BRAIN STIMULATION REWARD DERIVED FROM DOPAMINERGIC TERMINAL FIELDS IN THE RAT

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ABSTRACT

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Results of experiments dealing with the effects of lesions and of drugs that interfere with the metabolism and synaptic action of central catecholamines indicate that dopamine (DA), but not norepinephrine, is critically involved in the mediation of the rewarding effects of electrical stimulation of the brain. This hypothesis has received further support by the finding that self-stimulation is readily seen when the tissue contained within the layer of mesencephalic dopaminergic cells is stimulated, but not when stimulating electrodes are above, below, lateral, or caudal to this layer. Recent studies, however, suggest that the directly activated substrate in self-stimulation of the area of the medial forebrain bundle is not catecholaminergic.

In this experimental series, the dopaminergic terminal fields were mapped for self-stimulation effects in order to determine the type of correlations that exist (if any)

between DA density and self-stimulation characteristics. Moveable electrodes were implanted in eight regions of the caudate putamen, in four regions of the septal area, in the amygdaloid complex, nucleus accumbens, olfactory tubercle, pyriform cortex, medial and sulcal prefrontal cortex, and in the entorhinal cortex. Non-dopaminergic regions of the brain were mapped as well: the parietal cortex, corpus callosum, anterior commissure and olfactory tract.

Positive sites for self-stimulation were found in all catecholaminergic and non-catecholaminergic regions that were studied, except for the parietal cortex, but in no place was there a clear, significant, correlation between DA density and the four variables that defined the characteristics of self-stimulation (threshold, pressing rate, behavioral stability, and number of sessions to start self-stimulating).

These results suggest that DA neurons involved in brain stimulation reward in dopaminergic terminal fields are efferent to the directly activated substrate in the self-stimulation paradigm, and that that the rewarding effect of brain stimulation is mediated by the release of DA in a few, perhaps one, dopaminergic terminal fields, not necessarily at the site of stimulation.

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INTRODUCTION

More than twenty-five years ago, Olds and Milner (1954) reported their discovery that rats would learn instrumental tasks when their performances were followed by electrical stimulation of certain areas of the animals' brains. This discovery has led, not surprisingly, to a great amount of experimental work. It is believed that by understanding the underlying mechanisms of this phenomenon we will be able to better understand the physiological basis of a broad range of motivational processes and of other related processes (e.g., learning and memory) that occur both in the normal and in the diseased brain.

As is the case with most major discoveries, the history of intracranial self-stimulation (ICSS) is filled with many instances of theoretical controversy. A great deal of experimentation has been undertaken to determine whether or not the reinforcing properties of intracranial stimulation are qualitatively equivalent to those of conventional (natural) reinforcers. It is now well established that intracranial stimulation can sustain learned behaviors in much the same way as do natural reinforcers (Keesey and

Goldstein, 1968; Olds, 1956; Trowill and Hynek, 1970, Trowill, Panksepp, and Gandelman, 1967; etc.).

Two other major questions have emerged in this field. What is the neuroanatomy of ICSS? (i.e., which areas or systems of the brain are capable of supporting self-stimulation?). What is the neurochemistry of ICSS? (i.e., which endogenous chemicals mediate the rewarding properties of self-stimulation?). The results of mapping and lesion studies, together with those of pharmacological studies, have led to the proposition that brain stimulation reward (BSR) depends on direct activation of at least one of the two classes of catecholamine (CA) systems--a norepinephrine (NE) or a dopamine (DA) system.

The Catecholamine Hypotheses of Brain Stimulation Reward

What follows is a brief account of experiments related to the CA hypotheses of BSR, which were derived from the early work of Olds (Olds and Travis, 1960) and formally proposed by Stein (1964, 1968). In simple terms, these hypotheses state that activation of central CA-containing neurons (together, of course, with their normal efferent connections) mediates the reinforcing properties of

intracranial stimulation.

The original experiments on BSR dealt with the stimulation of tissue contained in some of the limbic areas of the brain. It later became evident that ICSS could also be readily obtained by electrical stimulation of most regions of this system (Olds, 1960; Olds and Olds, 1963). Further research showed that BSR could also be readily produced by the stimulation of other neural systems. In their 1974 review article, German and Bowden matched existing BSR maps with the recently available maps of the CA systems. They found that in almost every case in which BSR was obtained the electrode tip was located within (or near) at least one of the primary CA systems and concluded that the stimulation of either the NE or the DA systems was a sufficient condition for producing rewarding consequences.

Lesion studies also appeared to provide support for the CA hypothesis of reward. Electrolytic and chemical lesions of either, or both, of the CA systems generally produced various degrees of attenuation of ICSS (see reviews by German and Bowden, 1974, and by Lorens, 1976). Pharmacological studies showed that by treating self-stimulating animals with drugs that interfere with the metabolism of the CA's, or with their synaptic action, also induced a reduction of self-stimulation behavior. In

contrast, it was shown that pre-treatment with drugs that increase CA synthesis or synaptic efficacy produces an improvement of ICSS (for recent reviews see Fibiger, 1978; Wauquier, 1980; Wise, 1978a, b; 1981a).

Although most of these experimental data seem to fit nicely with the proposed CA hypothesis, careful analysis has led to more cautious conclusions. It can be reasoned, for example, that electrical stimulation within the CA systems might activate CA elements and other anatomically overlapping systems as well, so that the rewarding properties of such stimulation could be accounted for by the activation of non-catecholaminergic systems, and not necesarily by the activation of a CA system.

The first catecholamine hypothesis of brain stimulation reward stated that that NE was the critical neurotransmitter involved in ICSS (Stein, 1964, 1968). A second CA hypothesis, derived from the mapping of the mesencephalon, was advanced by Crow (1972, 1976); according to this hypothesis, either NE or DA can be critically involved in brain stimulation reward. As discussed below, there are some experimental results which have called into question the NE hypothesis as initially conceptualized by Stein (1964, 1968) and as later supported by Crow and others. First,

although there have been reports of self-stimulation from the noradrenergic nucleus locus coeruleus (LC)(Crow, Spear, and Arbuthnott, 1972; Ellman, Ackerman, Farber, Mattiace, and Steiner, 1974; Ritter and Stein, 1973), results of more recent studies where adequate histological materials were presented (lacking in the former studies), and where more precise mapping methods were also used, have yielded negative results with respect to self-stimulation from the nuclei of origin of the NE systems. BSR has not been obtained from the A1, A2, or A5 cell groups of origin of the ventral NE system (Clavier and Routtenberg, 1974), nor has it been obtained from the LC by some workers (Amaral and Routtenberg, 1975; Corbett and Wise, 1979; Simon, Le Moal, and Cardo, 1975).

Second, lesions of the locus coeruleus, of the caudal ventral bundle or of the central tegmental tract failed to attenuate brainstem self-stimulation (Clavier and Routtenberg, 1976). Lesions of the dorsal noradrenergic bundle (DNB) did not disrupt BSR from the region of the LC (Clavier, Fibiger, and Phillips, 1976) but, instead, produced a facilitation of self-stimulation from the region of the lateral hypothalamus (LH) (Clavier et al., 1976; Corbett, Skelton, and Wise, 1977; Farber, Ellman, Mattiace, Holtzman, Ippolito, Halperin, and Steiner, 1976; Koob, Balcom, and Meyerhoff, 1976).

Finally, there is an increasing awareness of problems associated with the interpretation of early experiments which dealt with the effects of drugs on ICSS. It is a fact that the performance maintained by BSR is impaired after giving the animals drugs that interfere with CA functions. This fact, nevertheless, cannot be interpreted as being necessarily due to a reduction of the rewarding quality of brain stimulation; rather, it could be that animals become less capable of executing the required responses to obtain the reward under some, or all, of these drugs (e.g., Fibiger, Carter, and Phillips, 1976; Roll, 1970; Rolls, Rolls, Kelly, Shaw, Wood, and Dale, 1974). In order to determine whether or not the CA's are involved in reward functions per se, more sophisticated methodologies must be employed. A number of studies have been specifically designed to deal with this problem.

Results of these recent experiments suggest that DA, but not NE, is critically involved in the rewarding effects of brain stimulation. Both NE and DA receptor blockers cause a reduction in self-stimulation behaviors, but only the latter class of drug produces effects that are similar to those that are seen in conditions of extinction, that is when rewards are withheld during the performance of a learned task. Self-stimulating animals that are pre-treated with

neuroleptics (drugs that block DA receptors) show normal response rates at the start of testing but decreasing rates as the testing session progresses (Fouriezos and Wise, 1976). In contrast, pre-treatment with NE receptor blockers produces low (but stable) ICSS throughout, thus suggesting that there is not a reduced quality of reward but, rather, an impaired capacity for responding (Fouriezos and Wise, 1976).

Subsequent experiments produced equivalent results across a variety of paradigms. Extinction-like decrements in ICSS were induced, in a dose-related fashion, by the neuroleptic pimozide. These decrements were very similar to those produced by lowering the stimulation current in non-treated animals (Fouriezos, Hansson, and Wise, 1978; Franklin and McCoy, 1979). Also relevant to the DA hypothesis of brain stimulation reward are experiments in which latency to initiate running, running speed, and ICSS rates were not affected during the early trials in a runway when rats had been treated with a DA receptor blocker. Performance declined only gradually as a function of repeated trials (Fouriezos et al., 1976; 1978; Franklin, 1978). Finally, threshold tests indicate that higher than normal stimulation currents are required to produce BSR in neuroleptic-treated rats (Esposito, Faulkner, and Kornetsky, 1979; Liebman and Butcher, 1973; Schaeffer and Michael, 1979; Zarevics and Setler, 1979a). Taken together, these

experimental findings indicate that the reported reductions in performance after neuroleptic treatment are not due to an impairment of the response systems but to an interference with the mechanisms that mediate reward.

It is of interest to note that DA receptor blockers not only interfere with the reinforcing properties of electrical stimulation of the brain but also with the rewarding value of natural reinforcers (Gerber, Sing and Wise, 1981; Xenakis and Sclafani, 1981; Wise et al., 1978a, b) and of some drugs of abuse (Bozarth and Wise, 1981; de Wit and Wise, 1978; Yokel and Wise, 1975, 1976).

If we grant on pharmacological evidence that dopaminergic activity is essential for BSR (at least with some electrode placements), then one might expect to most readily obtain ICSS when the stimulating electrodes are lodged within or near DA systems (near the cell bodies, dendrites, axons, or terminal fields). The work of Corbett and Wise (1980) shows that in the nuclei of origin and along the initial segments of their fiber tracts the boundaries of the DA ascending systems bear a close relation to the boundaries of the substrate of BSR. High pressing rates and low thresholds were obtained when the stimulating electrodes were in those areas with the highest DA cell body or fiber

density, whereas no self-stimulation was seen when the electrode tips were outside DA-containing regions; the boundaries of the BSR region corresponded precisely to the boundaries of the DA cell group.

To summarize, mapping and pharmacological studies lend strong support to the view that dopaminergic activation represents a critical link in the processes involved in brain stimulation reward.

The Directly Activated Substrate for Brain Stimulation Reward

As would be expected from the results of mapping, lesion and pharmacological studies reviewed above, many specialists in the field of brain stimulation reward agreed at first that this phenomenon was probably mediated by the activation of at least one of the CA systems. There has also been the implicit assumption that ICSS is brought about by the direct electrical activation of the catecholamine elements that are located at, or near, the tip of the stimulating electrode. However, this assumption has been questioned by Wise (1978a, b; 1981a), and recently it has been convincingly challenged by Gallistel and co-workers, based on their findings derived from ingenious combinations of classical electrophysiological and behavioral methodologies (Gallistel, Shizgal, and

Yeomans, 1981; Shizgal, Bielajew, Corbett, Skelton, and Yeomans, 1980; Yeomans, 1979). Given the important theoretical implications of these findings and the novelty of this combination of techniques, a brief description of some of these expetiments is warranted.

The experiments reviewed by Gallistel et al. (1981) deal with the study of the physiological properties of the neural tissue which, when stimulated, reinforces the behavior that preceded its stimulation. It should be remembered that the minimal interval at which two action potentials can be produced by an axon is determined by the absolute refractory period of that axon. Because of this, when a fiber, or a set of parallel fibers, is stimulated with a train of pairs of pulses, there is a critical inter-pulse interval below which only one of the pulses in each pair is effective in activating the axons and their post-synaptic contacts. By the same token when an axon is stimulated at two different sites along its length, there is also a minimal inter-pulse interval at which the latter pulse will be effective in producing an action potential. With a shorter interval only one of the two action potentials will induce an effect on the post-synaptic contacts; the simultaneously produced antidromic action potential will cancel the orthodromic one produced at the other electrode.

In the self-stimulation studies under consideration, it is assumed that when a given set of stimulation parameters is used a certain number of fibers within a "reward" bundle are activated and that the degree of this rewarding activation is reflected in the animal's degree of willingness to work for such stimulation. In this view a reduction in the number of stimulation-induced action potentials will reduce the rewarding impact of BSR (as evidenced by reduced response rates). In typical experiments, the tips of one or two electrodes are chronically fixed within an hypothesized reward fiber tract of a rat, and the behavioral output of the animal is observed when a train of pairs of pulses is delivered contingent upon the rat's performance. By reducing the interval between the first (conditioning) and second (test) pulses of each pair of pulses, refractory periods (one electrode paradigm) or collision intervals (two electrode paradigm) can be inferred from the critical inter-pulse interval at which there is an abrupt change in the animal's performance; with the two-electrode measures an estimate of conduction velocities of the activated fibers can be derived.

This type of experiment indicates that the electrophysiological properties of the neural elements that are directly activated through a self-stimulation electrode differ from the functional properties of the catecholamine

fiber systems. The known CA projections are represented by unmyelinated axons with refractory periods greater than 1.5 msec and conduction velocities of less than 1.0 m/sec, whereas those of the directly activated reward substrate range between 0.5 and 1.2 msec and between 2.0 and 8.0 m/sec, respectively (for references, see Gallistel et al., 1981). Thus, these experiments indicate that in the ICSS paradigm activation of some non-catecholaminergic system is the first in the series of events that is ultimately translated into reward. Given the supportive evidence for the hypothesis that DA is critically involved in brain stimulation reward, it can now be postulated that this monoamine is involved at some stage efferent to the directly activated fibers at the electrode tip.

These data have been integrated by Wise (1980a, b). He has hypothesized that, in the region of the medial forebrain bundle (MFB), the tissue directly activated by ICSS electrodes is a descending system of myelinated fibers that makes synaptic contact with dopamine cells in the ventral tegmental area (VTA). Hence, it may be the trans-synaptic activation of a dopaminergic system that mediates the rewarding properties of brain self-stimulation. These postulations lead to many important questions. It is important to determine (a). what is the neuroanatomical and

neurochemical identity of the proposed descending system that activates the tegmental dopaminergic cells , (b) which of the dopaminergic terminal fields are critically involved in the brain stimulation reward phenomenon, and (c) what is the mode of action on post-synaptic receptors of the dopamine that is presumably released during self-stimulation. Moreover, it is important to determine if non-dopaminergic cells are directly activated when electrodes are in other locations. Locations in dopaminergic terminal fields are particularly interesting, since DA efferents could be activated in such cases. BSR in dopamine terminal fields, where little is known about the directly-activated substrate, is the focus of the present Thesis.

