurred with both self-stimulators and non-self-stimulators with electrodes in the fornix, fornical comissure, lateral septum, corpus callosum, and medial hippocampus.
the SF0 itself was difficult because of the small size of the structure, (Akert, 1969), there was some indication that higher rates of responding occurred when the electrodes were in the lateral and dorsal regions. The sites of stimulation are shown in Fig. 1 and a photomicrograph showing the locus of one of the electrode tips is presented in Fig. 2A.

Eleven of 14 electrodes terminating in the commissural fibres dorsal to the SF0 were negative for self-stimulation (see Fig. 1 and Fig. 2B). Other investigators previously reported that commissural electrode placements were negative for self-stimulation (Olds, & Olds, 1963; Routtenberg, 1971). Furthermore, since the 3 commissural electrodes positive for self-stimulation terminated within 0.75 mm of the SF0 (Figs. 1 and 2C) and required higher current intensities and were associated with low rates of lever pressing (see Table 1), it is possible that self-stimulation of the 3 commissural sites was the result of spread of current to the SF0. In any case there is no evidence from the present study that self-stimulation of electrodes in the SF0 was due to spread of current to adjacent structures.

Self-stimulation was obtained from 8 of 10 electrodes in the dorsal fornix. An example of one such positive placement is shown in Fig. 2D. Although the rates of responding and intensities of stimulating current were similar to those for SF0 placements (see Table 1), it is unlikely that self-stimulation elicited from dorsal fornix placements resulted from spread of current to the SF0 or vice versa. This is indicated by the large number of negative electrode placements between the region of the dorsal fornix and the SF0 (Fig. 1). The behaviors associated with self-stimulation of the dorsal fornix, which included prominent motor effects, were quite
Fig. 1. Coronal sections from a stereotaxic atlas (Pellegrino, and Cushman, 1967) showing the sites of the electrode tips. Numbers in the upper left-hand corner of each section indicate mm anterior or posterior to bregma. Note that the center section, at the same rostrocaudal level (AP = 0.0) as the section immediately above it, is an enlargement taken from a photomicrograph of the SF0 and surrounding area. This section indicates positive and negative placements not shown on the smaller section above due to lack of space. The SF0 in this photomicrograph is the heavily-stained area projecting into the lateral ventricle. Each point on the map represents 1-3 placements, except for the points in the SF0 of the center section which represents up to 5 placements each. Abbreviations: CC, corpus callosum; CH, hippocampal (fornica commissure); FD, dentate gyrus; FL, fornix longus (of Forel); FX, fornix; HPC, hippocampus; LS, lateral septum; MD, mediodorsal nucleus of thalamus; TS, triangular nucleus of septum; V, ventricle; PV, paraventricular nucleus of thalamus; PT, paratenial nucleus of thalamus; SM, stria medullaris thalami.
NO. RESPONSES/15 min.

★ > 250
☆ 150-250
● 50-150
■ < 50
Fig. 2. Photomicrographs of representative coronal sections showing the sites of the electrode tips (arrows). A: lateral SF0 placement supporting self-stimulation (SS). B: two fornical commissural placements not supporting SS. C: fornical commissural placement supporting SS. This placement is one of 3 commissural placements which supported SS. Of the 3 it was furthest from the SF0 or dorsal fornix. D: dorsal fornix placement supporting SS.
different from those observed with self-stimulation of the SF0, where no elicited behavioral responses were observed and, in fact, the rats seemed hypoactive. The latter observation was subsequently confirmed when several SF0 self-stimulating animals were given programmed stimulation of 20-40 sec duration during which an arrest of ongoing activity was observed.

Self-stimulation from electrodes in other adjacent brain regions was also investigated. Ten of 11 electrodes placed in the corpus callosum were negative for self-stimulation, as were two lateral septal electrodes, one electrode located in the dentate gyrus of the hippocampus, and 9 electrodes in the third ventricle immediately ventral to the subfornical organ. Four electrodes located at the border between the mediodorsal and paraventricular nuclei of the thalamus supported high rates of self-stimulation although relatively high current intensities were required. The possibility that self-stimulation from the SF0 is due to current spread to this area or vice versa was considered. This seems unlikely, however, since the positive thalamic sites were about 1 mm ventral and more than 1 mm caudal to the sites in the SF0 positive for self-stimulation. Furthermore, several electrodes negative for self-stimulation were in the region between the SF0 and 4 positive electrodes in the area of the mediodorsal and paraventricular nuclei. A number of these negative electrodes terminated in the third ventricle and 3 in the dorsal thalamus.

Self-stimulation was also observed for each of 6 electrodes implanted into the lateral hypothalamus. In comparison to self-stimulation of the SF0, the rates of lever pressing elicited from rats with LH electrodes were considerably higher and the animals were hyperactive. These observed differences between self-stimu-
lation of the SF0 and the LH are of interest in view of recent suggestions that self-stimulation is mediated exclusively by noradrenergic (Crow, 1972; Ritter, & Stein, 1973; Stein, 1964; Stein & Wise, 1969) and dopaminergic (Clavier, & Routtenberg, 1974, Crow, 1972; Phillips, & Fibiger, 1973; Wauquier, & Niemégeers, 1972) pathways. Histochemical evidence indicates that the SF0 contains large quantities of acetylcholinesterase (Akert, 1969; Jacobowitz, & Palkovits, 1974; Lewis & Shute, 1967; Shute, & Lewis, 1966) and 5-hydroxytryptamine (Lichtensteiger, 1967), but is lacking in catecholaminergic cell bodies, fibers, or terminals (Jacobowitz, & Palkovits, 1974; Lichtensteiger, 1967; see Appendix 4.). Considered together, these data suggest that self-stimulation elicited from the SF0 and self-stimulation elicited from the lateral hypothalamus may be subserved by different neurobiochemical substrates. Recent pharmacological experiments (Robertson, Kucharczyk and Mogenson, unpublished) support this possibility and further indicate that self-stimulation elicited from the SF0 may be mediated by both serotonergic and cholinergic systems.
Self-Stimulation Of The Subfornical Organ and Lateral Hypothalamus: Differential Effects of Atropine and Methysergide

ABSTRACT

The effects of cholinergic blockade of neurons by atropine or serotonergic blockade by methysergide was investigated in rats responding for brain-stimulation reward. Bipolar stimulating electrodes were placed either in the subfornical organ (SF0) or the lateral hypothalamus (LH). Atropine sulphate and methysergide significantly suppressed self-stimulation of the SF0 but not of the LH, suggesting that cholinergic and serotonergic neurons are involved in brain-stimulation reward associated with this site.
The subfornical organ (SF0), which has gained prominence as the possible site of the dipsogenic effects of angiotensin II (Kucharczyk, Assaf & Mogenson, 1976; Simpson, & Routtenberg, 1973), has been shown recently to serve brain stimulation reward (Robertson, Kucharczyk & Mogenson, 1976). There is no evidence that this structure contains dopamine or noradrenaline (Jacobowitz, & Palkovits, 1974; Lichtensteiger, 1967; Saavedra, Brownstein, & Palkovits, 1976), both hypothesized to mediate self-stimulation (Mogenson, & Phillips, 1975; Phillips, & Fibiger, 1973). However the SF0 may contain acetylcholine (Akert, 1969; Jacobowitz & Palkovits, 1974; Lewis & Shute, 1967) and serotonin (Lichtensteiger, 1967). The possibility that these neurotransmitters play a role in self-stimulation of the SF0 was investigated by administering atropine, a cholinergic antagonist, and methysergide, a serotonergic antagonist, to rats self-stimulating through SF0 electrodes. For comparison, the effects of these drugs on self-stimulation of the lateral hypothalamus (LH) was also examined.
METHODO

Male albino rats weighing 250-300 g at the time of surgery were used in the study. The animals were individually housed in wire mesh cages in a temperature controlled room with lights on from 7:00 a.m. to 9:00 p.m. and with tap water and Purina rat chow available ad lib. Rats were anaesthetized with sodium pentobarbital (40-50 mg/kg IP) and were implanted under stereotaxic control with bipolar electrodes (Plastic Products Co., Roanoke, V), 127 μ in diameter and insulated except for the cross-sectional area at the tips. With the incisor bar 5.0 mm above the interaural line, electrodes were implanted into the subfornical organ (12° from the vertical midline, 0.0 mm anterior to bregma, 1.0 mm lateral, and 4.4-4.5 mm ventral to dura) and in the medial part of the lateral hypothalamus.

Following a one-week recovery period, the animals were tested for self-stimulation. Testing took place in a Plexiglas box (30 X 16 X 30 cm) with a lever at one end which could be depressed with a force of 16 g to deliver a 0.20 sec train of electrical stimulation. The stimulating waveform provided by a Grass S44 stimulator consisted of monophasic rectangular pulses of 0.20 msec duration presented at 80 Hz through a Grass stimulus isolation unit. Current was monitored continuously on an oscilloscope.

For animals in which self-stimulation occurred, testing was continued daily in one-half hr sessions and current was kept constant at suprathreshold levels which generated approximately equal response rates between groups of rats. Drugs were administered only after response rates were stable (i.e., a change from one day to the next of less than 10%) over a minimum of three daily consecutive tests.
A minimum of three days was allowed to elapse between drug administration and no animals received more than three different drug treatments.

Atropine sulphate and atropine methyl nitrate were dissolved in 0.9% isotonic saline and were administered (10, 15 and 20 mg/kg, IP) 20 min before testing. Animals with electrodes in SF0 received 10, 15 and 20 mg/kg of atropine sulphate or atropine methyl nitrate; animals with electrodes in LH received 15 and 20 mg/kg of atropine sulphate or atropine methyl nitrate. Methysérgide maleate dissolved to a concentration of 12.5 mg/ml in 0.9% saline and 0.1 N HCl and adjusted with NaOH to a pH of 7.0, was administered in a dose of 25 mg/kg body weight, IP, 20 min before testing. Additionally, three rats with SF0 electrodes were administered d-amphetamine and l-amphetamine (1 mg/kg, IP), 30 min before testing.

Upon completion of the behavioral tests, rats were sacrificed by an overdose of sodium pentobarbital. Brain sections of 50 μ thickness were cut using a freezing microtome and were stained with thionin and examined under a microscope. Only those rats with confirmed electrode placements in the SF0 and LH were included in the results. A representative electrode placement in the SF0 is shown in Fig. 1.
Fig. 1. Coronal section of a rat brain showing a representative electrode placement in the subfornical organ. The tip of the electrode, indicated by the arrow, lies in the lateral part of the subfornical organ.
RESULTS AND DISCUSSION

Rates of self-stimulation after various drug treatments were calculated as percentages of the average response rates for the two preceding test sessions. The results are presented in Fig. 2 and Table 1. Atropine sulphate (10 mg/kg) administered to rats with SF0 electrodes did not produce a significant suppression of responding compared to the effect of atropine methyl nitrate ($p > 0.05$). When given 15 mg/kg atropine sulphate, however, rats with SF0 electrodes showed a significant reduction of response rates (to 53% of predrug levels) in comparison to their rates when administered atropine methyl nitrate ($p < 0.05$) or in comparison to rats with LH electrodes given atropine sulphate ($p < 0.01$). When administered 20 mg/kg atropine sulphate, SF0 rats responded at 47% of preinjection levels, significantly lower than their rates following atropine methyl nitrate ($p < 0.02$) and lower than response rates of rats with LH electrodes administered 20 mg/kg of atropine sulphate ($p < 0.001$).