Role of Dopamine in Brain Stimulation Reward Involving Dopamine Terminal Fields

The histofluorescence techniques have proven to be invaluable tools for the demonstration and mapping of the catecholamine systems of the brain. These techniques can be used not only to visualize the cells of origin, fiber pathways and terminals of these systems but also as markers of the afferent fibers and efferent processes associated with CA systems.

Thus, the use of the glyoxylic acid-induced

histofluorescence method made it possible to determine the correlation between DA density in the region of cells of origin of the ascending dopaminergic systems and self-stimulation thresholds and between DA density and pressing rates (Corbett and Wise, 1980). As described above, the boundaries of the BSR region corresponded precisely to the boundaries of the DA cell group. This result, together with those which indicate that the directly activated reward-relevant neurons in LH self-stimulation are not catecholaminergic (Shizgal et al., 1980; Yeomans, 1979), suggests that the directly activated tissue is represented by the afferent terminals that synapse on the mesencephalic DA neurons. In this case the histofluorescence technique indicated the topography of termination of a non-dopaminergic projection.

In the case of the DA terminal fields, histofluorescence can again be used to map two potential populations of reward-relevant elements. Here sites of dense fluorescence mark, first, the DA terminals themselves and, second, the population of cell somata or dendrites on which the DA terminals make synaptic contact. If ICSS in the dopaminergic terminal fields involves activation of either the DA terminals or their efferent targets, then the ICSS boundaries and the boundaries of the region of DA fluorescence should correspond. In other words in regions where the DA terminal

fields have precise limits (e.g., in the caudal region of the septal area), ICSS characteristics when the electrode tip is within those limits should differ significantly from when the tip is outside those limits. By the same token in those terminal fields with a homogeneous distribution of DA fluorescence (as is the case of the striatum), self-stimulation effects should not differ from one region to another.

It thus seems important to determine the kinds of relationships that exist between BSR and the anatomy of the terminal fields of the ascending dopaminergic systems. If some relationships should be found in some of these terminal fields, they could shed some light on whether or not dopamine terminals or dopamine efferents might play a critical role in BSR.

Of particular importance for interpreting the anatomy of the reward circuitry in relation to DA terminal fields is the question of whether DA excites or inhibits its efferent target neurons. This knowledge would allow predictions about the direction of expected correlations between DA density and BSR measures. The question of whether DA terminals and their efferents are excited or inhibited in BSR is discussed below.

How does dopamine affect post-synaptic receptors? One possibility is that DA produces the activation of some neural processes, which mediate a rewarding state. It is not difficult to argue, however, that DA could produce the same effect via the inhibition of some neural process. Indeed, the latter alternative might be the most likely mode of action. There is a substantial body of evidence that leads to the conclusion that the effects of DA on its post-synaptic neuronal contacts are predominantly inhibitory in nature. This evidence largely involves the effects of iontophoretically applied DA on spontaneous or evoked unit activity.

Inhibitory effects of DA have been seen in units of the prefrontal cortex (PFC), located in those layers that are innervated by the A10 cell group (Bunney and Aghajanian, 1976b) and in the pyriform cortex (Legge, Randic, and Straughan, 1966). In the striatum both inhibitory and facilitatory effects on unit activity have been obtained, but in most cases the inhibitory effects predominate (Bloom, Costa, and Salmoiraghi, 1965; McLennan and York, 1967). In contrast with the inhibitory effects of DA on caudate neurons, this catecholamine has been found to exert mainly facilitatory effects on units of the putamen and globus pallidus (York, 1970). Electrophoresis of DA also inhibits cell firing in the mesolimbic projection fields, namely, the

accumbens (NAS) and olfactory tubercle (OT) (Bunney and Aghajanian, 1973), the amygdala (AMY) (Ben-Ari and Kelly, 1976; Straughan and Legge, 1965), and in the lateral septal nucleus, which receives the richest dopaminergic innervation in the septal area (Bunney, 1979).

Excitatory effects of DA are far less often seen in diencephalic areas, such as the hypothalamus (Moss, Urban, and Cross, 1972) and in various thalamic nuclei (Phillis and Tevecis, 1967; Phillis, Tevecis, and York, 1967). At more caudal levels of the brain, for example in the red nucleus (Davis and Vaughan, 1969), mesencephalic reticular formation and superior colliculus (Straschill and Perwein, 1971), and cerebellum (Kawamura and Provini, 1970), electrophoresis of DA elicits a less potent inhibition of cell firing than at more rostral levels. Weaker inhibitory effects of DA are seen at the most caudal levels of the brainstem (Hosli, Tebecis, and Schonwetter, 1971) and in the spinal cord (Biscoe, Curtis, and Ryall, 1966).

Taken together, the results of these studies show that DA has a differential rostro-caudal inhibitory potency on neural activity, with strongest inhibitory effects in the cortex and generally weaker ones in the spinal cord.

The experiments reviewed thus far lead to a few general conclusions. First, dopamine is critically involved in the process of brain stimulation reward; second, the main effect of dopamine on neurons of the ascending dopaminergic projection fields is inhibitory; third, the directly activated reward-relevant neurons in the medial forebrain bundle do not seem to be catecholaminergic. Hence, it should be expected that DA density or content around self-stimulation sites in cases of dopaminergic projection fields where dopamine is intimately involved in reward should correlate negatively with pressing rates and positively with thresholds for self-stimulation. These outcomes should be expected because electrical stimulation does not, in this case, seem likely to directly activate the dopaminergic fibers but, rather, their post-synaptic contacts. This post-synaptic activation would cancel the inhibitory effect that dopamine release is thought to exert on most post-synaptic targets. In other words, the underlying proposition is that a rewarding state is usually produced by the inhibitory effects of dopamine on some post-synaptic neurons, and that direct activation of those neurons should itself not be rewarding. Further elaboration on this point will be presented in the final paragraphs of this chapter.

Numerous authors have reported that animals will perform an instrumental task when their responses are rewarded with

electrical stimulation in the region of the DA terminal fields (see below). In these studies, however, no systematic correlations between DA density or content and ICSS parameters were made. Before reviewing these studies, a brief description of the neuroanatomy of the DA systems is in order.

Anatomy of the Dopaminergic Systems

Early biochemical studies (Bertler and Rosengren, 1959) and studies showing reduced dopamine levels in the striatum and substantia nigra (SN) of Parkinsonian patients (Hornykiewicz, 1966) pointed to the existence of a DA projection system in the brain. With the advent of the formaldehyde histofluorescence method developed by Hillarp's group (Falck, 1966; Falck, Hillarp, Thieme, and Torp, 1962), hitherto undetected CA systems were discovered (see, for example, Anden, Carlsson, Dahlstrom, Fuxe, Hillarp, and Larsson, 1964; Bertler, Falck, Gottfries, Ljunggren, and Rosengren, 1964; Dahlstrom and Fuxe, 1964; Fuxe, 1965; Ungerstedt, 1971). An improvement in the histofluorescence methodology (Lindvall and Bjorklund, 1974) and the use of autoradiographic and horseradish peroxidase tracing techniques (Fallon and Moore, 1978; Nauta, Pritz, and Lassek, 1974), the use of silver staining (Maler, Fibiger, and McGeer, 1973), and the use of immunohistochemistry (Pickel,

Joh, and Reis, 1975) further expanded our knowledge of the neuroanatomy of the CA systems. The DA systems can be subdivided into four basic groups.

The first group is comprised of small DA-containing neurons which are found in the retina and olfactory bulb, interconnecting the inner and outer plexiform layers and the mitral cells and adjacent glomeruli, respectively. In the second group there are three systems of DA cells with axons of intermediate length; the first of these systems projects from the arcuate and periventricular nuclei into the intermediate lobe of the pituitary and into the median eminence (the tuberoinfundibular system); the second system projects from the dorsal and posterior hypothalamus, connecting with the dorsal anterior hypothalamus and lateral septal nuclei (the incerto-hypothalamic system). The third system is comprised of DA cells in the area of the motor nucleus of the tenth cranial nerve, the nucleus tractus solitarius, and cells scattered in the tegmental radiation of the peri-aqueductal grey matter.

The third group is represented by a descending projection, which originates from two distinct cell groups. These are the A11 group, which includes cells in the periventricular hypothalamus and in the medial zona incerta

(Bjorklund and Skagerberg, 1979), and the substantia nigra, from which uncrossed axons descend to at least the level of the thoracic cord (Commisiong, Gentleman, and Neff, 1979).

A fourth set of DA systems is comprised of neurons with long axons which connect the brainstem with diencephalic and telencephalic structures. These systems are the nigro-neostriatal bundle, the mesolimbic projection, and the mesocortical system. It is these long fiber systems that are of primary current interest to those studying BSR.

The nigro-neostriatal bundle originates in the pars compacta (A9 cell group) of the SN, and some of its fibers arise from the A10 and A8 neurons. As axons leave the SN in a medial direction, they are joined by fibers from the A10 group and ascend dorsolateral to the medial forebrain bundle (MFB). In general the lateral aspect of the SN projects to the lateral neostriatum (the terms neostriatum, striatum and caudate nucleus will be used synonymously when referring to any mammalian species; when referring to rodents, the term caudate-putamen will also be used). The medial SN projects more medially to the caudate-putamen (CPU), and the neurons situated laterally in the A10 area send their axons to the medial and ventral aspects of the neostriatum. It appears that the A8 neurons innervate the ventral CPU. The nigro-neostriatal bundle also contributes terminals to the

interstitial nucleus of the stria terminalis and gives a relatively sparse innervation to the globus pallidus. In the CPU the dopaminergic projections form a relatively homogeneous, dense terminal network which extends without interruption into the nucleus accumbens and into the medial olfactory tubercle.

The mesolimbic projection originates in the A10 group (ventral tegmental area) and terminates in the NAS, OT, septum, bed nucleus of the stria terminalis, and AMY. The axons of this DA system run in the dorsal portion of MFB immediately ventromedial to the nigro-neostriatal bundle. Some of its fibers continue to the frontal cortex and others to the head of the CPU. There also seems to be a topographical arrangement in this system: the most medial cells of origin project mainly to the septum, those located more laterally terminate in the NAS, and the most laterally located cells innervate the OT.

Dopaminergic terminals are found mainly in the medial aspect of the OT and can be seen as a dense network of fine varicose fibers. In the septum there are two types of DA terminals. First, there are smooth fibers with few varicosities which often surround the cell bodies and proximal dendrites of the septum. These smooth fibers are

found in the most anterior aspect of the lateral nucleus and ventral to the hippocampal rudiment. Second, there are fine varicose fibers, which terminate more caudally, where the DA innervation increases progressively and where the highest density is seen as a conspicuous diagonal band outlining the medial border of the lateral septal nucleus. Smooth fibers and pericellular arrangements are found in the lateral and dorsal aspects of this nucleus. A sparse dopaminergic innervation is found in the most posterior regions of the septum. There is a dense projection to the central nucleus of the amygdala, seemingly an extension of that in the CPU, and also a rich innervation in the lateral and basolateral nuclei, as well as in the intercalated masses.

The mesocortical projection originates mainly in the VTA (there are some projections from the SN), ascends within the dorso-lateral MFB, and innervates the anteromedial-frontal, the anterior cingulate, the ventral aspect of the entorhinal and sulcal cortices. The density of dopaminergic terminals in the neocortex is not homogeneous. In the frontal cortex DA terminals are found throughout the second and sixth layers; in the anterior cingulate cortex dopaminergic innervation is confined to its three first layers; in the sulcal area terminals are found in the fifth and sixth layers; in the ventral entorhinal cortex DA terminals form clusters in the second and third layers.

Brain Stimulation Reward Derived From Dopaminergic Terminal Fields

Caudate Nucleus

Attempts to induce self-stimulation through electrodes placed in this region of dopaminergic terminals have produced controversial results. In the early mapping studies conducted by Olds' group, it was found that electrical stimulation of the CPU produced aversive consequences. In one study (Olds and Olds, 1963), it was reported that a high rate of escape responding was obtained in response to stimulation of the medial aspects of this structure, although some instances of mild escape or neutrality were also seen. The dorso-medial and ventro-lateral aspects of the CPU yielded mild ambivalent (approach-escape) responses. In a similar study by Wurtz and Olds (1963), the same picture emerged: eight of nine electrodes in this nucleus produced escape behavior, and four of these also induced approach responses (ambivalent effects); the ninth electrode was neutral.

In subsequent mapping studies of BSR from the striatum, aversive effects of electrical stimulation were not objectively determined. Using a stringent behavioral criterion, Routtenberg (1971) was able to demonstrate

self-stimulation from the most medial aspect of the CPU, while Phillips, Carter, and Fibiger (1976b) reported that ICSS could be obtained from all regions of the caudate, with the medio-ventral quadrant yielding the highest ICSS rates. Thus, the results of these two reports do not fit with the early findings of Olds and Olds (1963) that stimulation of the medial aspect of the CPU usually produces aversive consequences. The most discrepant results, however, are those of Prado-Alcala, Kent, and Reid (1975). Thirty-seven electrode placements scattered throughout the CPU and three placements in the globus pallidus appeared to be neutral with respect to self-stimulation.

Rats were used as experimental subjects in the studies mentioned above. In the late 1950's and during the 1960's, several investigators attempted to obtain self-stimulation with caudate nucleus (CN) electrode placements in cats. Again, there were no consistent results. Nielson, Doty, and Rutledge (1965) made the observation that their animals would press a lever to obtain electrical stimulation of the CN only if pre-training of lever pressing to obtain food had occurred. Naive cats did not learn to self-stimulate by pressing the same lever in the same box using the same CN stimulation parameters that were effective for the pre-trained cats. In 1963, Justesen, Sharp, and Porter

demonstrated that BSR could be elicited from the CN of naive cats. They used a Skinner box with a relatively large treadle switch located at floor level; in addition, several food deprivation schedules were imposed on the animals. Rates of responding were increased over operant levels by CN BSR, and ICSS rates seemed to be a direct function of the duration of food deprivation. The authors also noted that, unlike more "typical" cases of ICSS, their animals did not show resistance to extinction when responding was not followed by CN stimulation. They concluded that "...the reinforcing effects of caudate stimulation owed simply to induction of generalized motor activation" (p. 373). More recently O'Donohue and Hagamen (1967) published the results of a comprehensive mapping study of self-stimulation in the cat. More than 2000 sites were explored and it was concluded, among other things, that electrical stimulation of the antero-ventral aspect of the CN yielded positive reinforcement.

As is the case of many instances where inconsistent, often contradictory, results are obtained in studies of a particular problem, most of the inconsistencies found in the self-stimulation experiments reviewed in this section may be accounted for by the diverse methodologies that were employed by the different investigators. To illustrate this point, the analysis of three studies carried out in rats in which

different degrees of self-stimulation behavior were obtained will be presented. In each of these studies 60 Hz sine wave stimulation was given, with train duration of either 0.20 or 0.25 sec.