**TABLE 1**

THE EFFECT OF d- AND L- AMPHETAMINE* ON SELF-STIMULATION OF THE SF0 X

<table>
<thead>
<tr>
<th>Rat</th>
<th>d</th>
<th>l</th>
<th>Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>38</td>
<td>347</td>
<td>172</td>
<td>175</td>
</tr>
<tr>
<td>42</td>
<td>325</td>
<td>154</td>
<td>171</td>
</tr>
<tr>
<td>43</td>
<td>265</td>
<td>128</td>
<td>137</td>
</tr>
<tr>
<td>Mean difference</td>
<td>161</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* 1 mg/kg, IP.

x Results expressed as percentage of preinjection response rates.
Fig. 2. Response rates for electrical stimulation of the subfornical organ (SF0) or lateral hypothalamus (LH) upon administration of atropine sulphate or atropine methyl nitrate (10, 15 or 20 mg/kg IP, 20 min before testing) or methysergide (25 mg/kg IP, 20 min before testing). Response rates are expressed as percentages of preinjection rates. Vertical bars represent standard errors of the mean. The numbers of rats in each condition is indicated above each bar.
The administration of 25 mg/kg of methysergide did not reduce self-stimulation of the LH but did, in comparison, significantly reduce self-stimulation of the SF0 (p < 0.005).

The attenuation of self-stimulation from the SF0 when a cholinergic antagonist (atropine) or a serotoninergic agonist (methysergide) were administered suggests that both cholinergic and serotoninergic neurons are involved in self-stimulation of this area. A nonspecific effect (general behavioral debilitation) can be ruled out since self-stimulation of the LH was not similarly affected. It has previously been reported that atropine sulphate (Newman, 1972) and methysergide (Silverira Filho, & Graeff, 1977) have no effect on self-stimulation of the LH. Therefore self-stimulation of the SF0 can be differentiated from self-stimulation of the LH by the responses of animals to these drugs.

Methysergide, while it does have central antagonist effects on serotoninergic transmission, is probably not a universal antagonist (Haigler & Aghanjanian, 1974; Sofia & Vassar, 1975). Thus its lack of effect on self-stimulation of the LH does not indicate necessarily that serotoninergic transmission is not important in self-stimulation of the LH. It does indicate that the suppression of self-stimulation of the SF0 was not secondary to a general disruption of operant behavior.

Rates of self-stimulation of the SF0 were increased following the administration of both isomers of amphetamine, suggesting a possible role for catecholaminergic neurons (Phillips & Fibiger, 1973). The greater facilitation of self-stimulation following D-amphetamine than following L-amphetamine may indicate that noradrenergic neurons might be involved in self-stimulation of the SF0 (Herberg,
Stephens & Franklin, 1976; Phillips & Fibiger, 1975; Phillips & Fibiger, 1973). On the other hand, since self-stimulation of the SFO is suppressed by spiroperidol, a dopamine antagonist (unpublished observations), dopaminergic neurons might also be involved. Because of a recent report that the SFO contains both dopamine and noradrenaline (Saavedra, Brownstein, Kizer & Palkovits, 1976) it is possible that activation of catecholaminergic neurons contribute to self-stimulation of the SFO. Alternatively, the attenuation of self-stimulation by spiroperidol might be the result of motoric or other nonspecific behavioral effects.
PAPER IV

Evidence For A Role For Dopamine In Self-Stimulation Of The Nucleus Accumbens Of The Rat

Published By Canadian Journal Psychology, 1978, 32, 67-76.
ABSTRACT

The effects of centrally administered spiroperidol, a dopamine receptor or blocking agent, on self-stimulation of the nucleus accumbens and medial prefrontal cortex were investigated. Spiroperidol in a volume of 1 μl was micro-injected into the region of the stimulating electrodes. Self-stimulation of the nucleus accumbens was significantly attenuated by .75, 1.0, and 2.0 μg spiroperidol. Control micro-injections of the drug vehicle had no effect. Spiroperidol microinjected into the nucleus accumbens contralateral to the stimulating electrode, as a control for possible motor or non-specific effects, did not attenuate self-stimulation. Microinjections of spiroperidol into the region of the stimulating electrodes in the prefrontal cortex had no consistent effect on self-stimulation with the two lower doses, but did result in attenuation at the 2.0 μg dose. Self-stimulation of the nucleus accumbens was not changed by microinjections of spiroperidol into the ipsilateral or contralateral prefrontal cortex. Similarly, self-stimulation of the prefrontal cortex was not altered by microinjections of spiroperidol into the nucleus accumbens. By controlling for non-specific effects of spiroperidol, the results provide further evidence that dopaminergic neurons contribute to self-stimulation of the nucleus accumbens.
INTRODUCTION

Olds and his coworkers (1956) pioneered the pharmacological approach to the study of brain stimulation reward by observing the attenuation of self-stimulation of the lateral hypothalamus after the administration of chlorpromazine. Since that time, there has been increasing interest in identifying the neurochemical substrates of brain-stimulation reward. The mapping of monoaminergic neurons using histofluorescence techniques, and new methods of studying drug-brain interactions, provided an impetus to investigations in this field (Ungerstedt, 1971; Wauquier & Rolls, 1976). The early observation by Olds of the effects of chlorpromazine may be seen in retrospect as the first evidence implicating catecholamines in brain-stimulation reward.

Although both noradrenaline and dopamine have been implicated in brain-stimulation reward, recent evidence using techniques to selectively destroy dopaminergic neurons has tended to favor dopamine as a critical substrate at certain self-stimulation sites in the brain (Breese, Howard, & Leahy, 1971; Phillips, Carter & Fibiger, 1976). Evidence from drug studies, in which dopamine receptor blockers suppress self-stimulation, has also supported this view (Wauquier & Niemegeers, 1972; Phillips, Brooke, & Fibiger, 1975; Rolls, Rolls, Kelly, Shaw, Wood & Dale, 1974; Takigawa, Robertson, & Mogenson, 1978). Much of this pharmacological evidence, however, is inconclusive since it is not clear whether the suppression of self-stimulation represents a specific effect on central reward mechanisms or more general motor or other behavioral effects which disrupt operant performance (Fibiger, Carter, & Phillips, 1976; Fibiger, 1978; Fibiger & Phillips, 1978). In the present
study an attempt was made to circumvent this problem. Experiments were done in which spiroperidol, a dopamine receptor blocking agent (Andén, Butcher, Corrodi, Fuxe, & Ungerstedt, 1970), was microinjected directly into the area of the stimulation electrode. Electrode-cannula assemblies were implanted chronically into two dopamine-containing regions of the rat forebrain: the nucleus accumbens septi (NAS) and the medial prefrontal cortex (PFC). As a control for possible non-specific effects of spiroperidol, self-stimulation from the electrode contralateral to the site of drug administration was also investigated. In addition, spiroperidol was micro-injected into the prefrontal cortex in rats with self-stimulation electrodes in the ipsilateral nucleus accumbens or into the nucleus accumbens in rats with self-stimulation electrodes in the ipsilateral prefrontal cortex.
METHOD

Animals and Surgery

Male albino rats weighing from 250 to 300 gm at the time of surgery were used. They were housed in individual wire mesh cages with food and water provided ad libitum.

In preparation for stereotaxic surgery, rats were anaesthetized with sodium pentobarbital (Nembutal, about 50 mg/kg, i.p.). In the first and second series the animal was implanted using standard stereotaxic procedures with an electrode-cannula unit into the NAS and an electrode (bipolar Plastic Products, 127 μ in diameter at the tips) into the contralateral NAS, or with PFC electrode-cannula units and contralateral PFC electrodes. In the third series, some animals were implanted with cannulae (635 μ in diameter) into the NAS and electrodes and electrode-cannula units into the PFC ipsilateral and contralateral, respectively, to the cannula. Other animals were implanted with cannulae into the PFC and electrodes and electrode-cannula units into the NAS ipsilateral and contralateral, respectively, to the cannula. With the incisor bar 5.0 mm above the interaural line, NAS electrodes and electrode-cannula units were positioned 3.4 mm anterior to bregma, 1.7 mm lateral to the midline, and 8.2 mm ventral to the skull. Cannulae were implanted into the NAS using the same co-ordinates, except that the cannula was lowered 6.6 mm ventral to the skull. PFC electrode and electrode-cannula units were 4.5 mm anterior to bregma, 0.7 mm lateral, and 3.5 mm ventral to the skull. Cannulae aimed at the PFC were positioned using the same co-ordinates, except that the cannula was lowered 2.0 mm ventral to the skull.
An electrode-cannula unit is shown in Figure 1. The entire unit was insulated with epoxy (Epoxylite). An obturator, constructed from a 00 insect pin and cut so that it terminated slightly above the outer cannula, was left in place between injections.

Self-Stimulation Tests

Following a one-week recovery period from the surgery, rats were trained to bar press for electrical stimulation in a Plexiglass box (28 X 16 X 30 cm) with a lever at one end which could be depressed by a force of approximately 16 gm to deliver current for 0.2 sec. The stimulation, generated by a Grass 544 stimulation, consisted of monophasic rectangular pulses, 0.2 msec in duration, presented at 80 Hz through a Grass stimulus isolation unit. Current was monitored on an oscilloscope throughout testing and was kept constant for each rat throughout testing.

Rats which self-stimulated were tested for 15 minutes per electrode per day. Since each animal had 2 electrodes, it received a total of 30 minutes of testing per day. The order in which the 2 electrodes were tested (i.e., contralateral or ipsilateral electrode first) was varied from rat to rat. The mean baseline response rate for rats with NAS electrodes and electrode-cannula units was 367 responses per 15 min (at a mean current of 280 µA) and, for rats with PFC electrodes and electrode-cannula units, was 388 responses per 15 min (at 430 µA).

When response rates had stabilized (a variation of less than 10% over 3 days of testing), drug injections began. Animals received drug and control injections in random order with a minimum of 3 days between successive infusions.
Fig. 1. Diagram showing the electrode-cannula unit implanted into the nucleus accumbens and/or prefrontal cortex. The electrode was attached to the cannula by dental cement. The mean distance between the tip of the injector cannula and the electrode tips (indicated by 1) was 0.57 mm for prefrontal cortex placements and 0.62 mm for nucleus accumbens placements. The mean distance between the tip of the outer cannula and the tips of the electrode (indicated by 2) was 1.58 mm for prefrontal cortex placements and 1.61 for nucleus accumbens placements.
inner cannula (injector 310 μ)
outer cannula (635 μ)
dental cement
electrode (127 μ diameter wires)
Their response rates following drug infusions were expressed as a percentage of the mean response rates for the 2 to 3 day period preceding injections.

Central Administration of Drug

In preparation for injection of the drug, polyethylene tubing (PE10) attached to an injector cannula constructed from 30 ga stainless steel tubing was pre-loaded with the drug solution. A Hamilton microsyringe was used to inject the solution into the brain. Injections of the drug were made over a 2.5 to 3-min period. Rats were then placed in their home cages for 4.5 to 5 minutes before self-stimulation tests were begun.

In the first series of animals, spiroperidol was dissolved in a vehicle of tartaric acid and distilled water. Rats were injected through the cannula of the electrode-cannula unit with .25, .35, .45, and .75 µg of spiroperidol in volumes of .5 to 1.0 µl. Additionally, two vehicle control injections (.5 and 1.0 µl) were given to each rat. The solutions were adjusted by the addition of IN NaOH to a pH of 6.0 (except for one control solution and the .75 µg dose, in which the pH was 2.5). In the second series of animals, spiroperidol was dissolved in a vehicle of 1% lactic acid and distilled water, with pH adjusted to 6.0. All solutions were injected through the cannulae of the electrode-cannula units in a volume of 1.0 µl in the following doses: .5 µg, 1.0 µg, and 2.0 µg. Additionally, a vehicle control solution (1.0 µl) was injected into each animal. Several rats were also tested after the administration of atropine sulphate (1.0 µg dissolved in distilled water and infused in a volume of 1.0 µl). In the third series of animals, spiroperidol (in 1% lactic
acid and distilled water and 1N NaOH with a pH of 6.0 to 6.5) was administered through the cannula of the electrode-cannula unit or through the single cannula.

Histology

Upon completion of the behavioural tests, rats were sacrificed by an overdose of sodium pentobarbital. Brain sections of 50 μ thickness were cut using a freezing microtome and were stained with thionin and examined under a microscope. Only those rats with confirmed placements in the nucleus accumbens or prefrontal cortex were included in the results. Électrode placements are shown in Figure 2.
Fig. 2. Electrode placements in the prefrontal cortex and nucleus accumbens shown on three coronal sections redrawn from the Konig and Klippel (1970) atlas. The stippled areas in each section show the location of the electrode tips. Microns anterior to the interaural line are indicated on each section. The prefrontal cortex (section 11050) is outlined according to the area of the dopaminergic terminals shown by Berger, Thierry, Tassin and Moyne (1976). The nucleus accumbens is shown in sections 9820 and 8920. Abbreviations: AC, anterior commissure; CC, corpus callosum; CPU, caudate-putamen; NAS, nucleus accumbens, PFC, medial prefrontal cortex; SL, lateral septum; TO, olfactory tract; VL, Lateral ventricle.
RESULTS

In the first series of 11 animals, spiroperidol injected into the ipsilateral NAS reduced self-stimulation at all dose levels. After administration of .25, .35 or .45 μg of spiroperidol, response rates were 80-86% of pre-drug levels and after the highest dosage of .75 μg, response rates were 44% of pre-drug levels. Neither spiroperidol nor vehicle control injections had an effect on self-stimulation of the contralateral NAS. When response rates for the ipsilateral electrode were compared with the contralateral electrode at each dose, it was found that only the highest doses produced a significant suppression of responding for the ipsilateral electrode ($t_D = 2.73; p < .025$). Spiroperidol in the range of .25-.75 μg microinjected into the PFC did not significantly reduce self-stimulation of either the ipsilateral or contralateral PFC, but responding was more variable.

In the second series of 14 animals, a wider range of doses of spiroperidol was used (.5-2.0 μg in 1.0μl). Injection of spiroperidol into the NAS reduced self-stimulation at all dose levels tested (Fig. 3). After the administration of .5 μg, response rates were 83% of pre-drug levels. Response rates after microinjections of 1.0 and 2.0 μg of spiroperidol were 53 to 55% of pre-drug levels. Contralateral NAS response rates were not affected by spiroperidol administration. When response rates from the contralateral and ipsilateral electrodes were compared for each dose of spiroperidol administered, it was found that only the 1.0 and 2.0 μg doses, but not the .5 μg dose, produced a significant suppression of self-stimulation of the ipsilateral electrode ($t_D (1.0 \mu g) = 5.07; t_D (2.0 \mu g) = 4.08; p < .001$). Vehicle control injections had no effect on self-stimulation of the NAS, ipsilateral or contra-
Fig. 3. Effects of spiroperidol (0.5, 1.0 and 2.0 μg) microinjected into the nucleus accumbens on self-stimulation of the nucleus accumbens (NAS, top panel) and into the prefrontal cortex on self-stimulation of the prefrontal cortex (PFC, bottom panel). All injections were in a volume of 1.0 μl. Response rates are shown on the ordinate and drug doses on the abscissa. The effect of atropine (1.0 μg in 1.0 μl) microinfused into the nucleus accumbens and into the prefrontal cortex on self-stimulation of the prefrontal cortex is shown on the right. Cross-hatched columns represent self-stimulation from the electrode attached to the cannula (ipsilateral self-stimulation) and open columns represent self-stimulation from the contralateral electrode. The number of rats per group is indicated at the bottom of each column and standard errors are represented by vertical bars.
lateral to the site of the microinjection.

Self-stimulation of the PFC was not reduced by microinjections of the two smaller doses of spiroperidol (Fig. 3). After microinjecting 2.0 µg spiroperidol, however, there was a clear suppression of self-stimulation to 41% of pre-injection rates. This was significantly lower (tD = 2.58, p < .025) than self-stimulation of the contralateral PFC. Atropine (1.0 µg/1.0 µl) had no effect on self-stimulation of the NAS or PFC.

The results for the third series of 10 animals are shown in Figure 4. Microinjections of spiroperidol (1.0 µg) into the ipsilateral NAS reduced the rate of self-stimulation of this site by 58%. Compared with microinjections of spiroperidol into the contralateral NAS which did not change self-stimulation, the reduction was statistically significant (tD = 4.56, p < 0.01). Microinjections of spiroperidol (1.0 µg) into the PFC either ipsilateral or contralateral to the site of stimulation of PFC did not reduce self-stimulation, confirming the observations from the second series of animals. Self-stimulation of the NAS was not changed by microinjections of spiroperidol into the ipsilateral or contralateral PFC in five rats. Similarly, self-stimulation of the PFC was not reduced by microinjections of spiroperidol into the NAS in four rats.

The highest dose of 2.0 µg of spiroperidol did significantly suppress self-stimulation of the PFC when injected into that area. It was considered possible, therefore, that 1.0 µg injected into the PFC when self-stimulation of the NAS was tested, was not a sufficient dose to affect response rates. A dose of 2.0 µg was therefore injected into the PFC of 4 rats with contralateral and ipsilateral NAS
Fig. 4. Effects of spiroperidol (1.0 μg in 1.0 μl) microinfused into the nucleus accumbens (NAS, left side) or into the prefrontal cortex (PFC, right side) on self-stimulation of the NAS (top panel) and on self-stimulation of the PFC (bottom panel). Open columns represent self-stimulation from the electrode contralateral to the site of stimulation; cross-hatched columns from the electrode ipsilateral to the site of stimulation. The number of rats per group is indicated at the bottom of each column and standard errors are represented by vertical bars.
electrodes. Responding was not affected by this treatment (ipsilateral: 101% ± 6% of pre-drug rates; contralateral: 101% ± 4%). Similarly, 2.0 μg of spiroperidol injected into the NAS when self-stimulation of the PFC was tested in 5 rats, did not significantly alter response rates (ipsilateral: 82% ± 14%; contralateral: 104% ± 6%).
DISCUSSION

Microinjections of spiroperidol into the nucleus accumbens in doses of .75 to 2.0 μg significantly reduced self-stimulation of the nucleus accumbens. Self-stimulation of the contralateral nucleus accumbens was not altered at those doses, indicating that the effect was not due to motor or other non-specific performance deficits. When self-stimulation of the nucleus accumbens was tested following microinjections of spiroperidol into the prefrontal cortex, there was no change of rates. Microinjections of spiroperidol in doses up to 1.0 μg into the prefrontal cortex had no effect on self-stimulation of the prefrontal cortex. When the dose was increased to 2.0 μg, however, self-stimulation of the ipsilateral, but not the contralateral, prefrontal cortex was reduced. Self-stimulation of the prefrontal cortex was not altered by administration of spiroperidol into the nucleus accumbens either ipsilateral or contralateral to the site of self-stimulation. Microinjections of atropine, a cholinergic (muscarinic) receptor blocker, into the nucleus accumbens or prefrontal cortex had no effect on self-stimulation of these sites.

These observations suggest that self-stimulation of the nucleus accumbens and perhaps the prefrontal cortex depends on activation of dopaminergic neurons and provide further support for the hypothesis that dopamine has a role in brain-stimulation reward (Crow, 1972). Since self-stimulation of the two sites was not completely abolished by any of the doses of spiroperidol tested, however, other neurotransmitters may be involved. The observation that spiroperidol microinjected into the prefrontal cortex did not have the same effects on self-stimulation of the prefrontal cortex as did spiroperidol microinjected into the nucleus accumbens on self-stimulation of
the nucleus accumbens, and the observation that self-stimulation of the nucleus accumbens was not altered by spiroperidol microinjected into the prefrontal cortex or vice-versa, support the recent findings of Phillips and Fibiger (1978) that self-stimulation of the nucleus accumbens and prefrontal cortex does not depend on the same biochemical substrates.

The results of a number of previous studies using dopamine receptor blockers have implicated dopaminergic neurons in brain-stimulation reward of the nucleus accumbens and other sites (Phillips, Brooke, & Fibiger, 1975; Rolls, Rolls, Kelly, Shaw, Wood, & Dale, 1974; Takigawa, Robertson & Mogenson, 1978). In one of the earliest studies by Wauquier and Niemegeers (1972), haloperidol and pimozide reduced self-stimulation of the lateral hypothalamus in a dose-related manner. These investigators were, however, reluctant to conclude that reward mechanisms had been disrupted by these neuroleptic drugs and suggested that the attenuation of self-stimulation was the result of a motor or performance deficit. The results of other studies have suggested that dopamine receptor blockers, which produce catalepsy and akinesia in experimental animals and man (Chase, 1976; Honma & Fukushima, 1976) and disrupt shock-avoidance responding (Niemegeers, Verbruggen, & Janssen, 1969), might reduce brain self-stimulation because of motor impairments (Rolls, Rolls, Kelly, Shaw, Wood, & Dale, 1974; Fibiger, Carter, & Phillips, 1976).

In an attempt to circumvent this confounding effect, Mora and co-workers (1975) selected a tongue-licking response, not disrupted by spiroperidol when water was the reward. Since spiroperidol reduced the rate of tongue-licking to initiate self-stimulation of the lateral hypothalamus, it appeared that there was a specific effect
on the reward mechanisms. These findings may not be conclusive, however, since as pointed out by Fibiger (1978) and by Fibiger & Phillips (1978), baseline rates of licking for water and for brain-stimulation reward, which may have influenced the action of the drug, were not reported.

In the present study, spiroperidol was administered directly into the nucleus accumbens and medial prefrontal cortex, regions which receive dopaminergic projections from the ventral tegmental area (Berger, Thierry, Tassin, & Mayne, 1976; Ungerstedt, 1971). As a control for motor impairment or other non-specific behavioural effects, spiroperidol was administered to these regions contralateral to the site of stimulation. This seems an essential control in view of a report by Broekkamp & van Rossum (1975) that self-stimulation of the midbrain tegmentum was attenuated equally by microinjections of haloperidol into the contralateral and ipsilateral caudate-putamen. Our observation that spiroperidol microinjected into the ipsilateral nucleus accumbens significantly attenuated self-stimulation of this site whereas self-stimulation of the contralateral nucleus accumbens was not affected provides strong evidence in support of a role for dopaminergic neurons in self-stimulation of the nucleus accumbens.