In the first case where ICSS was obtained from all regions of the CPU, each rat was stimulated with a fixed intensity, determined during preliminary testing and selected for each animal on the basis of its apparent reinforcing effect; the intensities ranged between 6 and 180 uA. The animals were tested for 30 min per day until ICSS occurred, or until 14 days of shaping were completed (Phillips et al., 1976b). With this paradigm 87% of CPU placements yielded self-stimulation, with a mean of 7.7 days of training for this behavior to appear (25% of the rats that acquired the lever pressing response showed ICSS in less than 6 sessions, 50% needed between 6 and 10 training sessions to self-stimulate, and the remaining 25% required 11 to 14 days of training). In the second case , all rats were given free access (no shaping) to a lever during 28 daily sessions which lasted 25 min each. The pressing of the lever produced a current of 15 uA. In this study ICSS was obtained from the medial aspect of the CPU (Routtenberg, 1971). Finally in the case of the Prado-Alcala et al. (1975) study, each rat was shaped to approach and press a lever; shaping lasted for

10 min at each of seven intensities (30 uA to 90 uA) on each day until ICSS began or until five consecutive days elapsed with no evidence of ICSS. With this procedure the CPU appeared to be neutral with regard to self-stimulation.

At least three variables seem likely to account for the differences in the results of these three studies: duration of training with a given intensity of stimulation, number of training sessions, and use (or lack of) shaping. In the two reports in which ICSS was obtained, a fixed intensity was used for 15 or 30 min in each session; a relatively high number of training sessions were given (up to 28 days); in the study in which shaping was used, ICSS was obtained from more sites. In contrast, failure to obtain self-stimulation from the CPU involved training for only 10 min at a given intensity and a relatively low number (five) of training sessions. Given these considerations, it can be concluded that the caudate nucleus is capable of supporting self-stimulation when adequate stimulation currents and training are given.

There is an abundance of literature dealing with the effects on self-stimulation of systemic administration of drugs that alter dopaminergic transmission, and no attempt will be made to review it in detail (for reviews, see Fibiger, 1978; German and Bowden, 1974; Wauquier,

1980; Wise, 1978a, b; 1980a, b). Rather, experiments involving the effects of direct application of drugs into the brain on ICSS will here be analysed.

A prediction that can be derived from the CA hypothesis of BSR is that the destruction of a CA-containing system should result in the loss of self-stimulation. A straightforward test of this prediction was made by Phillps et al. (1976b); as discussed above, they found that most of the CPU proved to be positive for ICSS. In the same article they reported that injections of a neurotoxin which lesions CA-containing neurons (6-hydroxydopamine, 6-OHDA) into the ipsilateral or contralateral SN produced a significant reduction in CPU self-stimulation. The performance of the ipsilateral group dropped to about 10% of control rates and remained at this level for the duration of the experiment (21 days). In contrast, the contralateral lesion group recovered to about 70% of pre-lesion rates by the 21st test day. The fact that there was a reduction in ICSS in all animals, regardless of the side of the lesion, can be interpreted in terms of a generalized performance impairment. The long-lasting impairment seen in the ipsilateral group despite behavioral recuperation of the contralateral group, can be taken as evidence that DA is also critically involved in the rewarding effects of CPU stimulation.

Further support for the idea of critical involvement of striatal DA in the neural mechanisms of reinforcement was provided by Neill, Parker, and Gold (1975). They found that applications of crystalline 6-OHDA suppressed self-stimulation from the lateral hypothalamus (LH) when applied to the ventral-anterior striatum (VAS) but not when deposited into the dorsal CPU or septal area. On the other hand, crystalline applications of DA to the VAS reversed the suppressive effects of 6-OHDA applied to this area and improved self-stimulation in non-lesioned rats. In a follow-up study (Neill, Peay, and Gold, 1978), their earlier findings were confirmed, i.e., bilateral crystalline applications of DA or 6-OHDA to the VAS increased or suppressed, respectively, self-stimulation from the LH. Applications of these drugs to the dorsal or posterior CPU were without effect.

Consistent with these findings are their observations that bilateral applications of d-amphetamine facilitated, and unilateral applications of the neuroleptic haloperidol disrupted, self-stimulation from the LH when the treatments were applied to the VAS, while no important changes in self-stimulation were produced by the application of these agents to the dorsal or posterior striatum. The authors concluded that dopaminergic transmission in the

ventral-anterior striatum, alone among the striatal sites that were tested, is facilitatory in hypothalamic self-stimulation (Neill et al., 1978). Note that the ventral-anterior striatum is near to, and continuous with the nucleus accumbens, which would also be influenced by the treatments of Neill et al. (1978).

The facilitatory effects of d-amphetamine applications to the CPU on ICSS have been replicated in independent laboratories; Broekkamp, Pjinenberg, Cools, and Van Rossum (1975) reported enhanced ICSS with electrodes in the A10 cell group, and Stephens and Herberg (1979) reported similar findings with electrodes in the LH.

Involvement of CPU dopaminergic transmission in SN self-stimulation was also suggested by the experiments of Broekkamp and Von Rossum (1975). They showed that unilateral injections of haloperidol into the CPU depress self-stimulation from the ipsilateral SN. Similarly, contralateral application of the receptor blocker also reduces nigral self-stimulation. In the case of contralateral effects, the disruptive behavioral effects could be ascribed not solely to a reward deficit but, perhaps, to a performance debilitation as well.

Additional experimental tests of the idea that DA within the CPU is critically involved in brain stimulation reward have been carried out with the aid of other drugs that modify DA synaptic action. Intraperitoneal (I.P.) injections of the DA receptor blocker spiroperidol significantly attenuated self-stimulation from the head of the CN of rhesus monkeys, while pimozide only induced a non-significant reduction of CN self-stimulation. This weak effect of pimozide might have been due to the low doses that were administered (0.15 and 0.20 mg/kg). In contrast, apomorphine (a DA receptor agonist) facilitated the same CN self-stimulation. (Phillips, Mora, and Rolls, 1979). In a previous study these authors (Mora, Phillips, Koolhaas, and Rolls, 1976) described similar facilitatory effects of apomorphine (administered subcutaneously, s.c.) on ICSS elicited from the striatum of rats; the drug produced an increment in most animals' response rates at one or more of the doses that were studied; at other doses a decrement of ICSS was seen. The relative variability of these effects could perhaps be accounted for by the great dispersion of electrode locations within the CPU. As noted earlier, it seems that as far as self-stimulation is concerned the ventral-anterior aspect of the striatum is more sensitive to manipulations that alter DA transmission (Neill et al., 1978; 1979).

The inconsistency of the effects of apomorphine could

also be explained in terms of the mode of action of this DA agonist, which is different from the mode of amphetamine action on dopaminergic transmission. Amphetamine causes the release of CA's from nerve endings, and also prevents its reuptake; in addition, it further enhances the release of CA's when their containing fibers are stimulated (Von Voigtlander and Moore, 1973). Apomorphine, in contrast, acts in a non-stimulation-contingent manner by sustained and direct activation of post-synaptic DA receptors. Thus, it would be expected that low doses of apomorphine would facilitate DA transmission (by partially activating post-synaptic receptors), while relatively high doses would produce a generalized stimulation of DA sensitive neurons (precluding any integrative action). This generalized and unpatterned activity would be expected to result in the attenuation of ICSS, and other dopaminergic-mediated behaviors, since it would eliminate the motivation to work for stimulation.

Equivalent effects would be expected from local applications of the same doses in different ICSS DA-sensitive loci (e.g., ventral-anterior vs. posterior CPU) or from systemic applications of apomorphine on ICSS derived from regions with different density of dopaminergic elements (e.g., medial vs. lateral aspects of the LH).

Contrary to expectations 6-OHDA lesions of the nigro-neostriatal system, which produced 97% depletion of DA in the CPU, only induced a temporary reduction of SN self-stimulation, and virtually no effect on SN self-stimulation was seen after 6-OHDA lesions of the NE-containing dorsal tegmental bundle (Clavier and Fibiger, 1977). On the other hand, a drug that inhibits CA synthesis (alpha-methyl-p-tyrosine) significantly reduced SN self-stimulation (Clavier and Fibiger, 1977). From these results the authors concluded that the nigro-neostriatal bundle is not the critical neuronal substrate which mediates the rewarding properties of self-stimulation derived from electrodes in the SN. Although this is a sound conclusion, two points merit discussion. First, the suppression of SN self-stimulation produced by alpha-methyl-p-tyrosine suggests that this self-stimulation was mediated by direct or trans-synaptic activation of DA elements; second, the recovery of ICSS seen after 6-OHDA treatment might have been due to supersensitivity of striatal post-synaptic receptors. Hefti, Melamed, and Wurtman (1980) have shown that destruction of nigro-neostriatal neurons produced by 6-OHDA induced an enhancement in the synthesis and release of DA from surviving neurons; they also showed that supersensitivity occurred when 90% or more of these neurons were destroyed. Another explanation for the recovery

of SN self-stimulation after the lesion of the nigro-neostriatal system could be that self-stimulation from this region may be mediated by dopaminergic activity of other terminal fields (e.g., the accumbens or the ventral striatum).

Ettenberg and Wise (1976) implemented an interesting experimental design that is rarely seen in studies on BSR. They studied the effects of release from chronic I.P. administration of pimozide on locus coeruleus (LC) and SN self-stimulation. Two days after the 8 days of drug administration, ICSS increased in both structures to 25% above pre-pimozide base line levels. These authors suggested that the enhancement of ICSS was due to a state of dopaminergic supersensitivity induced by the drug treatment and that a dopaminergic substrate may be critical for self-stimulation even when dopaminergic elements are not directly (but only trans-synaptically) activated by the stimulating electrodes.

The evidence thus far reviewed indicates that (a) the integrity of the nigro-neostriatal system is probably important for the survival of BSR derived from the caudate nucleus, (b) dopaminergic synaptic activity within the CPU represents a critical component in self-stimulation from the

region of the LH and from the VTA, (c) the nigro-neostriatal dopaminergic system may not play a critical role in SN self-stimulation, and (d) SN self-stimulation may be mediated by dopaminergic activity of other DA terminal fields.

In the peripheral nervous system many functions are regulated through an interaction between different neurotransmitters. For example, cardiac functions are accelerated or slowed as a result of the relative activation of adrenergic and cholinergic systems. At higher levels it is also found that particular processes are mediated by the relative activation of different chemically-coded neural systems, which may act either in a synergistic or an antagonistic fashion. This point will be illustrated with the description of some of the neurochemical interactions that occur in the CPU-SN system, as well as their relation to a particular learning situation.

Nigro-neostriatal DA terminals represent an afferent input to the intrinsic cells of the striatum, where the interneurons use acetylcholine (ACh) as their neurotransmitter (McGeer, McGeer, Grewaal, and Singh, 1975). A number of experimental findings suggest that these cholinergic interneurons synapse with one striatal efferent system which, through the release of gamma-amino-butyric acid (GABA), regulates the activity of SN dopaminergic

neurons. As described below, it appears that both nigro-striatal and striato-nigral efferents exert inhibitory actions on each other's post-synaptic targets. Electrical stimulation of the SN inhibits the firing of some striatal cells (Connor, 1970), and the same effect is seen after intra-caudate electrophoresis of DA (Bloom et al., 1965) and of DA agonists (Guyenet, Agid, Beaujovan, Rossier, and Glowinski, 1975). Furthermore, administration of drugs that activate DA receptors, presumably located on the cholinergic interneurons, reduces ACh release and turnover, while drugs that block these receptors produce the opposite effect (Ladinsky, Consolo, Bianchi, Ghezzi, and Samanin, 1978).

On the other hand, SN neurons are inhibited monosynaptically by electrical stimulation of the caudate (Yoshida and Precht, 1971); there are also data which suggest that the activity of some nigral dopaminergic neurons is deppressed by GABA, which is released by caudato-nigral axons (Dray and Gonye, 1975; Precht and Yoshida, 1971), thus establishing a regulatory feedback loop.

It may be postulated that some behaviors are regulated by the activity of an output system efferent to the nigro-neostriatal system. The activity of this output system could be, in turn, dependent upon a dynamic balance between

the neural activity of the CPU and the SN. Thus, modifications of this balance would bring about modifications in behavior. The results of a series of experiments indicate that a functional balance between neurons of the striatum and neurons of the substantia nigra must be maintained for the development of some memory processes.

It has been shown that direct application into the CPU of the ACh receptor blockers atropine and scopolamine significantly impairs the retention of a passive avoidance response (Prado-Alcala, Cruz-Morales, and Lopez-Miro, 1980; Prado-Alcala, Signoret, and Figueroa, 1981). These findings support the hypothesis that cholinergic activity of the CN is critically involved in the processes that underlie passive avoidance learning. If this hypothesis is correct, then alterations in passive avoidance should occur when the neurochemical balance of the CPU-SN system is disturbed. Routtenberg and collaborators have found that electrical stimulation of the SN (Routtenberg and Holzman, 1973) and of the DA-containing MFB (Bresnahan and Routtenberg, 1980) results in a marked impairment in the retention of this task. These results could be explained as follows: electrical stimulation of the SN or of the MFB could increase the release of DA in the CPU, producing, in turn, inhibition of striatal cholinergic interneurons; decreased release of ACh (like blockade of ACh receptors by atropine or scopolamine)

would prevent or arrest the retention process. Retention deficits in passive avoidance would also be expected to occur if the dopaminergic-cholinergic balance is disturbed by other means, which is indeed the case. Retrograde amnesia is produced when DA is directly applied to the CPU (Kim and Routtenberg, 1976a) and also by injections of a GABA receptor blocker (picrotoxin) into the SN (Kim and Routtenberg, 1976b). Further support for the hypothesis under consideration is that choline injections into the CPU significantly improve passive avoidance (Fernandez-Samblancat, Solodkin, and Prado-Alcala, 1977).

Those studying the ICSS phenomenon are not only dealing with the neural basis of reinforcement but also with the learning processes associated with the behaviors that allow the inference that stimulation of some areas of the brain is rewarding. It is of interest to mention, in this context, the proposition derived from the work of Routtenberg's group:

> "...those areas of the brain that support ICSS overlap with the areas that, when stimulated, alter memory formation. A theoretical statement of these findings would be that ICSS pathways function as pathways of memory consolidation". (Clavier and Routtenberg, 1980, p. 96).

Apart from DA, ACh, and GABA, there is evidence suggesting a neurotransmitter role in the CPU for other

substances as well, such as serotonin (Saavedra, Brownstein, and Palkovits, 1974) and glutamate (Fonnum, Gottesfeld, and Grofova, 1978). The striatum also contains high concentrations of some peptide transmitter candidates, such as substance P (Kanasawa and Jessel, 1976) and methionine enkephalin (Hong, Yang, Fratta, and Costa, 1977) and low concentrations of neurotensin and somatostatin (Kobayashi, Brown, and Vale, 1977). It would not be surprising to find that BSR derived from the CPU, and from other areas of the brain, is dependent upon interactions among some of these chemicals.

After more than a quarter of a century of research on brain stimulation reward, very little is known about its dependence upon the interactions among different neurotransmitter systems, but it seems likely that if brain dopamine plays a significant role in reward, then acetylcholine may also be involved. Early findings (reviewed by Olds, 1977) suggeted that ACh could have opposite effects on BSR, depending on the type of ACh receptor that is stimulated. Activation of muscarinic receptors antagonized self-stimulation while blockade of these receptors reversed the effect. On the other hand, stimulation of nicotinic receptors promoted ICSS, and antinicotinic drugs opposed this action.

Recently, Stephens and Herberg (1979) reported that I.P. injections of the ACh receptor blockers scopolamine and benztropine enhanced LH self-stimulation, thus confirming some of the early findings mentioned above; they went further and showed that each of these drugs partially restored self-stimulation that had been suppressed by the DA antagonist spiroperidol. In addition, they studied the effects of bilateral injections of scopolamine into the CPU or into the NAS on spiroperidol-induced suppression of LH self-stimulation. Only the NAS injections proved effective in partially restoring ICSS. These investigators concluded that LH self-stimulation may be influenced by DA and ACh systems within the NAS. Before concluding that cholinergic activity of the striatum does not play a role in LH self-stimulation (or in self-stimulation of other sites), a cautionary note, provided by the authors of the study under analysis, is in order: "...the CPU compared with the ACB (nucleus accumbens) is a very large structure and a wider range of doses and injection sites would have to be sampled before any negative conclusions could be drawn, since the critical region may have simply been missed" (p. 337).

With respect to serotonin (5-HT), it is known that there is a monosynaptic projection that originates in the dorsal raphe nucleus (DRN), in which 5-HT neurons are found, and

innervates the caudate in a diffuse manner (Miller, Richardson, Fibiger, and McLennan, 1975). Electrical stimulation of the DRN produces a relatively long-lasting inhibition of cell firing in the striatum (Miller et al., 1975).

Phillips, Carter, and Fibiger (1976a) reported the effects of intragastric administration of an inhibitor of the biosynthesis of 5-HT (para-chlorophenylalanine) on LH and CPU self-stimulation. ICSS was suppressed in both areas when testing was conducted 24 h after the treatment; after 48 h self-stimulation of the LH increased to 115% reaching a level of 180% by the third post-treatment day, while CPU self-stimulation continued to decline to 48% of control levels on the sixth day of testing.

In an experiment conducted by Redgrave (1978), rats exhibiting self-stimulation from both MFB and ventral mesencephalic tegmentum (VTA) were perfused with DA, NE, or 5-HT in the NAS or in the CPU. It was found that, in general, DA infusions produced an improvement in ICSS, regardless of site of injection or locus of BSR. NE infusions produced response patterns similar to those produced by DA, and 5-HT had the opposite effect.

The results of these two studies are difficult to reconcile. In the experiment by Phillips et al. (1976a), a generalized reduction of 5-HT activity produced a reduction of LH and CPU self-stimulation; in the study by Redgrave (1978) BSR derived from MFB and VTA stimulation was impaired by 5-HT activation in the CPU and NAS. Even though there were obvious differences in procedures, the conclusions drawn in both cases were that BSR is critically dependent upon the interaction of DA and 5-HT systems.

It has been known for some time that the striatum contains opiate receptors (Pert, Kuhar, and Snyder, 1975) and that in some species morphine interacts with dopaminergic metabolism in the CPU; in the rat morphine does not seem to affect striatal levels of DA but increases its rate of synthesis and catabolism (Sugrue, 1974). In an interesting study by Broekkamp and Von Rossum (1975) in which self-stimulation from the SN-VTA area was depressed by application of haloperidol into the CPU, NAS, or cerebral ventricles, it was also found that morphine reduced ICSS when it was injected into the ventricles. When morphine was applied to the CPU or the NAS, however, there was no important change in ICSS. From these results the authors concluded that "...these results do not favor the hypothesis that morphine interferes with dopaminergic transmission within the neostriatum " (p. 110).

It is evident from the above that a great deal of research is needed in order to understand how the various chemically-coded systems interact with one another to produce the rewarding effects of stimulation of the striatum and other regions and to account for the frequent discrepancies seen in the literature.

Mesolimbic Terminal Fields

The septal area has been implicated in the regulation of various functions, such as homeostatic, emotional and autonomic functions, as well as in locomotor activity and learning processes (for reviews, see Grossman, 1976; Isaacson, 1974). It has been difficult to precisely define the mechanisms underlying those functions because, among other factors, the septum is not a homogeneous structure, and many fibers of passage traverse this region. Most studies on septal functions have involved lesions or electrical stimulation, and it is difficult to attribute the effects of such manipulations solely to interference with septal activity, since the effects could be a reflection of interference with the activity of structures that are connected to the septum.

With respect to the problem of BSR, the septal area is

historically important. The serendipitous finding that a rat returned to the place in the environment where it had received electrical stimulation of the septum inaugurated this prolific field of research. This discovery was made in November of 1953 by Olds and Milner and was formally reported in their 1954 article "Positive reinforcement produced by electrical stimulation of septal area and other regions of the rat brain".

In subsequent studies the reinforcing effects of septal stimulation were confirmed (Valenstein and Valenstein, 1964). BSR was obtained from the lateral (Olds and Olds, 1963; Routtenberg, 1971), medial (Routtenberg, 1971), dorsal (Gardner and Malmo, 1969), and ventral (O'Donohue and Hagamen, 1967) aspects of the septum.

The amygdala is a complex set of nuclei that has been described as having multiple functions. Electrical stimulation and lesion techniques have been used, as in the case of the septum, in most studies concerning the functions of the AMY. It appears that this structure participates in a variety of functions, including autonomic activity, orienting and habituation, emotionality, arousal, and learning and memory (Isaacson, 1974).

In the early days of self-stimulation, it was established that BSR could be obtained from the AMY (O'Donohue and Hagamen, 1967; Valenstein and Valenstein, 1964; Wurtz and Olds, 1963). In the study by Wurtz and Olds (1963), it was found that most electrodes yielding "pure" brain stimulation reward were located in the central and medial nuclei of the corticomedial division, whereas those electrodes in the lateral basal nuclei of the basolateral division produced escape behaviors. However, these effects were not strictly localized; that is, there was some degree of anatomical overlap between the reported reward and escape sites.

Self-stimulation derived from other terminal fields of the mesolimbic dopaminergic system was also described during the 1960's and early 1970's. The NAS was described as yielding moderate "approach-only" or mild ambivalent (approach-escape) behaviors (Olds and Olds, 1963). Olds and Olds (1963) gave the same behavioral descriptions of self-stimulation from the olfactory tubercle. Evidence for rewarding effects of electrical stimulation of the stria terminalis and its nucleus was provided by Routtenberg (1971).

As will become apparent from the following review, the role of dopaminergic activity in BSR from these regions is far from clear. In 1975, Phillips, Brooke, and Fibiger

reported the effects of I.P. injections of d- or l-amphetamine on self-stimulation from the NAS or the DNB. Both isomers produced significant increments in self-stimulation rates from both placements, although the d-isomer produced a greater increase than did the l-isomer in the DNB; d- and l-amphetamine were equipotent in facilitating BSR from the NAS. Pre-treatment with haloperidol or pimozide induced a reduction of stimulation rates from both NE and DA placements. Because of these findings, the authors suggested that the effects of the neuroleptics may be mediated by a disruption of operant behavior and that the decrement in ICSS does not necessarily implicate dopamine in the reward phenomena. These results must be re-interpreted, however, in light of the recent findings of Shizgal et al. (1980) and of Yeomans (1979). These authors' work suggests, as discussed above, that the directly activated reward-relevant tissue is non-catecholaminergic. Hence, in the study under consideration, regardless of where the electrodes were implanted, it is likely that the stimulating electrodes trans-synaptically activated DA neurons. DA release would mediate the rewarding effect of the electrical stimulation, and this effect would be counteracted by the neuroleptics. Congruent with the results of Phillips et al. (1975) are the results of Ettenberg and Wise (1976); they showed that chronic pimozide produces

an enhancement of self-stimulation in "typical" noradrenergic (LC) and dopaminergic (SN) sites. These two studies suggest that DA may be critically involved in BSR even when dopaminergic neurons are not directly activated.

In an interesting study by Robertson and Mogenson (1979), it was found that chronic (I.P.) administration of either spiroperidol or d-amphetamine produced significant increments in self-stimulation of the prefrontal cortex yet did not modify NAS self-stimulation. The results of this experiment seem to contradict the view that DA has a modulatory action on BSR involving NAS electrodes. Different conclusions were reached by Seeger and Gardner (1979). Rhesus monkeys with self-stimulation electrodes in the NAS were treated with I.P. haloperidol for 11 days, and release from this treatment produced a dose-related reduction of ICSS threshold, as measured by a rate-independent reward paradigm. These researchers also studied the effects of chronic treatment with haloperidol and clozapine on VTA self-stimulation in rats. These drugs induced a significant increase in ICSS rates. The data suggest that long-term treatment with neuroleptics, which induces receptor supersensitivity in the mesolimbic dopaminergic system, alters NAS BSR; this would support the view that DA is involved in the mediation of the rewarding consequences derived from electrical stimulation of this system.

Consistent with this latter view is a study showing that d-amphetamine, directly injected into the NAS, produces a facilitation of LH self-stimulation, whereas haloperidol produces the opposite effect (Stephens and Herberg, 1977). In this study it was also reported that both I.P. injections and local application of apomorphine into the NAS produced either a facilitation or a depression of LH self-stimulation; that is, there was a consistent effect on each rat, regardless of route of administration. Finally, they found that tyramine (which produces non-contingent release of CA's) increased ICSS when injected into the NAS. These authors concluded that the NAS (and the CPU, see preceding section) plays an important role in brain stimulation reward, and that DA is involved in this process. The facilitatory effect of d-amphetamine injections into the NAS on BSR had been described earlier in a study where self-stimulation was obtained with electrodes in the VTA (Broekkamp et al., 1975).

In an elegant experimental series where potentially interfering side effects were efficaciously controlled, Mogenson and co-workers showed that microinjections of the DA receptor blocker spiroperidol into the NAS had significantly reduced ICSS from the same nucleus, while ICSS from the

contralateral NAS and from the ipsilateral prefrontal cortex had been unaffected (Robertson and Mogenson, 1978). In a second study (Mogenson, Takigawa, Robertson, and Wu, 1979), they reported that when spiroperidol was applied to the NAS ipsilateral to VTA ICSS electrodes, it produced a suppression of this behavior but did not influence self-stimulation of the contralateral VTA. Also in line with these results are those of Broekkamp and Von Rossum (1975) and the findings of Mora, Rolls, Burton, and Shaw (1976). The former group reported that self-stimulation from the SN or the VTA was significantly reduced after ipsilateral injections of haloperidol into the NAS. Mora et al. (1976) showed that spiroperidol applications into this structure also reduced self-stimulation from the AMY.

Most of the studies cited above fit with the hypothesis that dopaminergic activity of the mesolimbic system plays an important role in BSR; nevertheless, some lesion studies provide data that do not seem to fit with such an hypothesis. In one report where the ratio between endogenous DOPAC and DA was measured, a significant activation of VTA neurons (as a result of VTA self-stimulation) was found in the nucleus accumbens (Simon, Stinus, Tassin, Lavielle, Blanc, Thierry, Glowinski, and Le Moal, 1979). Although this finding suggested that a dopaminergic mechanism within this nucleus might be participating in ICSS, in the same article it was

reported that near total destruction of the A10 cell bodies not only had failed to reduce self-stimulation from the NAS but in fact had facilitated it. It was concluded that DA of the VTA neurons is not critically involved in NAS self-stimulation. A complementary study was published by Phillips and Fibiger (1978) where 6-OHDA lesions of the MFB, which reduced DA levels to less than 5% in the accumbens-prefrontal cortex, only temporarily reduced self-stimulation from the NAS. Further, the lesions produced a reduction of DA levels in the CPU to less than 1% and a permanent suppression of VTA self-stimulation. This experiment indicates that the integrity of the A10-NAS projection is not essential for BSR obtained from this dopaminergic terminal field but suggests that "...dopamine plays an important role in self-stimulation in the ventral tegmentum" (p. 58). In each of these studies, it was also concluded that other non-catecholaminergic systems may be involved in BSR.

In summary, it seems from most studies that the functional integrity of the mesolimbic dopaminergic system is important for the maintenance of self-stimulation from the AMY and the VTA. On the other hand, there is conflicting evidence with respect to the involvement of this system when the stimulating electrodes are at or near the region of the

dopaminergic terminals and their synapses. In this case it might be expected that the rewarding stimulation is activating elements efferent to any DA link in the reward substrate.

Mesocortical Terminal Fields

As stated earlier, the mesocortical dopaminergic system originates, mainly, from the A10 dopaminergic cell group and projects to the dorsal bank of the rhinal sulcus, to the medial wall of the hemisphere (anterior and dorsal to the genu of the corpus callossum), to the entorhinal cortex, and to the anterior cingulate cortex. The first two cortices (sulcal and medial prefrontal) receive afferents from the medio-dorsal nucleus of the thalamus, as is the case in the monkey's brain. Because of this and other neuroanatomical similarities between rat and monkey (e.g., projection from the medial prefrontal cortex to the pretectal area and superior colliculus and from the sulcal cortex to the basal olfactory nuclei and lateral hypothalamus), the rodent sulcal and medial prefrontal cortex (PFC) has been thought of as equivalent to the monkey PFC (Leonard, 1969).

The dopaminergic projection to this system was first made manifest by the use of the histofluorescence method (see section 4 of this Introduction). The use of other procedures

has also permitted confirmation of the topographical projections of the A10 cell group to the PFC. Thierry, Tassin, Blanc, and Glowinski (1976) measured the activity of specific [3-H] DA uptake in cortical homogenates obtained from micro-discs punched out from frontal serial brain slices. As expected, DA terminals (inferred from [3-H] DA levels) were found in the sulcal and medial prefrontal cortex, as well as in the cingulate cortex. Bilateral electrolytic VTA lesions greatly reduced [3-H] DA uptake in frontal cortex homogenates and also decreased the amine uptake in the NAS.

In early mapping studies it was reported that BSR could be induced with electrical stimulation of the rat's PFC (Routtenberg, 1971) and from the cat's pyriform, prepyriform, anterior limbic and orbitofrontal cortices (O'Donohue and Hagamen, 1967). It was only later that systematic regional studies of the effects of self-stimulation in the cortex were conducted in Routtenberg's laboratory.

In 1972, Routtenberg and Sloan showed that both the medial and sulcal areas of the prefrontal cortex sustained ICSS, whereas no self-stimulation was observed from the central, dorsal or dorsolateral regions of the cortex. The highest rates of self-stimulation were obtained in the pregenual region of the medial cortex (mainly from the third

layer of both dorsal and ventral regions of this cortical area). Slightly lower rates were obtained from the sulcal cortex, located on the dorsal lip of the rhinal fissure. It is interesting to note that another line of evidence provides support for the notion that the PFC of the rat is involved in brain stimulation reward. There are units in both sulcal and medial prefrontal cortex that are driven (antidromically or trans-synaptically) by stimulation of many ICSS sites (e.g., LH, midbrain tegmentum, NAS), while units in other cortical areas do not seem to show this reactivity (Rolls and Cooper, 1973).

A second major mapping study of cortical self-stimulation was conducted by Collier, Kurtzman, and Routtenberg (1977), using both large (254 um wire) and small (78.7 um wire) bipolar electrodes. With the larger electrodes they found that the majority of positive sites were located in the ventral, ventromedial and deep layers of the dorsolateral entorhinal cortex. With the finer electrodes they were further able to differentiate some subregions in relation to BSR; there was a significant difference in pressing rates between the anterior lateral and posterior regions of the entorhinal cortex. There was also a reliable difference between the anterior dorsolateral and posterior dorsolateral aspects of the dorsal subzone of the

lateral entorhinal mantle. In summary, the best ICSS probes were located at anterior levels and also in the most superficial cortical layers. These authors made the important observation that moderate ICSS rates tended to be associated with the stimulation of cortical DA islands or aggregates and suggested that DA may play a role in entorhinal self-stimulation; at the same time they indicated that other non-dopaminergic systems may be involved in BSR from this area, since relatively high rates of self-stimulation had also been displayed by rats with electrodes in posterior levels of the dorsal lateral entorhinal cortex, an area low in DA and devoid of DA islands. In a related article Ott, Destrade, and Ruthrich (1980) reported that good self-stimulation rates were obtained with electrodes that had been implanted in the posterior part of the medial entorhinal cortex, an area which lacks dopamine (Palkovits, Zaborsky, Brownstein, Fekete, Herman, and Kanycsa, 1979).