The behavioral specificity of spiroperidol was controlled for in this study by comparing self-stimulation of sites contralateral and ipsilateral to the drug injection. It is possible, however, that the suppression of self-stimulation of the nucleus accumbens when spiroperidol was injected into this site was due to a non-specific 'anaesthetic' effect of the drug on neurons in the area of the microinjection. This possibility seems unlikely for two reasons. First, doses of .75 to 1.0 μg of
spioperidol, which produced a significant reduction of nucleus accumbens self-stimulation, had no significant effect on prefrontal cortex self-stimulation when spioperidol was microinjected into the prefrontal cortex. In fact, some rats showed increases in response rates after such treatment. If there was an anaesthetic effect, spioperidol should have produced the same consistent and uniform effect on both nucleus accumbens and prefrontal cortex self-stimulation. Second, there is evidence that spioperidol is a specific dopamine receptor blocking agent. It is one of the most potent neuroleptics known (Janssen, 1970, 1976), binds to dopamine-sensitive receptors in the rat brain (Fields, Reisine, & Yamamura, 1977; Leyson, Gommeren, & Laduron, 1978), has very little anti-cholinergic effect (Iversen, 1975), and blocks noradrenergic synapses only in very high doses (Anden, Butcher, Corrodi, Fuxe, & Ungerstedt, 1970). Spioperidol and other butyrophenones probably also have feedback actions on dopamine neurons since they increase the turnover of dopamine (Anden, Butcher, Corrodi, Fuxe, & Ungerstedt, 1970), increase the levels of homovanillic acid, the principal metabolite of dopamine (Asper, Baglioni, Burki, Launener, Ruch, & Stille, 1973; Lerner, Nose, Gordon, & Lovenberg, 1977) and increase the activity of tyrosine hydroxylase, the rate-limiting enzyme involved in dopamine synthesis (Lerner, Nose, Gordon, & Lovenberg, 1977). Dopamine receptor blockers also increase the firing of dopamine cells (Aghajanian & Bunney, 1973), compatible with the hypothesis that they act on dopamine receptors causing compensatory changes in dopamine neuronal activity, perhaps by presynaptic receptor activity. Therefore, it seems likely that, in the present study, spioperidol acted specifically on dopamine receptors in the nucleus accumbens to produce the observed reduction in self-
stimulation of that site.

Using a similar strategy of comparing contralateral and ipsilateral self-stimulation sites, Mogenson, Robertson, Takigawa & Wu (in preparation) have recently observed that microinjections of spiroperidol (1.0 µg/1.0 µl) into the nucleus accumbens suppresses self-stimulation of the ipsilateral but not the contralateral ventral tegmental area. This suggests that self-stimulation of both nucleus accumbens and ventral tegmental area is subserved in part by dopaminergic neurons originating in the ventral tegmental area and innervating the nucleus accumbens. Dopaminergic projections from the ventral tegmental area terminating in the prefrontal cortex are apparently not involved in self-stimulation of the nucleus accumbens since in the present study microinjections of spiroperidol into the prefrontal cortex did not alter self-stimulation of the nucleus accumbens, supporting the view that mesocortical and mesolimbic self-stimulation sites are not subserved by the same dopamine neurons (Phillips & Fibiger, 1978).

The role of dopamine in self-stimulation of the prefrontal cortex is less clear, since spiroperidol attenuated self-stimulation of the prefrontal cortex only at the highest dose (2.0 µg). Other studies, however, have implicated dopamine neurons of the prefrontal cortex in brain stimulation reward. Mora and co-workers (1976) observed the attenuation of self-stimulation of the lateral hypothalamus and other sites after microinjections of spiroperidol into the prefrontal cortex. Mora and Myers (1977) and Myers and Mora (1977) reported the release of dopamine in the prefrontal cortex during self-stimulation of the prefrontal cortex. These observations suggest that dopaminergic fibres projecting to the prefrontal cortex are
activated during self-stimulation of some brain areas. We have recently observed increased rates of self-stimulation of the prefrontal cortex for several weeks following chronic daily intraperitoneal administration of spiroperidol (unpublished observations). Further experiments are needed to account for the failure of the lower doses of spiroperidol to attenuate self-stimulation of the prefrontal cortex when the compound was microinjected directly to this site (Fig. 3). It is possible that spiroperidol has a different affinity for dopamine receptors in the prefrontal cortex than in the nucleus accumbens, since it has been suggested that spiroperidol has different effects on dopamine receptors in different areas of the forebrain (Bockaert, Tassin, Thierry, Glowinski, & Premont, 1977; Laduron, Janssen, & Leysen, 1978). It is also possible that self-stimulation electrodes were not in the region of dopaminergic terminals or that the injection failed to spread to the region of dopamine receptors. This seems unlikely, however, since the electrodes were in the area shown by Berger, Thierry, Tassin, & Moyne (1976) to receive dopaminergic projections, and the 2.0 μg dose, which significantly attenuated self-stimulation of the prefrontal cortex, was in the same volume (1.0 μl) as the lower, ineffective doses. Another possibility is that the 2.0 μg dose of spiroperidol affected the activity of other neurotransmitters in the frontal cortex. It has recently been suggested that spiroperidol may have anti-tryptaminergic effects, perhaps in the frontal cortex (Leysen, Gommeren, & Laduron, 1978). Spiroperidol may also, at this high dosage, block noradrenergic receptors which have been hypothesized to contribute to self-stimulation either independently or by interacting with dopaminergic neurons (Herberg, Stephens, & Franklin, 1976). Finally, it has been proposed that prefrontal cortex self-stimulation is at least part-
ially dependent on descending pathways from prefrontal cortex to midbrain (Routtenberg & Sloan, 1972; Rolls & Cooper, 1973; Phillips & Fibiger, 1978). This proposal would also account for the lesser effect of spiroperidol in self-stimulation of the prefrontal cortex compared to the nucleus accumbens.

In summary, experiments involving microinjections of spiroperidol into the nucleus accumbens, with a control for motor or behavioural effects, provides evidence that self-stimulation of this site is associated with activation of dopaminergic neurons. Further experiments are needed to clarify the role of dopaminergic neurons in self-stimulation of the prefrontal cortex.
PAPER V

Facilitation of Self-Stimulation of the Prefrontal Cortex in Rats Following Chronic Administration of Spiroperidol or Amphetamine
SUMMARY

The effects of chronic administration of spiroperidol, a dopamine-receptor blocking agent, on self-stimulation of the prefrontal cortex and nucleus accumbens were investigated. For comparison, self-stimulation of the supracallosal bundle, caudate-putamen, ventral tegmental area and subfornical organ were also observed. When spiroperidol (0.10 mg/kg, i.p.) was administered daily for nine days, two hours before the test session, self-stimulation of the prefrontal cortex was attenuated. When the administration of spiroperidol was discontinued, self-stimulation of the prefrontal cortex increased significantly above control levels. When spiroperidol was administered after, instead of before, the self-stimulation sessions every day for nine days, self-stimulation of the prefrontal cortex began to increase after two or three days and remained elevated for as long as testing was continued. Vehicle control injections for the same period of time did not alter self-stimulation of the prefrontal cortex. Self-stimulation of the nucleus accumbens was attenuated when spiroperidol was administered two hours before testing every day but was not changed when spiroperidol was administered after testing every day, either during or after withdrawal of the drug. Similarly, self-stimulation of the caudate, ventral tegmental area and subfornical organ was not significantly altered by spiroperidol administration. When D-amphetamine (1.50 mg/kg, i.p.) was administered to rats with prefrontal cortex electrodes daily after testing for nine days, self-stimulation rates began to increase after two or three days and remained elevated for the duration of testing. Self-stimulation of the nucleus accumbens and cingulate cortex was unaffected. Self-stimulation of the supracallosal bundle-
began decreasing after the first three to four days and remained at significantly depressed levels for the remainder of testing. The results provide evidence that dopamine is involved in self-stimulation of the prefrontal cortex although its role may not be essential, merely modulatory. Additionally, the site-specific effects of spiroperidol on self-stimulation of the prefrontal cortex may involve mechanisms similar to those involved in the therapeutic effects of neuroleptics in humans.
INTRODUCTION

Dopamine has been implicated in brain-stimulation reward partly on the basis that administration of neuroleptics, which block dopaminergic transmission, tends to suppress self-stimulation (see review by Fibiger, 1978). Most of these pharmacological studies have involved administration of single doses of the drug. When used clinically to alleviate psychotic symptoms in humans, neuroleptic drugs must be administered chronically for therapeutic effects to occur (Baldessarini, 1977). Chronic administration of dopamine-receptor blockers to self-stimulating rats may offer, therefore, information regarding the mechanisms through which such drugs exert therapeutic effects in man. This is particularly relevant in view of recent reports suggesting that chronic administration of neuroleptics produces effects on self-stimulation of sites in the lateral hypothalamus and midbrain markedly different from the effects observed with single doses (Ettenberg & Milner, 1977; Eichler, Antelmann & Fisher, 1976). Therefore the present experiments were designed to assess the effects of chronic administration of spiroperidol, a relatively potent and specific receptor blocker (Janssen, 1976), and of amphetamine, which releases catecholamines, on self-stimulation of several different forebrain sites in the rat. The medial prefrontal cortex was given special attention since it has been implicated in brain-stimulation reward (Mora, 1978) and in psychotic behavior (Berger, 1978; Hokfelt, Ljungdahl, Fuxe & Johansson, 1974; Snyder, Banerjee, Yamamura & Greenberg, 1974).
METHOD

Subjects and Electrode Implantation

Male albino rats weighing from 250–300 gm at the time of surgery were used. They were housed in individual wire mesh cages with food and water provided ad libitum. In preparation for implantation of electrodes, rats were anaesthetized with sodium pentobarbital (Nembutal, about 50 mg/kg, i.p.). Under stereotaxic control, bipolar electrodes (Plastic Products) made of stainless steel wire (127 μ in diameter and insulated except for the cross-sectional area at the tip) were lowered into the brain. Dental acrylic was used to attach the electrode to jewelers’ screws placed in the skull. Each rat was implanted with three electrodes aimed at three of the following sites: supracallosal bundle (SCB), médial prefrontal cortex (PFC), nucleus accumbens septi (NAS), caudate-putamen (CPU), ventral tegmental area (VTA), or subfornical organ (SF0).

Self-Stimulation Training

After a one-week recovery period, rats were tested for self-stimulation. Testing took place in a Plexiglas box (28 x 16 x 30 cm) with a lever at one end which could be depressed with a force of about 16 gm to deliver a 0.2 sec train of electrical stimulation. The source of stimulation was a Grass S44 stimulator set to deliver monophasic rectangular pulses of 0.2 msec duration at 80 Hz. The current was passed through a Grass stimulus isolation unit and was monitored continuously on an oscilloscope. Once self-stimulation was established in a rat, current was kept constant thereafter at a level which produced approximately equal response rates for the different sites (200 to 350 responses/10 min.). Rats were tested daily
at about the same time every day for 10 min. per electrode.

Drug Administration

Spiroperidol was dissolved in tartaric acid (0.01M) with the pH adjusted to 6.5 to 7.0 with 1 N NaOH. It was administered in a dosage of 0.10 mg/kg, i.p. D-Amphetamine HCl was dissolved in isotonic saline and administered in a dosage of 1.50 mg/kg, i.p. Drug administration was begun after each rat’s baseline rate had stabilized (a change of less than 10% over three days of testing).

The animal’s baseline rate for the three-day period preceding the first day of drug administration was averaged and used as a baseline measure. Daily response rates during and after drug administration were expressed as a percentage of this baseline rate. All drugs were administered for a period of nine days.

In the first experiment, rats with electrodes in the NAS and CPU, or in the PFC, were given 0.05 mg/kg of spiroperidol two hours before their daily test session. Thereafter, they were administered 0.10 mg/kg spiroperidol for 8 more days, two hours before their test session. Testing was continued daily for the period of administration and for 17 days thereafter.

In the second experiment, two separate series of animals were run. In the first series, rats were implanted with electrodes in the PFC, CPU and SFO, or with electrodes in the NAS, SCB, and SFO. In the second series, animals were implanted with NAS and PFC electrodes, or with PFC-VTA-SFO electrodes. Four rats with PFC electrodes and four rats with NAS electrodes received control injections. All other groups received 0.10 mg/kg spiroperidol immediately after testing.
every day for 9 days. All groups were tested for 18 to 27 days thereafter. Additionally, several rats were retested 48 to 51 days after the start of drug administration.

In the third experiment, the effects of chronic administration of \( \text{D-amp} \) amphetamine on self-stimulation of the PFC, SCB, and NAS was tested. Rats were implanted with electrodes in the SCB, PFC, and NAS and were trained to self-stimulate. When response rates had stabilized, drug administration began. For 9 days, rats were injected with 1.50 mg/kg \( \text{D-amp HCl} \), immediately after they were tested. Response rates were measured during this 9 day period and for 7 days after.

Data Analysis and Histology

Results were analyzed by analyses of variance, followed by Newman-Keuls tests for individual comparisons. After completion of testing, animals were killed with an overdose of sodium pentobarbital and their brains were removed and sectioned with a freezing microtome. The sections were stained with thionin and examined under a microscope to determine the site of electrode tips.
RESULTS

Effects of Spiroperidol Administered Before Daily Tests

The results from rats with NAS or CPU electrodes were similar and were therefore combined. When spiroperidol was administered two hours before the daily tests there was a decrease in self-stimulation of the NAS, CPU and PFC (Figure 1). For NAS electrodes, the decrease was statistically significant on the second day and for PFC electrodes, on the fifth day. \( p < .05 \). When the administration of spiroperidol was discontinued, self-stimulation of the PFC increased for the next four days and remained at a level about 70% above pre-drug rates of self-stimulation. Self-stimulation of the NAS and CPU returned to pre-drug rates when the daily injections of spiroperidol were discontinued and for the remainder of the experiment were significantly below rates for self-stimulation of the PFC.

Effects of Spiroperidol Administered After Daily Tests

Spiroperidol administered daily to rats with PFC electrodes two hours after the test session caused an enhancement of responding, which was first apparent after 5 days (Figure 2). Response rates continued to increase for 12 days after the first injection (about 3 days after the final injection) and thereafter remained at 80 to 100% above pre-drug levels. The increase in rates was still observed 48 to 52 days after the start of the experiment. In comparison, control injections had little effect on responding. By the 10th day, response rates of the control group were significantly different \( p < .05 \) than response rates of the group receiving spiroperidol.

Spiroperidol administered daily two hours after the test session to
Fig. 1. Effect of spiroperidol (0.05 mg/kg, i.p. on Day 1 and .10 mg/kg, i.p. on Days 2 to 9) on self-stimulation rates of rats with PFC, NAS and CPU electrodes. Injections were given two hours before the daily test session. The period of drug administration is indicated on the abscissa. Response rates are expressed as percentages of the baseline response rates before drug administration was begun. Standard errors are indicated by vertical bars.
Fig. 2. Effect of spiroperidol (0.1 mg/kg, i.p.) or control injections on self-stimulation rates of rats with PFC electrodes. Injections were given after the daily test session. The period of drug administration is indicated on the abscissa. Response rates are expressed as percentages of the baseline response rates before drug administration was begun. Standard errors are indicated by vertical bars.
rats with NAS electrodes did not significantly alter response rates (Figure 3). Similarly, spiroperidol administered to rats with SCB, SFO, CPU or VTA electrodes caused no significant effects although, during the 9 day period of administration response rates of rats with SCB, SFO and VTA electrodes were about 80% of pre-drug levels and rats with CPU electrodes showed an even greater but insignificant suppression of responding.

Effects of Amphetamine Administered After Daily Tests

The results were analyzed according to the site of the electrode. Five different neuroanatomical loci were identified: (1) the SCB, where electrodes bordered on the corpus callosum and were therefore probably stimulating NE fibres (SCB-NE, n=3); (2) the SCB where electrodes bordered on the cingulate DA-containing area (SCB-cing, n=3); (3) the PFC DA-containing area (PFC-DA, n=3); (4) the PFC non-DA-containing area (PFC-non DA, n=2) and (5) the NAS (n=3).

Within one to two days, response rates of rats with SCB-NE electrodes began declining and remained at significantly suppressed (p < .05) levels (about 20% of pre-injection rates from Days 8 to 14) for the duration of testing (Figure 4). Rats with PFC-DA electrodes showed a significant increase (p < .05) in response rates from Days 7 to 14. Animals in the other three groups—the SCB-cing; PFC-non DA; and NAS—showed no changes in rates throughout the experiment. Twenty-two to twenty-three days after the start of the experiment (13 to 14 days after withdrawal of amphetamine), PFC SS rates were still enhanced and SCB rates were still suppressed.
Fig. 3. Effect of spiroperidol (0.1 mg/kg., i.p.) or control injections on self-stimulation rates of rats with NAS electrodes (top panel) or CPU, SCB, SF0 or VTA electrodes (bottom panel). Injections were given after the daily test session. The period of drug administration is indicated on the abscissa. Response rates are expressed as percentages of the baseline response rates before drug administration was begun. Standard errors are indicated by vertical bars.
Fig. 4. Effect of amphetamine (1.5 mg/kg. i.p.) on self-stimulation rates of rats with SCB, PFC and NAS electrodes (see text for explanation of terms). Injections were given after the daily test session. The period of drug administration is indicated on the abscissa. Response rates are expressed as percentages of the baseline response rates before drug administration was begun. Standard errors are indicated by vertical bars.
DISCUSSION

Spiroperidol administered daily before self-stimulation tests caused a suppression of response rates in rats with PFC, NAS or CPU electrodes during the period of administration. Since all three groups of animals showed an attenuation of responding, there may have been some general or non-specific deficit in operant behavior. After the period of drug administration, rats with NAS or CPU electrodes responded at normal pre-drug rates. In contrast, rats with PFC electrodes had significantly higher rates of responding, confirming the recent findings of Antelman, Eichler and Fisher (1977) and Eichler and Antelman (personal communication). When spiroperidol was administered after the daily tests, response rates of animals with PFC electrodes increased after five to seven days of administration without the initial decrease. This increase persisted after drug administration ceased. Administration of the drug vehicle for nine days had no effect on self-stimulation of the PFC, which indicates that the facilitation of response rates after spiroperidol administration was not due to any tendency for PFC self-stimulation rates to increase with time, regardless of drug treatment. Amphetamine administered daily after testing caused much the same facilitative effects as did spiroperidol on response rates of rats with PFC electrodes after three days of administration, suggesting that chronic treatment with this drug also alters the reinforcing aspects of stimulation of the PFC. Non-specific behavioral activation appears unlikely since, as was the case after chronic administration of spiroperidol, NAS self-stimulation rates were essentially normal.

The most striking observation from these experiments is the long-term
changes in self-stimulation of the PFC during and after administration of sprioperidol or amphetamine and the lack of similar changes in self-stimulation of other dopaminergic areas (NAS and CPU) or other sites. Chronic administration of amphetamine probably enhances dopaminergic transmission (Rebec & Groves, 1976; Costentin, Protas & Schwartz, 1975) and may induce an increased receptor sensitivity (Jenner, Pycock & Marsden, 1978). Since sprioperidol, a fairly selective dopaminergic antagonist, also produced a facilitation of self-stimulation of the PFC, the data indicate that modification of dopaminergic transmission is sufficient to produce this effect. However, in previous experiments we (Robertson & Møgenson, 1978) have observed that sprioperidol microinjected directly into the region of stimulating electrodes in the PFC does not alter self-stimulation of the PFC in doses, which, when microinjected into the NAS, suppress self-stimulation of the NAS. These data might suggest that dopamine partly maintains self-stimulation of the NAS but is of lesser importance in self-stimulation of the PFC. But chronic sprioperidol administration facilitated self-stimulation of the PFC but not the NAS. Additionally, Phillips and Fibiger (1978) recently reported that destroying the dopaminergic projections to the NAS by 6-OHDA disrupts self-stimulation of this structure but only temporarily; in contrast, self-stimulation of the PFC is more severely disrupted. One explanation of these studies is that self-stimulation of the NAS is partly maintained by dopamine neurons but with prolonged changes in dopaminergic activity (by chronic blockade or 6-OHDA lesions), self-stimulation of the NAS can be maintained by other neurotransmitters. In contrast, self-stimulation of the PFC may not initially depend on dopaminergic activation but, with prolonged drug treatment, dopamine
activity may increase and add to the positively reinforcing effects of PFC stimulation.

After administration of a single dose of a dopamine-receptor blocker, there is an immediate increase in dopamine synthesis and metabolism in all dopamine-containing regions of the brain (Lerner, Nose, Gordon & Lovenberg, 1977; Scatton, Glowinski & Julou, 1976a, b; Laduron, Dibie & Leyson, 1977; Crow, Deakin, & Longdén, 1977; Bowers & Rozitis, 1974; Anden, 1976; Plotscher, 1976; Post & Goodwin, 1975). These changes may represent a feedback mechanism operating to overcome the dopamine receptor blockade. With repeated administration, the drug exhibits less of a facilitatory effect on dopamine activity in the caudate (Lerner, Nose, Gordon & Lovenberg, 1977; Scatton, Glowinski & Julou, 1976a; Bowers & Rozitis, 1974; Anden, 1976) and probably also in the NAS (Scatton, Glowinski & Julou, 1976b). In the frontal cortex, however, there is evidence that this biochemical "tolerance" does not develop in that the drug continues to elicit large compensatory changes in synthesis and turnover (Scatton, Glowinski & Julou, 1976b). Although the mechanism of these selective changes is unclear, it is possible that receptor binding activity increases after long-term treatment (Seeman, Tedesco, Lee et al, 1978).

Thus spiroperidol administered chronically may increase the activity of dopaminergic neurons terminating in the PFC, thus increasing the amount of positive reinforcement obtained through stimulation. The increases in self-stimulation were long-lasting (up to five or six weeks after withdrawal of the drug). This may be because the changes in dopaminergic activity represent a relatively permanent
re-adjustment of this system to a new level. Alternatively, it may be due to the nature of excretion of neuroleptic drugs. There is evidence that these compounds accumulate in the body (in lipid and connective tissue pools) and are not fully excreted for weeks or months after discontinuation of administration (Baldessarini, 1977; DiMascio, 1972). The long-lasting effects of spiroperidol may reflect a slow release of spiroperidol from such stores.

The time course of changes observed in PFC self-stimulation after administration of spiroperidol form an interesting parallel to the time course of the therapeutic effects of neuroleptics administered to humans with psychotic disorders. Neuroleptics do not exert their therapeutic effects for days or weeks after administration is begun and do not lose their therapeutic effects over long periods of administration or even for weeks or months after discontinuation of drug therapy (Baldessarini, 1977). Similarly, the changes in PFC self-stimulation rates did not occur for a few days after administration started and did not disappear over long periods of testing, even after administration ceased.