During the last seven years, a number of lesion studies have been published which give support for the idea that the prefrontal cortex is critically involved in BSR. Rolls and Cooper (1974) found that bilateral injections of the local anesthesic procaine ("reversible lesion") into the sulcal cortex of the rat attenuated LH and pontine tegmentum self-stimulation. Two years later Clavier and Corcoran

(1976) studied the effects of bilateral electrolytic lesions of the sulcal cortex on self-stimulation derived from the SN or the DNB. The lesions induced a permanent reduction of SN self-stimulation (about 33% of pre-lesion rates) but failed to disrupt DNB self-stimulation. In this article they also reported that there was a descending fiber system originating in the sulcal cortex, which was very dense in the region of the SN but only scattered in the DNB region. They concluded that there may be several independent systems in the area of the SN, each of which could be sufficient to support self-stimulation in the absence of the others, and that a non-catecholaminergic system may participate in BSR. In a related experiment (Clavier and Gerfen, 1979), it was seen that 6-OHDA injections into the ascending trajectory of the A10 mesocortical system had produced a marked suppression of ipsilateral sulcal self-stimulation; contralateral sulcal BSR was only transiently reduced. These results suggest that sulcal self-stimulation is mediated by the ascending mesocortical dopaminergic system.

A lesser contribution of this aminergic system to prefrontal ICSS became apparent in an experiment which also dealt with the effects of 6-OHDA lesions of the MFB (Phillips and Fibiger, 1978). DA post-lesion levels were reduced to less than 1% in the CPU and were reduced to less than 5% in

the accumbens-prefrontal cortex. As a consequence of the lesion, VTA self-stimulation was permanently suppressed, and medial PFC ICSS suffered a mild reduction, followed by a partial recovery (62% of pre-lesion rates); NAS ICSS was the least affected with pressing rates approaching pre-lesion rates within six days after the lesion. In addition, d-amphetamine (I.P.) did not produce increments in BSR at any of the sites after the lesions. These results suggested to the authors that "...dopamine plays an important role in self-stimulation in the ventral tegmentum and contributes to this behavior in the prefrontal cortex. These findings also show that non-dopaminergic systems contribute to the phenomenon of brain-stimulation reward" (p. 58).

Studies where changes in dopaminergic activity have been induced pharmacologically (I.P., s.c., or localized application of drugs) and those where dopaminergic activity has been inferred from measurements of DA release in cortical areas have provided complementary evidence that the mesocortical dopaminergic system is involved in BSR. Much of this evidence has been provided by Mora and co-workers (Mora, 1978).

Rats that self-stimulated through electrodes located in the medial and sulcal prefrontal cortex and in the CPU were given s.c. injections of several doses of apomorphine. A

dose-dependent suppression in both medial and sulcal self-stimulation was observed, and this effect was consistent in all animals. CPU self-stimulation, on the other hand, was affected in a non-systematic way: some doses induced a facilitation of ICSS while other doses produced a decrement. The consistency of the effects of apomorphine on sulcal and medial prefrontal cortex self-stimulation was taken as evidence for the involvement of DA in this cortically-mediated behavior. The rationale for this conclusion is that the release of DA, contingent upon the animal's performance, would be less reinforcing (or no longer reinforcing) because of the continuous activation of DA receptors by apomorphine (Mora, Phillips, Koolhaas, and Rolls, 1976). Equivalent effects of apomorphine on brain stimulation reward were seen in the rhesus monkey; that is, application of this dopaminergic agonist attenuated orbitofrontal self-stimulation (as discussed earlier, this region of the monkey's brain is analogous to the prefrontal cortex of the rat). Spiroperidol and pimozide, administered systemically, also attenuated orbitofrontal self-stimulation in the same monkeys. In this paradigm BSR was obtained by licking a tube. The drugs were ineffective in altering this response when licking had been rewarded with blackcurrant juice (Phillips, Mora, and Rolls, 1979).

A dose-related attenuation of orbitofrontal self-stimulation by I.P. injections of spiroperidol had previously been reported (Mora, Rolls, Burton, and Shaw, 1976). These treatments also produced a dose dependent reduction of self-stimulation from the LH and the LC. When intracranial injections of the neuroleptic had been made into the NAS or the LH, attenuation of ICSS from the AMY was seen, and injections into the monkey's orbitofrontal cortex similarly reduced self-stimulation derived from the LH and the AMY. In addition to the conclusions derived from the experiments described above, these results also suggest that dopamine receptors in the NAS and orbitofrontal cortex are involved in self-stimulation involving the LH and the AMY.

Robertson and Mogenson (1979) tested the effects of chronic (9 days, I.P.) administration of haloperidol and of d-amphetamine on ICSS with electrodes in several areas. Surprisingly, each of these treatments induced a significant enhancement of medial PFC self-stimulation, which was seen even after drug administration had been discontinued. In contrast, chronic spiroperidol did not significantly change brain stimulation behavior derived from the NAS, supracallosal bundle, CPU, VTA, or subfornical organ; NAS self-stimulation was not altered by chronic amphetamine, but supracallosal self-stimulation was suppressed. The effects of chronic spiroperidol on PFC self-stimulation could be

explained in several ways: by an increase in DA receptors, by an alteration of feedback control of DA metabolism, by a slow release of spiroperidol accumulated in lipid or connective tissue, etc. No interpretation of the effects of chronic treatment of amphetamine on ICSS from the prefrontal cortex was offered by the authors. Whatever the mechanisms of action of these treatments may be, the results of this study suggest that DA modulates self-stimulation involving electrodes in this mesocortical projection field.

In two closely related articles, it was described that electrical stimulation of a region of the medial prefrontal cortex, which supported self-stimulation, induced the release of DA (Myers and Mora, 1977) and of DA and its associated metabolites (Mora and Myers, 1977). These results indicate that DA fibers play an important synaptic role in the processes that are activated in the case of PFC BSR, but they do not indicate whether this activation accounts for or alters the rewarding impact of the stimulation.

In parallel with these publications, there were other reports from which an almost opposite picture emerged. Goodall and Carey (1975) found that neither the d- nor the l-isomers of amphetamine affected PFC BSR; moreover, injections of haloperidol into this region did not produce changes in LH

or VTA self-stimulation (Mogenson, Takigawa, Robertson, and Wu; 1979; Mora, Myers, and Sanguinetti, 1977). Doses of spiroperidol which reduced accumbens self-stimulation when injected into this nucleus proved ineffective in altering medial PFC BSR after injection into this structure; only after injection of a relatively high dose of the neuroleptic was there an attenuation of prefrontal cortex self-stimulation (Robertson and Mogenson, 1978). Near total destruction of the A10 dopaminergic cell bodies did not alter self-stimulation derived from the medial PFC (Simon et al., 1979).

Several inferences can be made from the results of this set of studies. Dopaminergic synaptic activity within the prefrontal cortex may not be necessary for brain stimulation reward produced by stimulation of the nucleus of origin of the mesocortical system (VTA) or from other areas of the brain, such as the LH. Also, dopaminergic activity of the mesocortical system may not be critically involved in brain stimulation reward derived from stimulation of the medial prefrontal cortex; rather, other non-catecholaminergic (descending?) systems may be involved in self-stimulation of this cortical region.

Purpose of the Present Study

The neural mechanisms of brain stimulation reward remain unknown despite the hundreds of studies summarized above. In only one place in the brain can the substrate of brain stimulation reward be localized with identical boundaries to a defined anatomical system. This place is the region of DA cell bodies in the ventral tegmental area, where it appears that DA afferents, not the cells themselves, are being directly activated by the stimulation. The origin and trajectory of the directly stimulated fibers is not determined, nor is it certain that these fibers are direct dopaminergic afferents; the pharmacological work which implicates brain dopamine in reward function does not tell us how many synapses intervene between the directly-activated elements and the critical dopaminergic elements. The mesolimbic system appears implicated, but it is far from clear whether it plays a role in brain stimulation reward involving many or only some brain sites, and it is far from clear whether other dopaminergic systems are involved in reward at all.

The general purpose of the present study was to extend the anatomical analysis of brain stimulation reward in an attempt to find other sites in the system where the boundaries of some anatomically-identified set of neurons correspond to the boundaries of positive sites for brain

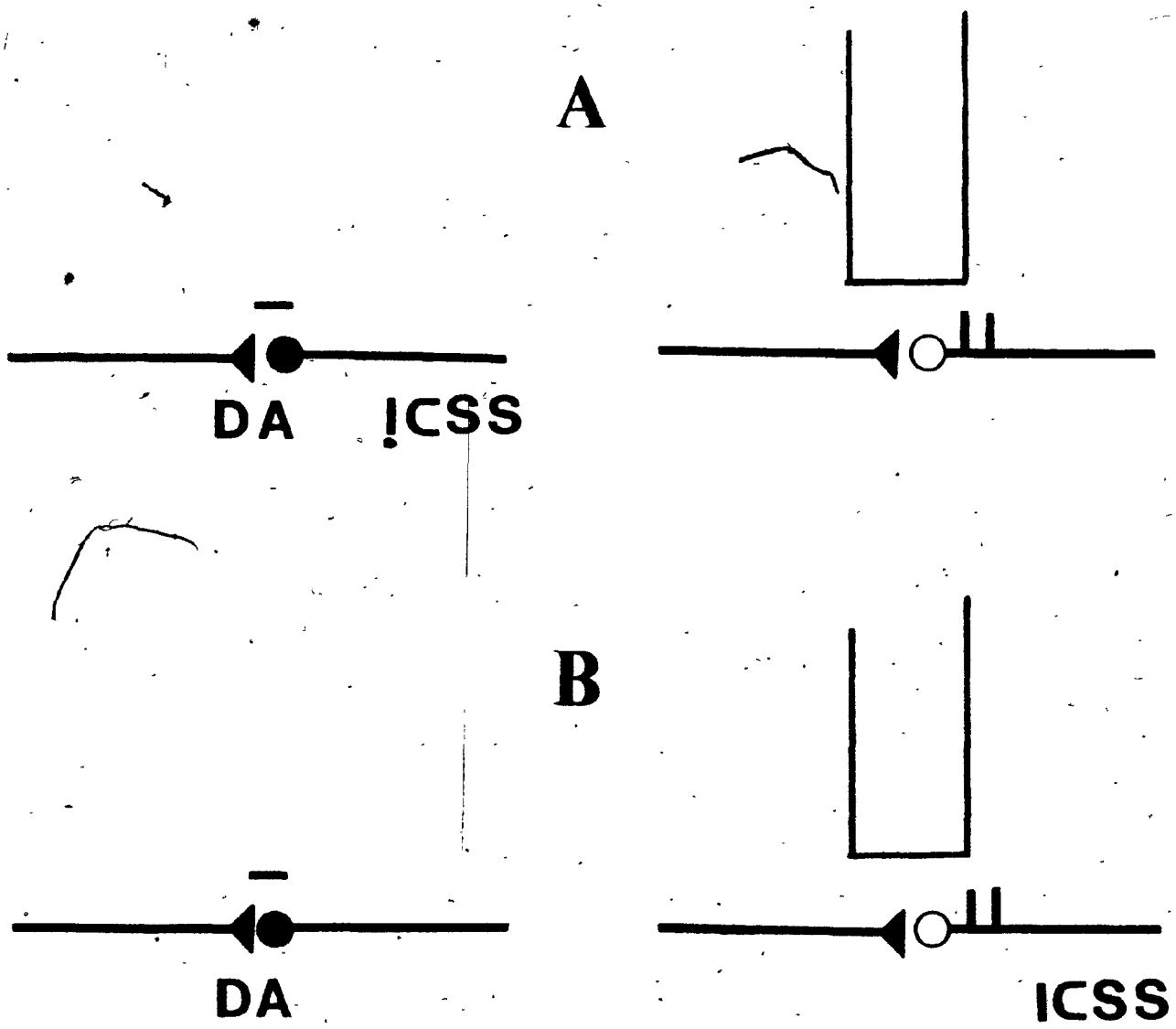
stimulation reward. Because fluorescence histochemistry allows visualization of dopaminergic neurons, one can explore the possibility that they define a system with unique anatomical relations to BSR sites. Corbett and Wise (1980) and Wise (1981b) have mapped the region of DA cell bodies and found an important correspondence between the boundaries of DA elements in this region and the boundaries of the positive sites of BSR in the same region. Inasmuch as the fluorescent dopamine cell bodies mark both their own boundaries and also the boundaries of their population of input fiber terminals, the anatomical data suggest two populations as primary candidates for the substrate of BSR in this region, and other studies (Gallistel et al., 1981) suggest which of the two is likely. Similar procedures and similar logic can be used to map the regions of dopamine terminal projections. Catecholamine fluorescence will mark the relative density of the catecholamine fibers themselves, though they are unlikely to be the directly activated substrate of brain stimulation because of their apparent insensitivity to normal parameters of BSR (Shizgal et al., 1980; Yeomans, 1979) and will also mark the location of any population of cells which receive dopaminergic afferents. The specific aim of the present study was to determine if brain stimulation reward bears any special anatomical relation to the terminal fields of forebrain dopamine projection systems or to the non-dopaminergic cells in these terminal fields upon which

dopamine makes synaptic contact.

The way in which the relationship between BSR and the topography of DA terminals was approached was to compute the correlations between self-stimulation characteristics and dopamine terminal density around different stimulation sites. The self-stimulation characteristics included: intensity thresholds, pressing rates, and number of sessions needed before animals started self-stimulating or reached a criterion of stability. Both positive and negative correlations were of interest. If brain stimulation reward in these regions of the brain were to excite dopamine fibers themselves, then the boundaries of brain stimulation reward should correspond to the boundaries of the dopaminergic terminal fields, and the goodness of BSR should be positively correlated with dopamine terminal density. The same should be true if stimulation activates dopaminergic efferents and if activation of these efferents accounts for any reward message conveyed to them via the dopaminergic fibers connecting these regions to other portions of the reward circuitry. On the other hand, it is possible that dopamine fiber terminals are the directly activated reward substrate in these regions of the brain but that they normally inhibit rather than excite their efferent targets. If this were the case, then brain stimulation reward should correlate

negatively with dopaminergic terminal density, since stimulation very near the dopaminergic terminals would override the effects of stimulation-released dopamine by directly activating the cells that rewarding dopamine normally inhibits. This direct activation of dopamine efferents would, in this case, effectively reverse the rewarding effects of dopamine terminal activation in proportion to its proximity to the target cell bodies.

Fig 1. Hypothetical mode of action of dopamine in self-stimulation regions of the dopaminergic projection fields. Drawings on the left represent the physiological inhibition, produced by dopamine release from dopaminergic terminals, of post-synaptic neurons. Direct activation of these post-synaptic neurons, by a stimulating electrode, is represented in the drawings on the left. A. Dopamine-induced post-synaptic inhibition is required for a rewarding state to occur. Electrical stimulation, by activating the post-synaptic neurons, inhibits self-stimulation. B. The opposite effect is produced in areas where dopamine-induced post-synaptic inhibition blocks reward.