In view of these similarities, it is particularly interesting that the antipsychotic effects of neuroleptics have been hypothesized to be due to the continued ability of neuroleptics to alter dopaminergic activity in the mesocortical dopamine system terminating in the PFC (Hokfelt, Ljungdahl, Fuxe, & Johansson, 1974; Laduron, DiBié & Leyson, 1977; Scatton, Glowinski & Joulou, 1976b; Snyder, Banarjee, Yamamura & Greenberg, 1974; Thierry, Tassin, Blanc & Glowinski, 1976b). Therefore the antipsychotic effects of neuroleptics and the effects on self-stimulation
of the PFC may be similar not only in time course (delayed onset with a lack of behavioral tolerance to the drug over long periods of administration or after administration has ceased) but also in mechanism (increased activity of PFC dopamine neurons). In this way, chronic administration of spiroperidol to self-stimulating rats is not only a useful way of examining the neural substrates of brain-stimulation reward in the PFC but may serve as a model to assess the neural basis of drug-induced behavioral changes in humans.

When amphetamine was administered to rats with SCB electrodes, a significant suppression of responding was observed both during (after about four days of administration) and after amphetamine treatment in rats with electrodes in the noradrenaline-containing SCB. It is possible that self-stimulation of the SCB would have decreased with time, regardless of drug treatment. However, chronic administration of spiroperidol did not alter SCB self-stimulation rates, in spite of longer testing periods. Spiroperidol is a potent dopaminergic antagonist but a very weak noradrenergic antagonist (Janssen, 1970) with little effect on noradrenergic transmission even when administered chronically (Scatton, Glowinski & Julou, 1976b). On the other hand, amphetamine does alter noradrenergic transmission (Axelrod, 1970). Therefore it seems likely that the changes in SCB self-stimulation after amphetamine administration were due to actions on noradrenergic transmission. Thus these data may support the recent proposal that self-stimulation of the SCB depends at least partly on the activation of noradrenergic neurons (Takigawa, Robertson & Mogenson, 1978). Noradrenergic receptor sensitivity reportedly decreases following chronic
amphetamine treatment (Baudry, Martres & Schwartz, 1976) and whole brain noradrenalin levels drop (Anderson, Leith & Barrett, 1978). This may be due to the formation of p-hydroxynorephedrine, which acts as a false transmitter in noradrenergic neurons (Freeman & Sulser, 1972; Clay, Cho & Robefroid, 1971). Thus it is possible that noradrenergic neurons in the SCB are hypofunctional following chronic amphetamine treatment and can no longer maintain self-stimulation of this site.
GENERAL DISCUSSION

The mechanisms of brain-stimulation reward, studied intensively for the last 25 years, are still poorly understood. Although it has been clearly established that electrical stimulation of sites in the hypothalamus, limbic forebrain and midbrain and extrapyridnal structures is a potent reinforcer of lever pressing and other operant responses, the neural bases of such reinforcing effects are still largely unknown. In recent years, there has been a good deal of interest in the possibility that catecholamine-containing neurons have an important, and perhaps even critical or exclusive role in brain-stimulation reward (German & Bowden, 1974; Mogenson & Phillips, 1976). This was suggested initially by the observation that self-stimulation of the lateral hypothalamus and other sites is increased after the administration of amphetamine, a drug which causes the release and blocks the reuptake of dopamine and noradrenaline from axon terminals (Stein, 1964, ). Further support for the catecholaminergic hypothesis of brain-stimulation reward was obtained after the catecholamine-containing neurons were visualized and mapped using the technique of histofluorescence (Dahlstrom & Fuxe, 1964; Ungerstedt, 1971). The most effective sites of self-stimulation were those that contained heavy projections of catecholamine-containing neurons (German & Bowden, 1974). The chemical anatomy of the catecholamine pathways has served as a valuable aid in studying the neural substrates of brain-stimulation reward.

Neuropharmacological techniques, used in the experiments presented in this thesis, have been used extensively in recent years to investigate the neural mechanisms of brain-stimulation reward. The major objective of this discussion is
to assess the contributions of the experimental findings presented above and to assess the current state of knowledge in this field. It is appropriate, however, to begin with a brief consideration of the research strategy of using drugs to study the neural mechanisms of brain-stimulation reward and to note the limitations and possible pitfalls of this approach.
The Use of Pharmacological Methods to Investigate Self-Stimulation

Although a specific and unique role for noradrenalin and dopamine in brain-stimulation reward has been proposed (e.g. Stein, 1968a, b; Crow, 1972b), it has not been easy to provide definitive evidence. A widely used approach has been to administer drugs that block noradrenergic or dopaminergic systems. However when such drugs are administered systemically, the changes in response rates for brain-stimulation reward may not reflect a change in reward mechanisms. For example, in an early study, Olds and Travis (1960) concluded that chlorpromazine, which suppressed self-stimulation of the lateral hypothalamus, disrupted operant behavior and did not necessarily alter reward per se.

In order to circumvent possible non-specific effects of systemically administered drugs, injections have been made directly into brain structures, either unilaterally or bilaterally. This technique has an important advantage over peripheral administration in that it is possible to implicate specific neural structures in self-stimulation. With bilateral injections, however, non-specific side-effects may obscure any specific effect on brain-stimulation reward. For example, Stephens and Herberg (1977) injected haloperidol, amphetamine and other drugs bilaterally into the nucleus accumbens or caudate-putamen while observing self-stimulation of the lateral hypothalamus. Haloperidol suppressed self-stimulation and amphetamine facilitated self-stimulation when injected into either site. Similarly, Brökkamp, Pijnenberg, Cools, and Van Rossum (1975) injected D-amphetamine bilaterally into the nucleus accumbens or caudate-putamen and observed increases in self-stimulation of the ventral tegmental area in both cases. The problem with interpreting these
effects is that the drugs could be altering the animal's sensorimotor capabilities.

Unilateral injections may reduce the probability of motor impairments. Mora, Sanguinetti, Rolls and Shaw (1975) used neurological tests to assess non-specific deficits after unilateral injections of spiroperidol into the nucleus accumbens while rats were self-stimulating the lateral hypothalamus. Self-stimulation of the lateral hypothalamus was suppressed after spiroperidol injections into the nucleus accumbens although no deficits on neurological tests appeared. However, very subtle deficits may not have been measured, and therefore the basis of the suppression of self-stimulation is unclear. An important control in experiments involving central administration is to test self-stimulation contralateral as well as ipsilateral to the site of injection. If contralateral self-stimulation is normal, then the capacity for operant performance of the animal must be intact. These controls seem necessary if one is to accurately assess the effect of a drug specifically on the positively reinforcing aspects of electrical stimulation.

Even when behavioral controls are rigorously used, one other major problem of interpreting drug effects is that the drug itself may be having several actions, any or all of which could mediate the behavioral changes. For example, chlorpromazine, a commonly used drug in self-stimulation studies, has both anti-noradrenergic and anti-dopaminergic activity so is of little use if one wishes to implicate one of these neurotransmitters to the exclusion of the other in brain-stimulation reward. The synthesis of relatively specific dopamine blockers such as haloperidol, pimozide and spiroperidol in recent years has minimized this problem to
some extent (see Janssen, 1970, 1976). These drugs have little or no noradrenergic activity. However, their effects on the activity of other neurotransmitters in the central nervous system is still under investigation (e.g. Leyson, Gommeren & Laduron, 1978). Therefore the effects of drugs must be interpreted with caution, particularly when one wishes to implicate a specific neurotransmitter in self-stimulation.
The Role of Acetylcholine in Self-Stimulation

The results reported in Paper 111, in which it was shown that atropine, a muscarinic cholinergic blocker, suppressed self-stimulation of the SF0 but not the lateral hypothalamus, suggest that ACh normally has an excitatory role in self-stimulation of some but not all brain areas. Previous pharmacological experiments have also implicated ACh in self-stimulation but these studies suggest that a muscarinic cholinergic system inhibits self-stimulation since muscarinic cholinergic agonists suppress self-stimulation (Stark & Boyd, 1963; Jung & Boyd, 1966; Newman, 1972; Olds & Domino, 1969 a, b). However the effects of these agonists are not limited to self-stimulation behavior—they also inhibit other operant activity (see introduction). This suggests that cholinergic neurons do have an excitatory function in self-stimulation but that this has been obscured in previous studies by the presence of non-specific effects. In the present study, any such effects may have been circumvented since self-stimulation of the lateral hypothalamus was unimpaired by the administration of atropine. However, another possibility should be considered that would account for this apparent discrepancy. Cholinergic neurons may have excitatory, inhibitory, or no effects on self-stimulation depending on the site of stimulation and the precise way in which these neurons interact with other neural systems maintaining self-stimulation.
The Role of Serotonin in Self-Stimulation

The results of the third study, in which self-stimulation of the SFO was attenuated by methysergide, suggest that serotonergic neurons normally serve an excitatory role in self-stimulation of this structure. Other investigators have also postulated an excitatory role for 5-HT on the basis of the observations that administration of PCPA (which depletes 5-HT) is associated with a suppression of self-stimulation of the caudate-putamen (Phillips, Carter & Fibiger, 1976b), the hippocampus (Van Der Kooy, Fibiger & Phillips, 1977) and the median raphe nucleus (Millarreissis, 1977). However 5-HT has also been hypothesized to have an inhibitory role in self-stimulation since administration of PCPA facilitates self-stimulation of the lateral hypothalamus (Van Der Kooy, Fibiger & Phillips, 1977; Phillips, Carter & Fibiger, 1976b) and sites along the medial forebrain bundle (Poschel & Ninteman, 1971). In the present study, methysergide did not alter self-stimulation of the lateral hypothalamus. This discrepancy might be due to the nature of the compounds administered. Methysergide may have different effects on serotonin receptors in different brain areas (Sofia & Vassar, 1975) and so it is possible that 5-HT neurons involved in self-stimulation of the lateral hypothalamus were not affected by methysergide. Another possibility is that the effects of PCPA on self-stimulation of the lateral hypothalamus were mediated through alterations in noradrenergic transmission since PCPA has been reported to change noradrenergic activity for several days following administration (Miller, Cox, Snodgrass & Maickel, 1970).

On the whole, the data do not indicate a direct or exclusive role
for 5-HT in mediating self-stimulation. However, altering 5-HT transmission does appear to have specific effects on self-stimulation in some regions of the brain. It has been suggested that, in some brain areas, 5-HT neurons normally interact with other systems—perhaps dopaminergic (Phillips, Carter, & Fibiger, 1976b) or noradrenergic (Poschel & Ninteman, 1971)—to maintain self-stimulation. This might account for the finding that altering 5-HT transmission can have inhibitory, excitatory, or no effects on self-stimulation depending on the site of stimulation.
The Role of Noradrenalin in Self-Stimulation

The results concerned with self-stimulation of the SCB suggest that noradrenergic neurons contribute to self-stimulation of this structure. However, as indicated earlier, this was not demonstrated conclusively. The anatomical and electrophysiological evidence is clearly inferential. Differences in the potency of D- and L-amphetamine were also used as evidence that noradrenergic neurons were involved in self-stimulation of the SCB but the significance of the D-L differential is unclear and appears to be of limited value (see Fibiger, 1978).

Recent results from lesion studies are clearly inconsistent with the hypothesis that NE neurons subserve self-stimulation. Thus self-stimulation of the locus ceruleus is not permanently disrupted by 6-OHDA lesions of the dorsal noradrenergic bundle (Clavér, Fibiger & Phillips, 1976) or electrolytic lesions of the dorsal noradrenergic bundle (Corbett, Skelton and Wise, 1977). Self-stimulation of the dorsal noradrenergic bundle is not eliminated by electrolytic lesions of the locus ceruleus (Clavér & Routtenberg, 1976) and, similarly, self-stimulation of the hippocampus (a termination area for dorsal noradrenergic bundle fibres) is not permanently altered by 6-OHDA lesions of the dorsal noradrenergic bundle (Van Der Kooy, Fibiger & Phillips, 1977).

Taken together, these data suggest that NE is not critical in areas where NE neurons are probably directly stimulated. Of course, such evidence does not preclude the possibility that NE does serve an important role in self-stimulation of the SCB or other areas so far untested. Indeed the temporary disruption of re-
sponding in several rats after colchicine injections into the locus ceruleus (Paper 1) suggests that locus ceruleus neurons projecting ipsilaterally to the SCB maintain in part self-stimulation of this structure. It is possible that NE neurons do contribute initially to self-stimulation of noradrenergic structures but, when these neurons are permanently destroyed by 6-OHDA or electrolytic lesions, other neuronal systems can take over to maintain self-stimulation. However, it seems probable that NE does not play an exclusive or essential role in the mediation of central reinforcement processes. It may serve a modulatory role. Perhaps, as suggested by Franklin (submitted for publication), NE might act through a general effect on cerebral metabolism since endogenous and exogenous NE do alter cerebral blood flow and metabolism. Thus by affecting the general substrates of central reinforcement, NE might play an indirect non-essential role in brain-stimulation reward.
The Role of Dopamine in Self-Stimulation

In considering the possible roles of DA, NE, 5-HT or ACh in self-stimulation, the evidence is strongest for a role for DA. Selective destruction of DA pathways by the injection of 6-OHDA has implicated the nigrostriatal and mesocorticolimbic DA neurons in self-stimulation of the caudate-putamen (Phillips, Carter & Fibiger, 1976b) and the ventral tegmental area (Phillips & Fibiger, 1978). Evidence from studies using central administration of blocking drugs also strongly suggests a role for DA. The experiments reported in this thesis concentrated on the role of mesocorticolimbic DA neurons in self-stimulation of the NAS and PFC. The finding that spiroperidol microinjected into the NAS suppressed self-stimulation of the NAS suggests that DA neurons projecting to the NAS are important in self-stimulation of this structure. These same neurons, moreover, may be important in self-stimulation of other structures. Recently we (Mogenson, Robertson, Takigawa & Wu, in preparation) have observed that spiroperidol microinjected into the NAS suppresses self-stimulation of the ipsilateral but not the contralateral ventral tegmental area, which suggests that the DA neurons originating in the ventral tegmental area and terminating in the NAS are important in self-stimulation of the ventral tegmental area.

Mora, Rolls, Burton and Shaw (1976) have found that spiroperidol microinjected into the NAS in monkeys suppresses self-stimulation of the amygdala and NAS. Broekxkamp and Van Rossum (1975) observed a suppression of self-stimulation of the ventral tegmental area after microinjections of haloperidol into the NAS. Mora, Sanguinetti, Rolls and Shaw (1975) reported that self-stimulation of the ventral tegmental area was suppressed following injections of spiroperidol into the NAS. There
are some problems in the interpretation of these latter two experiments since non-specific effects were not strictly controlled (see Fibiger, 1978). However, since DA neurons from the ventral tegmental area to the NAS appear to be important in self-stimulation of these two structures, it seems likely that they mediate or modulate self-stimulation of other points along their trajectory, including the lateral hypothalamus.

DA neurons from the ventral tegmental area to the NAS may not be critical in self-stimulation of the PFC since spiroperidol microinjected into the NAS did not change self-stimulation of the PFC (Paper IV). Mora, Rolls, Burton and Shaw (1976) also failed to disrupt self-stimulation of the homologous orbitofrontal cortex in monkeys following spiroperidol injections into the NAS. Since neurons from the ventral tegmental area innervating the PFC are at least partly separable from DA neurons projecting from the ventral tegmental area to the NAS (Carter & Fibiger, 1977), it may not be surprising that blockade of NAS DA receptors fails to disrupt self-stimulation of the PFC or that blockade of PFC DA receptors does not alter self-stimulation of the NAS.

The role of mesocortical DA neurons innervating the PFC in self-stimulation of the PFC is unclear. The results reported in Paper IV showed that only in a higher dose did spiroperidol block PFC self-stimulation. Moreover, spiroperidol microinjected into the PFC does not alter self-stimulation of the ventral tegmental area (Mora, Myers & Sanguinetti, 1977; Mogenson, Robertson, Takigawa & Wu, in preparation), suggesting that these neurons are not important in self-stimulation of the ventral tegmental area. This conclusion receives support from the observation
that electrical stimulation of the ventral tegmental area is not accompanied by an increased release of labelled DA from the PFC (Myers & Mora, 1977). However, DA has been indirectly implicated in self-stimulation of the PFC. Thus apomorphine suppresses self-stimulation of the PFC (Mora, Phillips, Koelhas & Rolls, 1976) as well as decreasing the firing rate of a certain percentage of PFC neurons (Mora, Sweeney, Rolls & Sanguinetti, 1976). Additionally, electrical stimulation of the PFC is accompanied by an increased release of labelled DA from areas adjacent to the electrode (Mora & Myers, 1977). Further experiments are needed in order to determine what role DA plays in self-stimulation of the PFC. The possibility that self-stimulation is subserved by descending pathways to the midbrain deserves serious consideration, particularly in view of evidence that electrical stimulation of rewarding points in the lateral hypothalamus, midbrain tegmentum and locus ceruleus antidromically activates neurons in the PFC and cingulate cortex (Rolls & Cooper, 1973, 1974). On the other hand, the results reported in Paper V, using chronic administration of spiroperidol, suggest that DA plays some role, perhaps not essential, in self-stimulation of the PFC since response rates were markedly altered by chronic administration of this DA receptor blocker. In contrast, NAS response rates were not altered by chronic spiroperidol treatment. This might mean that functionally separate neural systems mediate self-stimulation of these two areas, although a cautionary note must be added since it is possible that DA receptors in the two areas are differentially sensitive to spiroperidol (Laduron, Janssen & Leyson, 1978).

In summary, the evidence suggests that self-stimulation of the ventral
Tegmental area and NAS is subserved in part by DA neurons projecting from the ventral tegmental area to the NAS. These same neurons are implicated in self-stimulation of the amygdala and lateral hypothalamus. This system may not, however, be involved in self-stimulation of the PFC. It is possible that a functionally and perhaps anatomically separate system of DA neurons projecting from the ventral tegmental area–substantia nigra area to the PFC contributes to self-stimulation of the PFC, but this conclusion is tentative at present. Thus, although it seems unlikely that DA plays an exclusive role in self-stimulation, it is clearly implicated in self-stimulation of several structures along the trajectory of the DA neural pathways.
Concluding Remarks

For many years, there was a great deal of interest in identifying a single neural substrate or neural pathway mediating self-stimulation. This endeavour has not been wholly successful, and two major reasons can be identified. First, the experimental techniques used have often not allowed unequivocal interpretation of the data. Second, although DA and NE were favoured candidates, it would now appear that there is no single or exclusive neural system mediating self-stimulation. In this thesis, the roles of DA, NE, 5-HT and ACh were examined and it was suggested that each might participate in mediating self-stimulation, depending on the site of the stimulating electrode. Additionally, other putative neurotransmitters such as Substance P (Goldstein & Malick, 1977) and enkephalins (Stein, 1977) may participate in central reinforcement mechanisms. Therefore, it seems probable that non-catecholaminergic systems serve mediatary or modulatory roles in self-stimulation, perhaps by interacting with catecholaminergic systems (Phillips & Mogenson, 1978). If so, the direction of future research will be to identify the specific components and the nature of interaction of these systems.
SUMMARY

1. There is considerable evidence that brain-stimulation reward is associated with the activation of dopaminergic and/or noradrenergic pathways. Since much of this evidence is inconclusive, experiments were undertaken using pharmacological techniques to investigate the contribution of dopaminergic, noradrenergic and other transmitter-specific pathways in brain-stimulation reward.

2. The effects of drugs which influence dopaminergic, noradrenergic, cholinergic, and serotonergic transmission were investigated. Electrodes for brain-stimulation reward were implanted in sites containing dopaminergic projections (nucleus accumbens (NAS), caudate-putamen (CPU), prefrontal cortex (PFC)) noradrenergic connections (supracallosal bundle (SCB)) and cholinergic and serotonergic projections (subfornical organ (SF0)).

3. The role of noradrenergic projections in self-stimulation of the SCB, a site containing noradrenergic neurons from the locus ceruleus, was investigated. Self-stimulation of the SCB was demonstrated and then the role of noradrenaline in self-stimulation of this site was studied.

4. Electrophysiological experiments implicated noradrenergic neurons of the locus ceruleus in self-stimulation of this site. Microinjections of colchicine into the locus ceruleus to temporarily block neuronal transmission suppressed self-stimulation of the ipsilateral but not the contralateral supracallosal bundle. Systemic injections of D- and L-amphetamine also suggested the involvement of noradrenaline in self-stimulation of the supracallosal bundle.

5. When spiroperidol, a dopamine-receptor blocking agent was administered,
self-stimulation of the SCB as well as the NAS and CPU were all suppressed to the same degree, which might suggest that dopamine is involved in self-stimulation of the SCB. It is possible, however, that the drug was having non-specific behavioral effects and was not selectively attenuating self-stimulation of these sites.

6. The role of acetylcholine (ACh) and serotonin (5-HT) in self-stimulation of the SF0 was investigated. Self-stimulation of the SF0 was demonstrated for the first time. Self-stimulation of the SF0 was suppressed after the administration of atropine (a muscarinic cholinergic blocking agent) or methysergide (a serotonergic blocking agent), implicating serotonin and ACh in self-stimulation of this area. As a control for non-specific effects of these drugs, it was shown that neither had an effect on self-stimulation of the lateral hypothalamus.

7. The effects of amphetamine and spiroperidol suggested the possibility that catecholamines might also be involved in self-stimulation of the SF0. Since it was again unclear whether spiroperidol was having a specific effect on self-stimulation, however, the exact role of dopamine was hard to determine.

8. In order to circumvent possible non-specific effects of spiroperidol, the drug was administered centrally into the region of stimulating electrodes in two dopamine-containing areas, the PFC and NAS. Self-stimulation of the NAS was significantly attenuated by spiroperidol. Control microinjections of the drug vehicle had no effects. Spiroperidol microinjected into the NAS contralateral to the stimulating electrode, as a control for possible non-specific effects, did not attenuate self-stimulation. The results provide evidence that dopamine neurons contribute to
self-stimulation of the NAS.

9. Microinjection of spiroperidol into the region of stimulating electrodes in the PFC had no consistent effect on self-stimulation except at a high dose. Self-stimulation of the NAS was not changed by microinjections of spiroperidol into the ipsilateral or contralateral PFC. Similarly, self-stimulation of the PFC was not altered by microinjections of spiroperidol into the NAS.

10. In order to further investigate the role of dopamine in self-stimulation of the PFC and NAS, and to provide information about the role of dopamine in long-term effects of neuroleptics, spiroperidol was administered systemically every day for 9 days to self-stimulating rats with electrodes in the NAS and PFC. For comparison, self-stimulation of the SCB, CPU, SFO, and VTA was also investigated.

11. When spiroperidol was administered either after testing every day or before testing every day for 9 days, self-stimulation rates of rats with PFC electrodes were significantly elevated following withdrawal of the drug and remained so for at least 43 days thereafter. Self-stimulation of the other sites tested was not altered, suggesting that the increased self-stimulation of PFC was not due to increased motor activity.