A
DA
ICSS
B
DA
ICSS

METHODS

Animals.

One-hundred and four experimentally naive male hooded rats weighing between 250 and 350 g were used. They were individually housed and had free access to solid food and tap water in their home cages. While under sodium pentobarbital anesthesia (60 mg/kg), a unipolar moveable electrode was implanted in each of 88 rats. The stereotaxic coordinates were selected from the Pellegrino and Cushman atlas (1967) in order to stimulate the tissue of the terminal fields of the ascending dopaminergic systems. The selected areas were bounded by the coordinates presented in Table I as determined from bregma (A-P = 0) and from the dural surface (D-V = 0).

The monopolar moveable electrode consisted of a 254 um stainless steel wire concentrically soldered into a male Amphenol connector that had been threaded externally with a 2-56 thread die. The electrode was insulated with Formvar except at the cross section of the tip. The threads of the Amphenol pin were covered with stopcock grease (Corning) prior to screwing the electrode into a threaded nylon receptacle 10 mm in length and 4 mm in diameter thereby

TABLE I

DA SYSTEM	TARGET	A-P	M-L	D-V
Nigro-neostriatal	CPU	-0.8 to 4.0	1.5 to 5.4	3.0 to 7.6
Mesolimbic	Septum	0.6 to 3.2	0.1 to 1.2	2.8 to 6.0
	Accumbens	2.0 to 4.0	0.1 to 1.2	2.8 to 6.0
	Amygdala	-0.4 to 1.6	4.0 to 5.0	6.2 to 8.6
	Olfactory Tubercle	3.0 to 4.2	1.9 to 3.6	5.5 to 8.2
Mesocortical	Medial prefrontal	3.2 to 5.2	0.2 to 2.0	0.4 to 6.0
	Sulcal	4.0 to 4.8	3.9 to 5.0	3.0 to 6.0
	Entorhinal	-3.4 to -2.6	6.2 to 7.6	5.6 to 9.0

Each moveable electrode was implanted using a set of coordinates obtained from the anterior-posterior (A-P), medial-lateral (M-L) and dorsal-ventral (D-V) limits shown in this Table.

insulating 2-3 mm of the protruding electrode shaft. When the assembly was implanted, the skull-nylon interface was sealed with stopcock grease thus preventing (a) dental cement from making contact with the electrode shaft or (b) cerebrospinal fluid from seeping up the nylon receptacle to the uninsulated threads of the Amphenol pin and creating an undesirable low resistance pathway.

Once implanted, the electrode could be lowered in steps of 250 um by grasping the threaded Amphenol pin with a pin vice and rotating it one-half revolution. The maximal ventral travel of the moveable electrode was 3.0 mm. A skull screw served as the indifferent electrode. Each animal was allowed 4-5 days to recover from surgery before ICSS testing was initiated.

Testing Procedure.

Testing for ICSS was conducted in eight identical Skinner boxes, each of which was equipped with a lever which, when depressed, delivered a 500 msec train of 60Hz sine wave stimulation. Each box was installed inside a sound-attenuating chamber with constant illumination, and a background masking noise was produced by an exhaust fan.

Testing was divided into three phases. During the first

phase, the constant current stimulators were set at 40 uA, and each rat was put inside a Skinner box for 15 min; if a rat spontaneously self-stimulated at a rate of 5 or more lever presses per min, it was shifted to phase three. If during phase one, a rat did not self-stimulate, then the experimenter proceeded to phase two--shaping the animal's behavior to approach and to press the lever by manually delivering electric current. During this phase a current that seemed rewarding was selected (i.e., one that would elicit forward locomotion and approach reactions). In addition, 2 to 3 current levels (10 uA steps) above and below the one that had been selected were also tested. Shaping lasted for about 15 min at each intensity level. If no approach and forward locomotion behaviors were elicited, shaping was conducted at 60, 40, 20, and 10 uA; if aversive reactions were produced by the stimulation, the intensity was reduced until these reactions were no longer evident. If a rat failed to self-stimulate after five consecutive sessions of testing under phases one and two, the electrode was lowered 250 uA and the same testing sequence was repeated.

In phase three, self-stimulating rats were tested using a descending series of current intensities starting with 60 uA. The current was progressively lowered, every five min, to 56 and then to 52 uA; from this level,

the current was lowered in steps of 2 uA until pressing rates fell below 5 responses/min during the last three min of two consecutive 5 min periods. The highest intensity that failed to maintain a response rate of 5 presses per min was considered to be the threshold value for that animal at that site.

Rate-intensity testing at each site lasted until threshold values did not change more than 4 uA across three consecutive days or until 10 consecutive sessions elapsed; then the electrode was lowered 250 um. The number of sites tested in each animal ranged from 2 to 11.

Histology.

At the conclusion of testing, all implanted animals were treated with the glyoxylic acid method of Battenberg and Bloom (1975) for the demonstration of catecholamines. Briefly, the animals were injected (I.P.) with an anesthetic dose of sodium pentobarbital and perfused transcardially with an ice-cold phosphate-buffered Ringer's solution containing 4% glyoxylic acid. The brains were removed, blocked and frozen on dry ice before being placed in a cryostat. Serial 20 um sections were cut in the coronal plane and thawed onto pre-chilled glass slides which were then immersed, for 0.5 to 1.5 min, in a 2% glyoxylic acid solution containing 7%

sucrose, dried in a stream of warm air and incubated in a covered glass container at 80-90 degrees C for 10 min. The sections were then examined in a Leitz fluorescence microscope, using epi-illumination from a 200 W mercury lamp with 355-425 nm excitation and 460 nm barrier filters. Photomocrographs were taken using Kodak Tri-X film and processed normally in Microdol-X developer. Before staining the slides with thionin for further examination, tracings of the brain slices showing the electrode's track were made from a projected enlargement of the sections.

The remaining 16 rats were used to determine the content of NE and DA after an attempt to deplete cortical NE. Eight of these rats were anesthetized with sodium pentobarbital (60 mg/kg) and then lesioned with 6-OHDA, dissolved in a cold Ringer's solution which contained ascorbic acid (0.2 ug/ul). The 6-OHDA solution (4 ug/ 0.5 ul) was prepared immediatelly before it was injected into each hemisphere through a 32 ga stainless steel injector; the infusion time was 2 min. The injections were delivered to the region of the dorsal noradrenergic bundle (A-P = 2.6, M-L = 1.1, D-V = 3.7 from the ear bars; bregma and lambda were positioned in the same horizontal plane, perpendicular to the injector shaft). After delivering the drug, the injector was left in place for an additional 3 min. The other eight rats were similarly operated on but only injected with the vehicle solution.

Twenty days after surgery these rats were decapitated and their brains quickly removed, rinsed in ice-cold Ringer's, and dissected. A coronal cut was made at the level of the pituitary stalk, and the cerebral cortex was separated from the rest of the brain in that portion posterior to the cut, and the subcortical tissue was discarded; the entire portion of the brain that was rostral to the cut was also kept for chemical analysis. After the dissection all samples were stored at -70 degrees C for 24 h and were then processed according to the fluorometric method of Shellenberger and Gordon (1971) using the extraction procedure described by Holman, Angwin, and Barchas (1976). These techniques allow for the quantification of NE and DA with a sensitivity of 5 ng/g of tissue.

After testing for ICSS, seven of the animals with moveable electrodes implanted in the mesocortical projection fields and five with electrodes in the region of the olfactory tubercle were also submitted to 6-OHDA lesions, as described above. After a survival period of 20 days, these rats' brains were processed for histological analysis with glyoxylic acid-induced histofluorescence and thionin staining.

The 6-OHDA injections into the region of the DNB produced an almost total disappearance (1.3% of controls) of NE in the cortical tissue that was dissected from the caudal half of the lesioned brains. This reduction in NE levels occurred in spite of the fact that in about 50% of the cases the injection sites were ventral to the DNB (in the region of the tectospinal tract- interstitial nucleus of Cajal). The reduction of cortical NE was very likely due to direct application of 6-OHDA to the DNB; and in the case of the ventrally located injection sites, to diffusion of the neurotoxin along the track left by the injector to the region of the NE bundle. In the anterior half of the lesioned brains, which contained all cortical and subcortical structures (with the exception of the olfactory bulbs), NE was reduced to only 12% of control samples. Residual levels of this amine were expected to be found, because the innervation of the ventral noradrenergic bundle to subcortical structures (part of the anterior-medial amygdaloid complex, ventral-medial septum and cingulum) was spared.

Reductions in DA, produced by 6-OHDA, to 75.5% and 74.2% of control values were found, respectively, in the same anterior and posterior samples that were assayed for NE content. This reduction could be accounted for by those cases in which the toxin was injected more ventrally than had

been intended. This reduction in DA content, however, would not be expected to significantly alter the ratings of DA density that were carried out (see below). In order to detect changes in fluorescence intensity, there must be a reduction of about 60% in the endogenous content of catecholamines (Jonsson, 1969).

In the sections to follow, where noradrenergic fluorescence would normally be mixed with dopaminergic fluorescence, dopamine density was inferred from animals that underwent 6-OHDA lesions.

Statistical analysis.

One of the objectives of this experimental series was to determine the characteristics of ICSS elicited by the stimulation of the different dopaminergic projection fields. In order to explore as much of these fields as possible, the stereotaxic coordinates were varied from one rat to the next and, in some cases, the number of sites that were stimulated varied as well. Thus, the use of inferential statistics to determine potential differences between scores of any two regions was considered to be inappropiate, since no two rats had equivalent stimulation sites.

From an anatomical point of view, each of the

stimulation sites was considered to be an independent sample, because, as the moveable electrode was lowered, different neural elements were stimulated. There is, however, the possibility of a carry-over (learning) effect from the stimulation of one site to the next, thus potentially invalidating the notion of independence among the different stimulation sites within each animal. This problem will be dealt with in the discussion section.

Working under the assumption of "independence of sites", Pearson correlation coefficients were computed between each of the A-P, M-L and D-V values that defined the location of the stimulation sites and each of the dependent variables that were studied. Correlations were also determined among all the dependent variables, which will be defined, again, here.

Threshold was taken as the highest stimulation intensity that failed to maintain a response rate of at least five presses per min. The second dependent variable was the highest pressing rate per min during any of the last three sessions of testing at each stimulation site. Also measured were the number of sessions that were needed to reach the criterion of behavioral stability and to start self-stimulating. Behavioral stability was defined as the number of sessions needed to maintain a threshold value that

did not change more than 4 uA during three consecutive sessions. If an animal did not reach this criterion within ten consecutive sessions, a score of 10 was assigned, and the electrode was lowered. The electrode was also lowered if a rat did not start self-stimulating in five consecutive sessions.

The fifth variable was the relative density of dopamine around each of the stimulation sites. A four-point subjective scale (Arbuthnott, Fuxe, and Ungerstedt, 1971) was used to define this relative density. A score of 0 reflects a lack of DA, and a score of 3 indicates the highest density of DA that can be visualized.

In order to avoid committing a Type I error, the data derived from the rats that did not self-stimulate were excluded from the correlations, because non-stimulators would contribute the most extreme values for some of the variables (pressing rates ranging from 0 to 4.9/min, highest "thresholds", etc.), thus artificially increasing the likelihood of obtaining high and significant r values.

For each brain region that was studied a total of 25 different correlations was computed, and a level of significance of 0.05 was used. In addition, the percentage

of sites that yielded self-stimulation within a particular brain region was computed.

Since there is not a universally accepted way of measuring how reinforcing the electrical stimulation of the brain is, a scoring system was used that permitted the comparison, among structures, of the "goodness" of self-stimulation. Each structure was rank-ordered, from best to worst, on each of the dependent variables that had been studied and on the percentage of positive sites, and its scores were added together. These operations were carried out on the assumption that lower final scores would reflect the location of the best self-stimulation regions. In some instances this procedure was followed using only two, three or four of the dependent variables.

RESULTS

Caudate-Putamen.

Two-hundred and eight stimulation sites were tested within the CPU, from which 135 (64.9%) proved to be positive for self-stimulation. Figures 1, 2, and 3 represent these and the rest of stimulation sites that were studied.

Thresholds were positively correlated with number of sessions required to start self-stimulating and, as expected, thresholds correlated negatively with pressing rates. There was also a negative correlation between pressing rates and number of days needed to reach the criterion of behavioral stability and with number of days needed to start self-stimulating. Finally, as the number of days to reach stability increased, so did the amount of training needed to begin lever pressing. Since all regions of the CPU had shown the same (the highest) score on relative density of dopamine, no correlations between this parameter and the rest of the variables were computed. Tables showing the correlation coefficients and their associated P values, computed for all brain regions that were studied, are found in Appendix III.

Fig 1. Schematic representation of stimulation sites. Small letters represent moveable electrode penetrations, and each dot and triangle show the stimulation sites. Numbers under each coronal section (modified from Pellegrino and Cushman (1967)) refer to the A-P plane. Self-stimulation threshold and highest pressing rate/min derived from each stimulation site are presented in Appendix II.

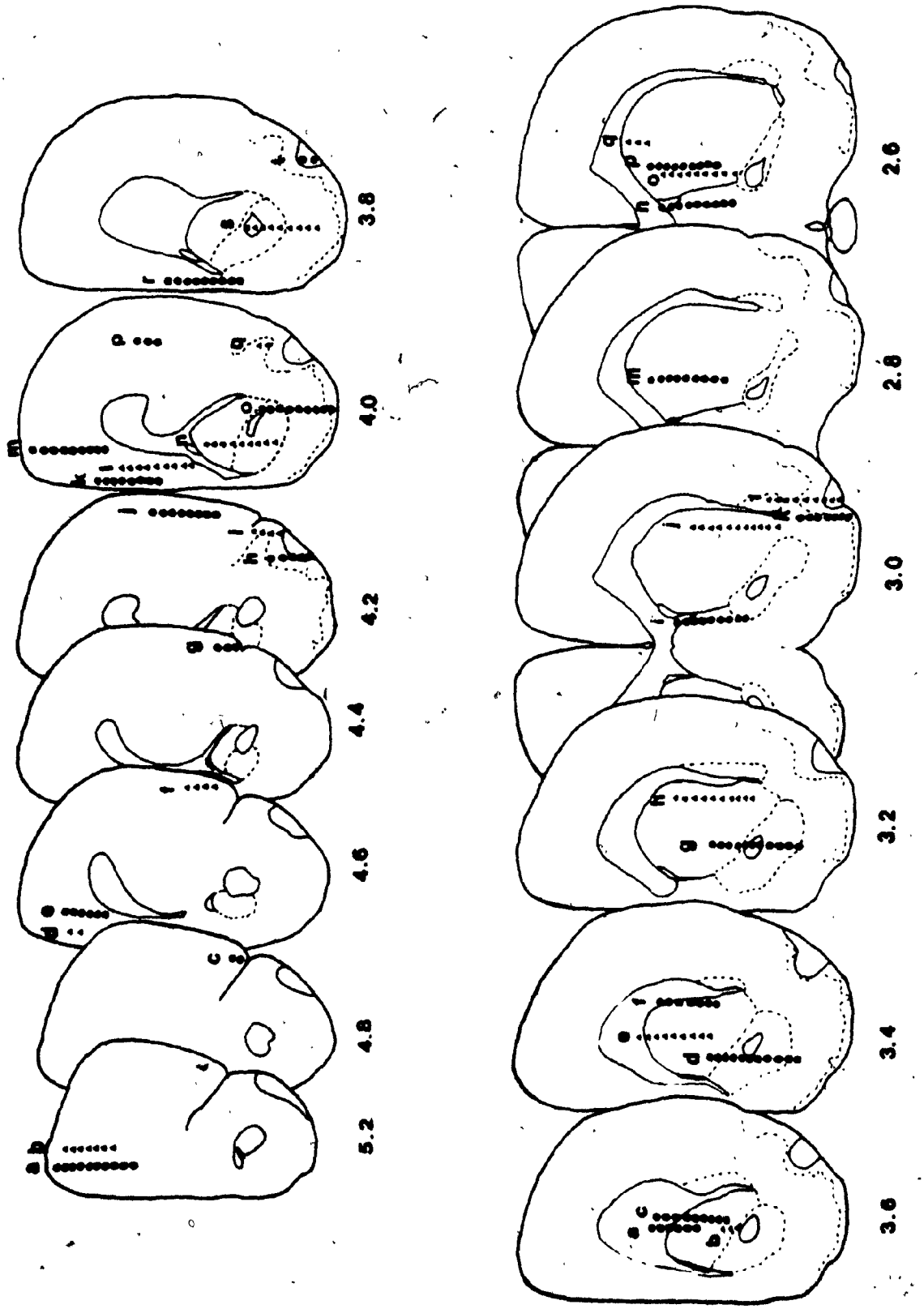

3.8
4.0
4.2
4.4
4.6
4.8
5.2
2.6
2.8
3.0
3.2
3.4
3.6

Fig 2. Schematic representation of stimulation sites. Small letters represent moveable electrode penetrations, and each dot and triangle show the stimulation sites. Numbers under each coronal section (modified from Pellegrino and Cushman (1967)) refer to the A-P plane. Self-stimulation threshold and highest pressing rate/min derived from each stimulation site are presented in Appendix II.

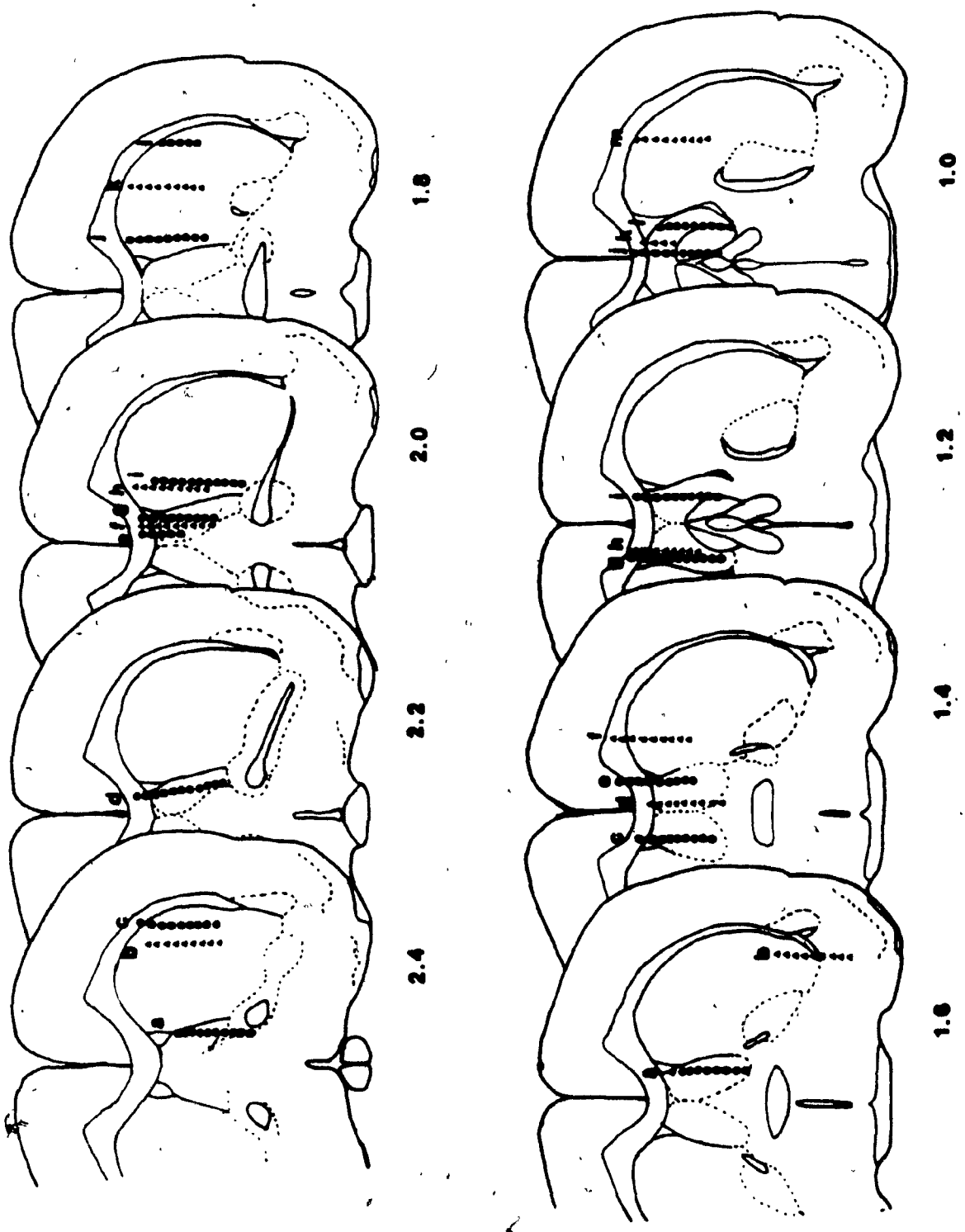
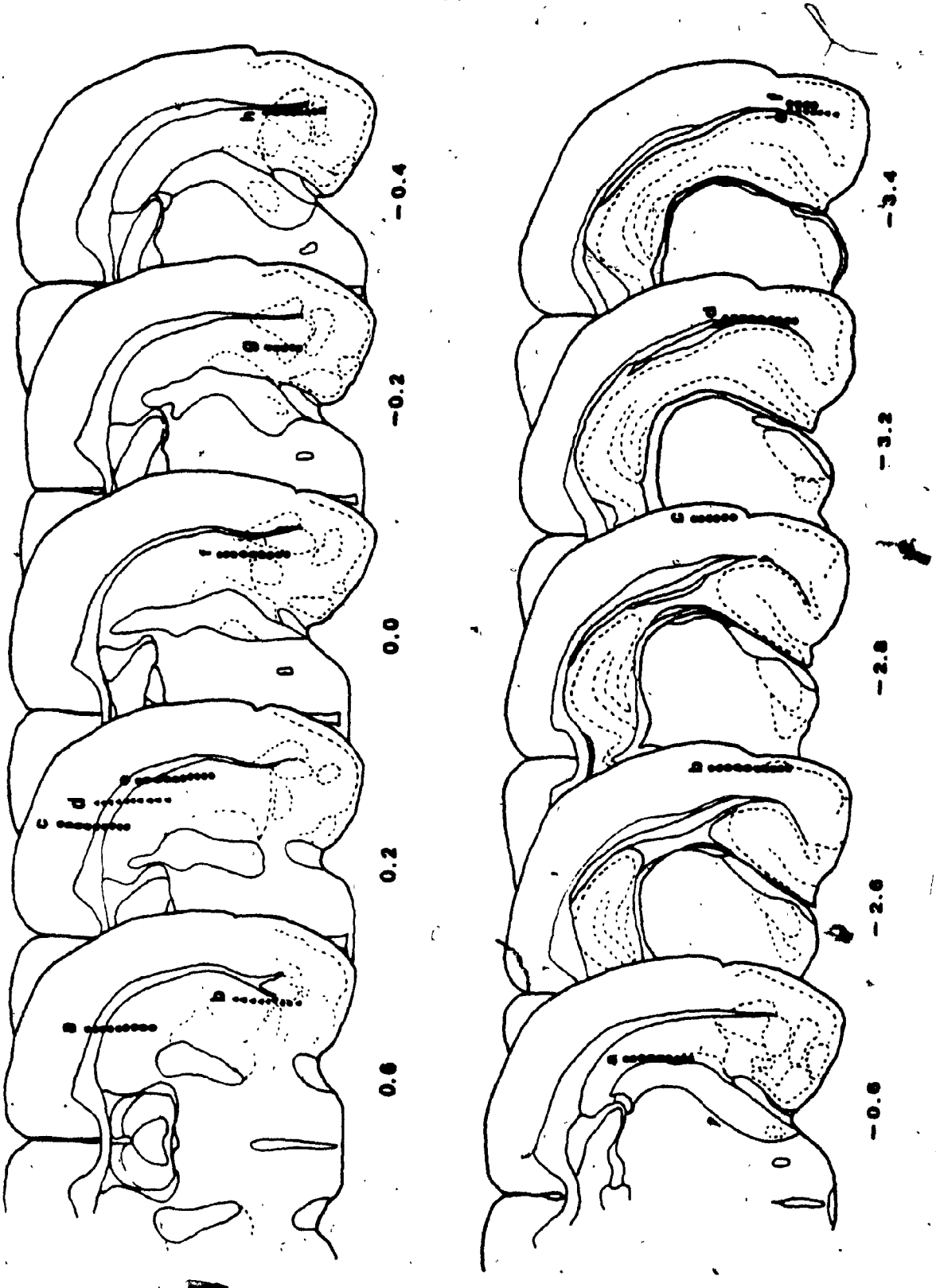

1.8
2.0
2.2
2.4
1.0
1.2
1.4
1.6

Fig 3. Schematic representation of stimulation sites. Small letters represent moveable electrode penetrations, and each dot and triangle show the stimulation sites. Numbers under each coronal section (modified from Pellegrino and Cushman (1967)) refer to the A-P plane. Self-stimulation threshold and highest pressing rate/min derived from each stimulation site are presented in Appendix II.


-0.4
-0.2
0.0
0.2
0.6
-3.4
-3.2
-2.8
-2.6
-0.6

When the positions of the stimulation sites were analysed, small but significant correlations were obtained which indicated that higher pressing rates, lower thresholds, fewer days to reach the criterion of stability, and fewer training sessions to start self-stimulating are associated with the most anterior, ventral, and medial placements. This finding, coupled with the results of experiments showing a topographical differentiation within the CPU with respect to other positively (Bermudez-Rattoni and Prado-Alcala, 1979; Divac, Rosvold, and Swarcbart, 1979) and negatively (Neill and Grossman, 1970; Prado-Alcala et al., 1980, 1981; Prado-Alcala, Maldonado, and Vazquez-Nin, 1979; Winocur, 1974) reinforced conditioned behaviors, led us to subdivide the CPU into several regions in order to perform a finer analysis of the data. These regions were defined as follows: antero-ventro-medial (AVM, n = 30 sites), antero-ventro-lateral (AVL, n = 26), antero-dorso-medial (ADM, n =39), antero-dorso-lateral (ADL, n = 26), postero-ventro-lateral (PVL, n = 25), postero-dorso-medial (PDM, n = 35), and postero-dorso-lateral (PDL, n = 26). Only one stimulation site was tested in the postero-ventro-medial (PVM) region, and no self-stimulation could be induced from this location.

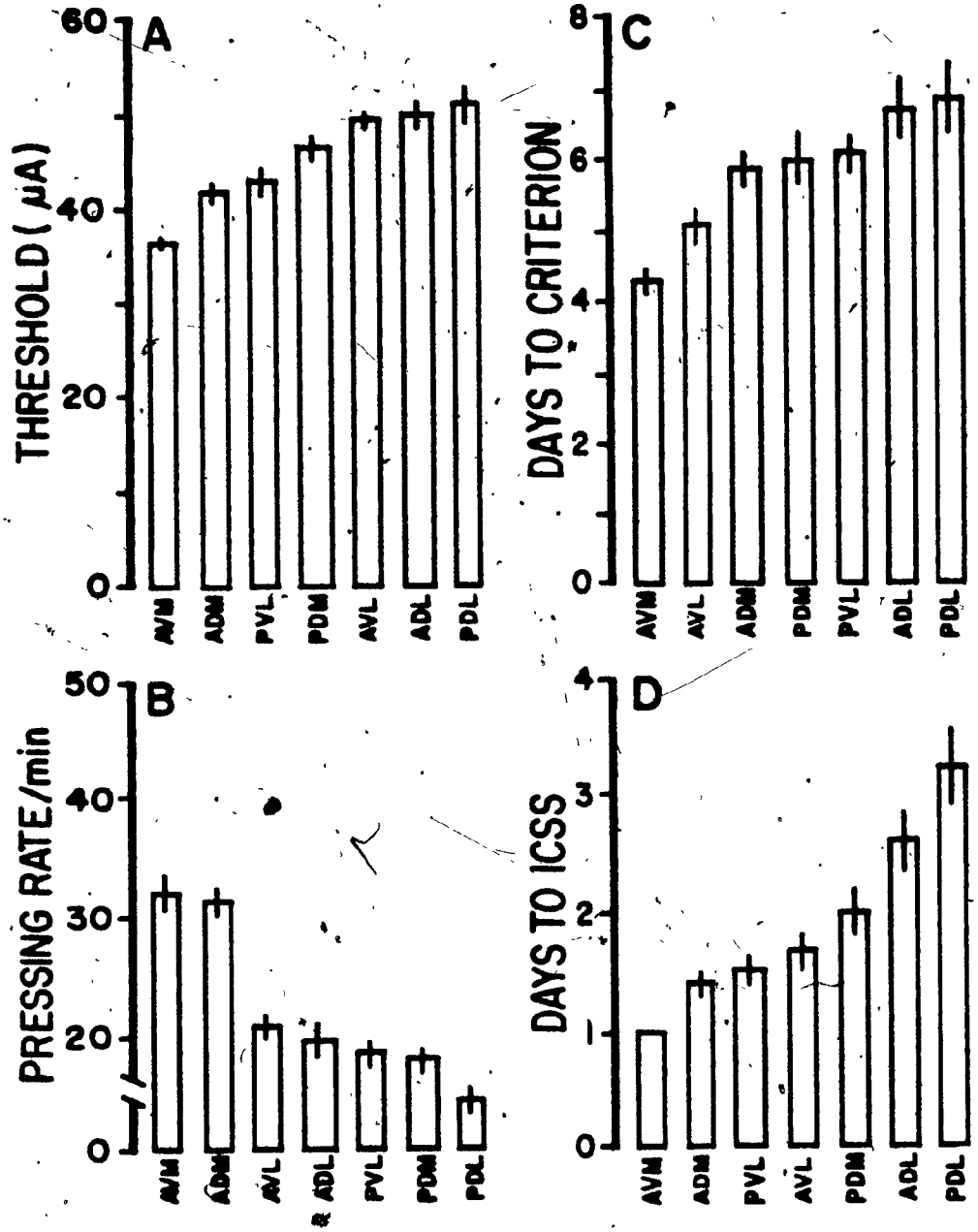
The boundary between the anterior and posterior regions

was defined as in previous work (Prado-Alcala, et al., 1979) and corresponds to the coronal plane at the A-P = 2.0 level of the Pellegrino and Cushman atlas (1967). At any given A-P level, the medial-lateral limit was taken as half the distance between the most medial and most lateral borders of the CPU; likewise, the dorsal-ventral boundary was defined as the horizontal line half way between the most dorsal and the most ventral borders of the CPU.