12. Chronic administration of D-amphetamine, which alters both dopamine and noradrenaline transmission, had effects on both PFC and SCB self-stimulation. PFC self-stimulation rates were increased and SCB self-stimulation rates were decreased.

13. The results of chronic administration of spiroperidol and amphetamine suggest that dopamine is involved in self-stimulation of the PFC. Since acute
central administration of spiroperidol had no clear effects, however, it is possible that dopamine serves a non-essential modulatory role in self-stimulation of the PFC. Additionally, the effects of chronic spiroperidol on self-stimulation of the PFC may model the therapeutic effects of neuroleptics in humans.

14. In conclusion, the data support the idea of multiple neurochemical substrates which mediate or modulate self-stimulation of forebrain areas in the rat.
Appendix 1

Distribution of Monoamine Neurons in the Brain

The first detailed localization of monoamine cell bodies and terminals came after the development of the histochemical formaldehyde fluorescence technique (Carlsson, Falck & Hillarp, 1962; Falck, 1962). This method is based on the reaction of formaldehyde with biogenic amines to produce fluorophores (serotonin or 5-HT shows as a yellow fluorescence; dopamine (DA) and noradrenaline (NE) as a green or yellow-green fluorescence). In the intact animal, cell bodies and axon terminals could be visualized but fibres were not shown. By use of electrolytic lesions, it was possible to increase the intra-axonal concentration of the monoamines sufficient to visualize the fibres (Anden, Carlsson, Dahlstrom, Fuxe, Hillarp & Larsson, 1964; Dahlstrom & Fuxe, 1964; Hillarp, Fuxe, & Dahlstrom, 1966). Since it was not possible to distinguish DA from NE by this method, however, other techniques were supplemented (for example, determination of the tissue content of DA and NE). Although much of the chemical anatomy had been detailed by these methods in the 1960's, Ungerstedt (1971) produced a detailed comprehensive map of the distribution of monoamines in the rat brain which has served as the basis for many subsequent behavioral studies.

The development of the glyoxylic acid fluorescence technique (e.g. Lindvall & Bjorklund, 1974) was advantageous in that it was possible to fluorescence monoamine neurons completely without prior ablation of the neurons. The development of immunohistochemical techniques also permitted the cellular localization of the enzyme dopamine-beta-hydroxylase, permitting the separation of DA and NE
neurons (Fuxe, Hokfelt & Ungerstedt, 1970). Additionally, sensitive microdissection and microassay techniques (e.g., Kizer, Palkovits & Brownstein, 1976) have added to our present knowledge of the distribution of DA, NE and 5-HT.

5-HT neurons shown in Figure 1A are drawn according to the work of Dahlström, Haggendal, & Atak (1973). NE-containing neurons mapped according to the histofluorescence study of Ungerstedt (1971) are shown in Figure 1B, although attention should be drawn to a later study by Lindvall and Björklund (1974), using the glyoxylic acid method, where these pathways are detailed in more complexity and with slight variations from the results reported by Ungerstedt (1971).

DA-containing neurons mapped according to work by Lindvall & Björklund (1974) are shown in Figure 1C. Ungerstedt's (1971) study did not show an important component of the dopamine projections to the forebrain: the mesocortical DA neurons. The presence of a mesocortical DA system was first suggested by a study by Thierry, Stinus, Blanc and Glowinski (1973) who noted that high levels of dopamine remained in the cortex after destruction of the NE fibres innervating the cortex, suggesting that the DA present there is not simply a precursor to NE. Thierry, Hirsch, Tassin, Blanc and Glowinski (1974) further demonstrated that, in synaptosomes from the cat cerebral cortex, DA could be synthesized from tyrosine. Additionally, they suggested that a specific DA uptake mechanism and a DA-sensitive adenyl cyclase was present. This was later confirmed by Bockaert, Tassin, Thierry, Glowinski, and Premont (1977). Fuxe, Hokfelt, Johansson, Lidbrink and Ljungdahl (1974) and Lindvall and Björklund (1974) demonstrated the presence of DA-containing
Fig. 1. Distribution of monoamine neurons in the rat brain. A. Serotonergic neurons redrawn from Dahlstrom, Haggendal, and Atak (1973). B1 cells stem from the raphe pallidus; B2-raphe obscurus; B3-cells around the pyramidal tract; B5-raphe pontis; B7-raphe dorsalis; B8-raphe medianus. B. Noradrenergic neurons redrawn from Ungerstedt (1971). The noradrenergic cell groups are labelled according to the nomenclature of Dahlstrom and Fuxe (1964). C. Dopaminergic neurons redrawn from Lindvall and Björklund (1974). Abbreviations: AC-anterior commissure; ACC-nucleus accumbens; ALC-anterior limbic cortex (cingulate); CB-cerebellum; CC-corpus callosum; DNB-dorsal noradrenergic bundle; FC-frontal cortex; HPC-hippocampus; HT-hypothalamus; MCG-mesencephalic cell groups; OB-olfactory bulb; ON-olfactory nucleus; OT-olfactory tubercle; S-septum; VNB-ventral noradrenergic bundle.
neurons to the frontal cortex and to the anterior cingulate cortex in the rat. Both groups of investigators suggested that cortical DA originates from A10 and A9 neurons in the ventral tegmental area. Berger, Thierry, Tassin and Moyne (1976) demonstrated that the projection pattern of DA neurons in the frontal cortex is identical to the projection pattern of mediodorsal thalamic neurons—comprising two zones, the sulcal PFC and the medial PFC. Lindvall, Bjorklund and Divac (1977) extended these findings to demonstrate that, in the oppossum, tree shrew, or rat, the DA projections overlap mediodorsal thalamic projections and both are divisible into three groups: a pregenual anteromedial (or medial PFC) group, a suprarhinal (or sulcal PFC) group, and a supragenual (or anterior cingulate or limbic) group. With respect to the exact origin of DA fibres, there is general agreement that the cingulate area received projections primarily from the A9 DA cell bodies in the substantia nigra and the medial PFC and the sulcal PFC probably receives projections from A10 cells in the ventral tegmental area (Carter & Fibiger, 1977; Emson & Koob, 1978; Lindvall, Bjorklund & Divac, 1977).
APPENDIX 2

Effects of D- and L-Amphetamine on Self-Stimulation of the Supracallosal Bundle

The original study by Phillips and Fibiger (1973) showing that D and L amphetamine have differential effects on self-stimulation if electrodes are in a noradrenergic area but not a dopaminergic area was based on a report by Coyle and Snyder (1968). These investigators had observed that D and L amphetamine were equipotent in inhibiting DA uptake by presynaptic neurons. These observations could not be replicated, however (Ferris, Tang & Maxwell, 1972). While this discrepancy might make the interpretation of the results of Phillips and Fibiger difficult, it is probable that the rate-increasing effects of amphetamine reflect an increased release rather than a decreased uptake of catecholamines (Phillips & Fibiger, 1975). In view of this possibility, the observation by Ziancé, Azzaro & Rutledge (1972) that D is more potent than L in releasing NE from the cortex may be relevant. Until the biochemical basis of the D and L potency differences is established, however, the significance of the D and L differential on self-stimulation remains in doubt. What does seem clear is that there is a good correlation between stimulation of noradrenergic sites (with a clear D-L differential) and stimulation of dopaminergic sites (with no D-L differential). Thus the presence of a D-L differential can be interpreted as evidence that noradrenergic fibres are being stimulated, but does not indicate the importance of noradrenergic fibres in maintaining self-stimulation response.
APPENDIX 3

Effects of Neuroleptics on Behavior

The problem of determining whether the suppressive effect on self-stimulation is specific to self-stimulation or whether it is due to a more general effect on operant behavior is pertinent for several reasons. Firstly, neuroleptics do, by definition, cause neurological disorders—specifically extrapyramidal side effects (catalepsy and hypokinesia in rats; akathisia and pseudo-parkinsonism in humans; Crow & Gilibe, 1974; Levitt & Krikstone, 1975; Honima & Fukushima, 1976; Shader, 1972; Merlis & Carone, 1975). Secondly, neuroleptics decrease responding in a shock avoidance situation (Niemegeers et al., 1974) and for food or water reinforcement (Rooks, Kelly, 1974b; Wise, Shaw, Wood & Dale, 1978; Fibiger, Carter & Phillips, 1976). These deficits in operant behavior may be due to the extrapyramidal side effects. The observation that spiroperidol decreases bar-pressing for self-stimulation so far from all regions of the brain tested, is not surprising in view of these points. Additionally, in the present experiments, where the effect of spiroperidol on self-stimulation of the SCB, NAS, and CPU was tested, systematic behavioral observations were taken of the animal while in the test chamber (see Table 1). It was observed that, compared to behavior after the injection of the drug vehicle or after the two lower doses of spiroperidol tested (.01 and .05 mg/kg, i.p.), behavior after the highest dose of spiroperidol (0.10 mg/kg) was quantitatively different. The frequency of appearance of all "voluntary" behaviors (bar-pressing, rearing, locomotion, jumping) went down markedly and, concomitantly, the frequency of appearance of involuntary "reflexive" behaviors (grooming, scratching, sniffing, shaking) and

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<table>
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<tr>
<th>Site of Electrode</th>
<th>Dose of Spiroperidol (mg/kg.i.p.)</th>
<th>Percentage of Time Spent bar-pressing, rearing, walking, or jumping</th>
<th>Percentage of Time Spent grooming, scratching, shaking, sniffing, or immobile</th>
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<td>73</td>
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<td></td>
<td>.01</td>
<td>73</td>
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<tr>
<td>SCB (n=6)</td>
<td>control</td>
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<td>25</td>
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1 The rat's behavior was observed every three minutes for 15 minutes while self-stimulation response rates were being measured in the test chamber.
immobility increased. In view of these observations, it seems likely that the effect of dopamine receptor blockade by spiroperidol was primarily to decrease the frequency of operant and other voluntary behaviors. Under these conditions, it is difficult to determine whether spiroperidol also decreased the positively-reinforcing value of electrical stimulation. Thus the role of dopamine in self-stimulation of the SCB could not be accurately assessed.
APPENDIX 4

Catecholaminergic Innervation of the Subfornical Organ

A study published by Säävedrä, Brownstein, Kizer & Palkovits (1976), after the experiment reported in Paper 11 was completed, reported moderate amounts of dopamine and noradrenalin, as well as the synthesizing enzymes for these catecholamines, in the SFO. 5-hydroxytryptamine (serotonin) and its synthesizing enzyme, plus histamine and choline acetyltransferase were also identified in this structure. Since the SFO is well-vascularized and devoid of a blood-brain barrier (Akert, 1967), it is possible that the catecholamines are taken up from the circulation. But the presence of the synthesizing enzymes suggests a local synthesis of dopamine and noradrenalin. Swanson, Connelly and Hartman (1977) recently reported, however, ultrastructural evidence for catecholaminergic innervation of vasculature in the SFO. This might therefore be the source of the catecholamines in the SFO. Additionally, it seems unlikely on the basis of the histofluorescence mapping studies (See Appendix 1) that the catecholamine cell bodies from the diencephalon, midbrain or hindbrain innervate the SFO or are directly stimulated by SFO electrodes. Of course, this does not exclude transynaptic involvement of catecholaminergic neurons in self-stimulation of the SFO.
REFERENCES


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