Except for the negative case in the PVM region, self-stimulation behavior was induced by electrical stimulation in all regions. The percentage of positive sites and the characteristics of ICSS varied, however, from one region to the next. The percentages of sites which yielded ICSS behavior were 86.7% in the AVM, 80.0% in the PVL, 76.9% in the AVL, 71.8% in the ADM, 46.2% in the ADL, 28.6% in the PDM, and 19.2% in the PDL.

As shown in Fig 4 A, the AVM sites had the lowest thresholds and the PDL region the highest, but the ordering of the rest of the regions did not follow the order described above of percentage of positive sites. It should be noted that within each region, threshold measures (as well as the rest of the measures to be described below) showed very little variance, as evidenced by their small standard errors of the mean.

Fig 4. Mean scores for self-stimulation threshold (A), highest pressing rate/min (B), number of sessions to reach behavioral stability (C), and number of sessions to initiate self-stimulating (D) are represented for each of the regions of the striatum that were mapped. The vertical lines in each column, in this and in subsequent Figures, represent the standard error of the mean. See Appendix I for abbreviations of regions of the striatum.


A
THRESHOLD (μA)
60
40
20
0
AVM
ADM
PVL
PDM
AVL
ADL
PDL
C
DAYS TO CRITERION
8
6
4
2
0
AVM
AVL
ADM
PDM
PVL
ADL
PDL
B
PRESSING RATE/min
50
40
30
20
0
AVM
ADM
AVL
ADL
PVL
PDM
PDL
D
DAYS TO ICSS
4
3
2
1
0
AVM
ADM
PVL
AVL
PDM
ADL
PDL

The animals with stimulation sites in the regions with the lowest thresholds displayed, as expected, the highest pressing rates (AVM and ADM), while those with electrodes in the higher-threshold zones showed lower rates (Fig 4 B). Again, the rats with electrodes within the PVL ranked last, and those with electrodes in the AVM ranked first with fewer number of days of training needed to start self-stimulating and with the lowest number of sessions to reach the criterion of behavioral stability (Fig 4 C and D).

There was a significant negative correlation in each of the regions between thresholds and pressing rates; different degrees of correlations between the rest of the variables were obtained for the various CPU regions, but there did not appear to be any consistent trend.

It is important to note that the difference between the lowest and the highest threshold values for a particular electrode penetration was as large as 114% (from 28 to 60 uA) in the ADL, 108% (24 to 50 uA) in the PVL, 75% (32 to 56 uA) in the PDM, 71.0% (28 to 48%) in the ADL, 56.0% (36 to 56 uA) in the AVM, 44.0% (36 to 52% uA) in the AVL, and 36% in the PDL (44 to 60 uA).

In summary, the correlation analyses between each of the stereotaxic coordinates defining the stimulation sites and each of the behavioral variables studied, pointed to the existence of a region within the CPU where the "best" self-stimulation could be found. This prediction was confirmed when the data had been analyzed in each of seven regions, since in every instance the AVM region produced the best measures of ICSS: lowest thresholds, highest pressing rates, fewer sessions needed to start self-stimulating, fewer sessions to achieve behavioral stability, and the highest percentage of positive sites. Exactly the opposite was true for the PDL region.

In order to compare the relative efficacy among all the regions for yielding ICSS, each of the regions was ranked on every behavioral measure, from best to worst, and its scores added. The resulting ordering was as follows: AVM, ADM, AVL and PVL, PDM, ADL, and PDL.

It should be remembered that several sites were tested in each animal; in most cases various extra-caudate structures and different dorso-ventral regions of the CPU were stimulated. This circumstance allowed for comparing ICSS characteristics between different brain regions in individual rats. Before making these comparisons, the characteristics that accompanied self-stimulation of the NAS

and of the region of the AMY will be analyzed; these two structures are in close anatomical relationship with the CPU and both were tested, in several animals, after stimulating the CPU.

Accumbens and Amygdala.

Self-stimulation was obtained from 29 (96.7%) of the 30 different sites that were tested in the NAS. The Pearson correlation coefficient indicated that, as in the case of the CPU, the higher pressing rates and the fewer training sessions needed to start self-stimulating were associated with the most medial placements. It also was found that depth of stimulation was not only significantly (positively) correlated with pressing rates but also with number of sessions to reach stability. Another significant correlation was the positive one between days to criterion of behavioral stability and days to begin self-stimulating; finally, a low positive correlation was found between pressing rates and thresholds.

No correlations between DA density and the other variables were computed since the ratings for DA density were the same for all NAS stimulation sites (score = 3).

Table II gives the characteristics of ICSS obtained from

TABLE II

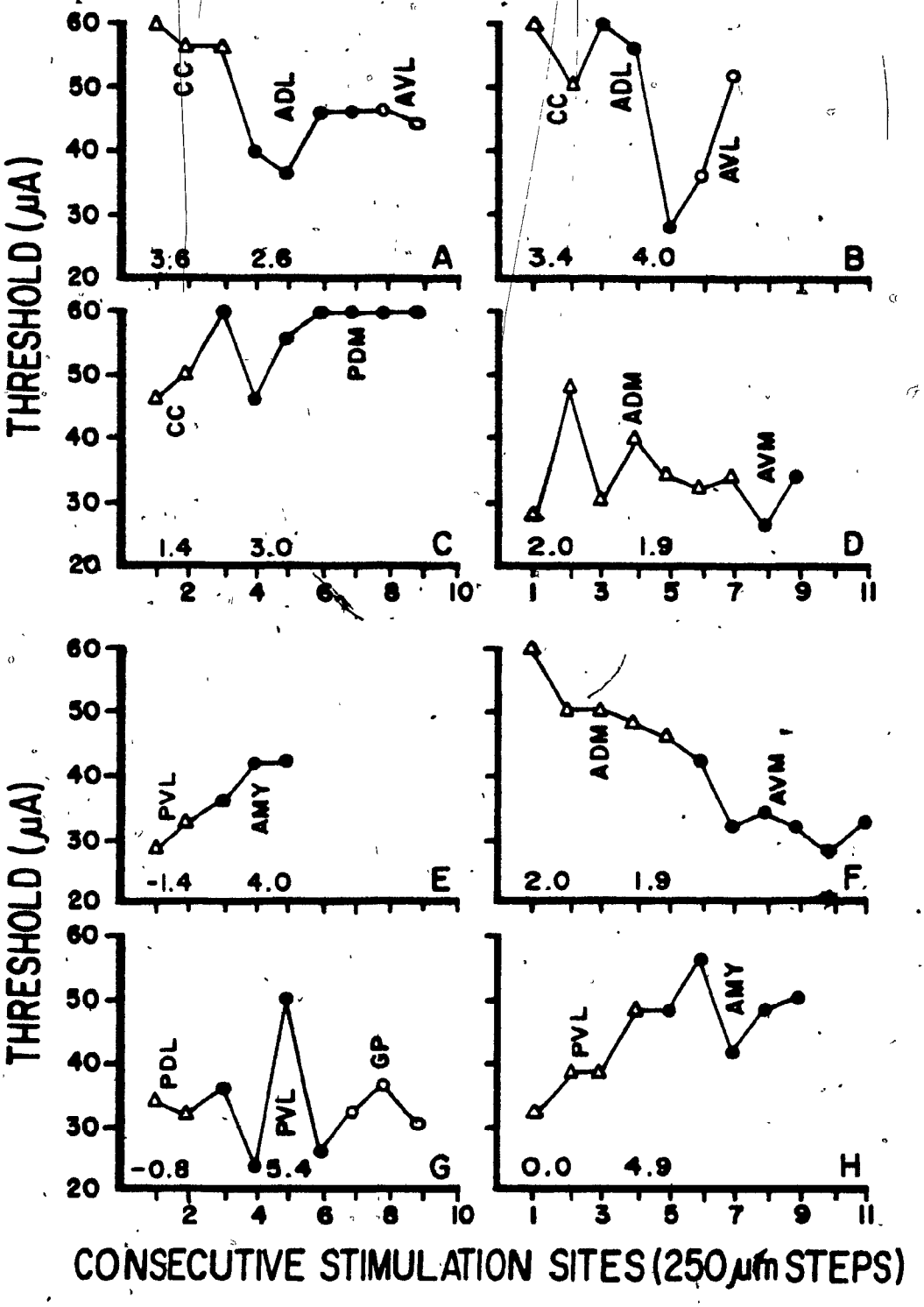
SITE	n	% S-S		TH (uA)	PR/min	DAYS CRIT	DAYS PR
AAA	5	100	X	40.0	18.7	5.6	1.0
			SEM	4.9	3.5	1.3	0.0
ACE & AL	7	100	X	43.1	23.6	4.6	1.0
			SEM	2.8	3.4	0.6	0.0
ABL	7	100	X	46.0	22.6	4.9	1.1
			SEM	1.7	2.1	1.0	0.1
AL	5	60	X	52.7	19.9	6.0	2.3
			SEM	3.7	5.0	2.1	1.3

ICSS characteristics of the amygdaloid nuclei. Abbreviations are as follows: n, number of sites; % S-S, percentage of sites that yielded self-stimulation behavior; TH, threshold; PR/min, highest pressing rate per minute; DAYS CRIT, number of days needed to reach the criterion of behavioral stability; DAYS PR, number of days needed to start self-stimulating; X mean score; SEM, standard error of the mean; AAA, anterior amygdaloid area; ACE, central amygdaloid nucleus; AL, lateral amygdaloid nucleus; ABL, lateral part of the basal amygdaloid nucleus.

the four regions of the AMY that had been stimulated--the anterior amygdaloid area (5 sites), the lateral amygdaloid nucleus (5 sites), the basal amygdaloid nucleus (7 sites) and seven cases where both the central and lateral amygdaloid nuclei were activated by the electrodes. In only two cases (8.3 %) was there no ICSS, and in both of these cases the stimulated tissue was within the lateral nucleus. When self-stimulation had been induced in three other animals with electrodes in this nucleus, low rates (X = 19.9/min) and the highest thresholds (X = 52.7 uA) were found. This latter observation fits well with the correlation analysis wich showed that the stimulation of the areas more laterally situated within the AMY had the higher thresholds. Also significant was the negative correlation between thresholds and pressing rates and the positive one between relative density of catecholamines and thresholds.

Working on the assumption that the combined measures of thresholds and pressing rates represent a reasonable index of how effective a self-stimulation site is and given that in all regions of the CPU and of the amygdaloid complex there was a significant negative correlation between these two measures, threshold scores were used to determine whether or not there had been changes as the electrodes were lowered through different brain regions in individual animals.

Fig 5. Self-stimulation current threshold data from consecutive stimulation sites of representative animals (A-H). Numbers above the abscissa refer, respectively, to the A-P and M-L stereotaxic coordinates used for the implantation of the electrodes (Pellegrino and Cushman, 1967). The change of symbols (open-filled-open) represents the stimulation of one region of the brain, followed by stimulation of regions situated more ventrally. Abbreviations are presented in Appendix I.


THRESHOLD (μA)
60
50
40
30
20
CC
ADL
AVL
3.6
2.6
A
CC
ADL
AVL
3.4
4.0
B
CC
PDM
1.4
3.0
C
ADM
AVM
2.0
1.9
D
2
4
6
8
10
1
3
5
7
9
11
PVL
AMY
-1.4
4.0
E
ADM
AVM
2.0
1.9
F
PDL
PVL
GP
-0.8
5.4
G
PVL
AMY
0.0
4.9
H
CONSECUTIVE STIMULATION SITES (250 μm STEPS)

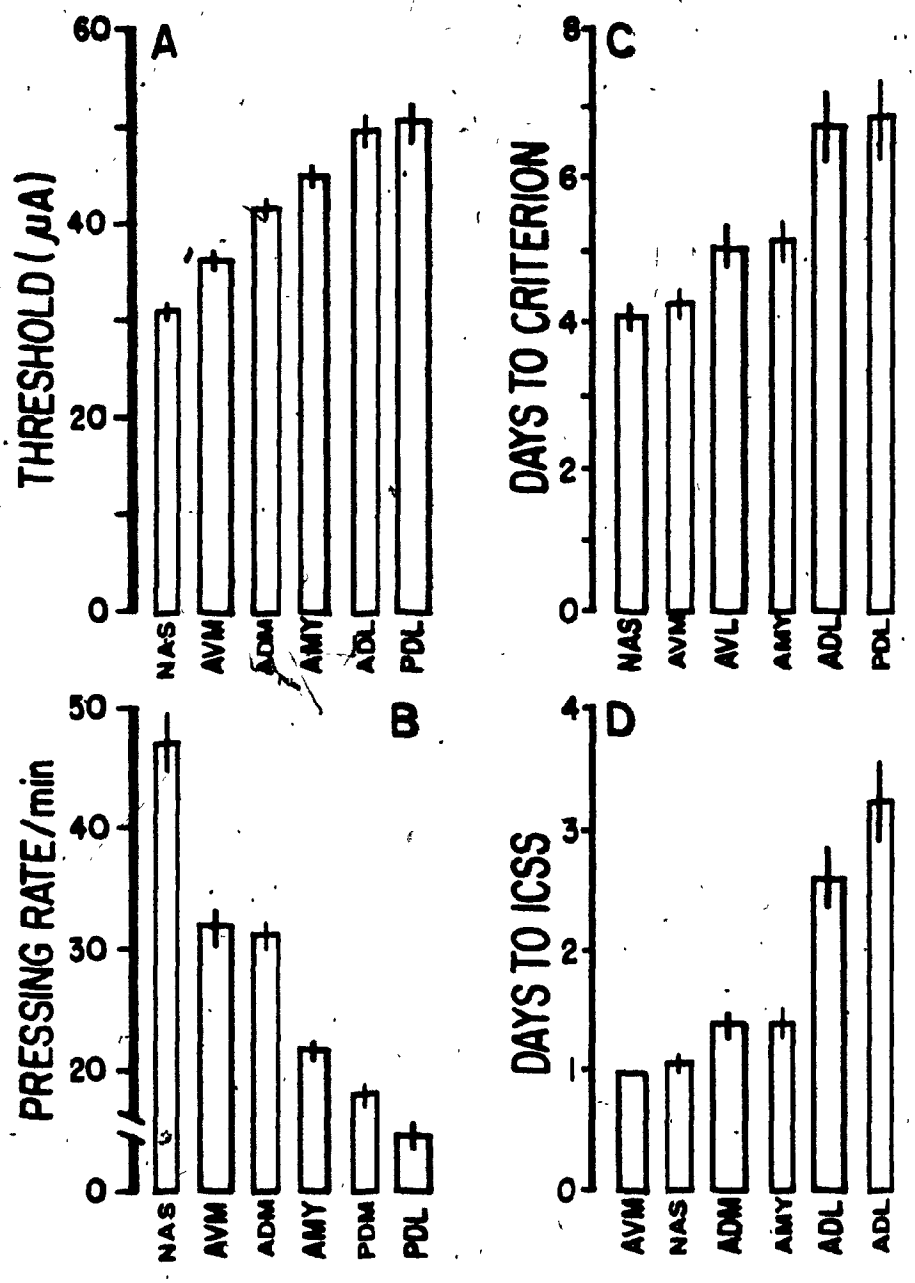
As seen in Fig 5, when moving an electrode from any region to a more ventral region (e.g., from the corpus callossum to the dorsal aspects of the CPU, from the ventral regions of the CPU to the NAS or the AMY), there was about the same probability of finding increments, decrements, or no change in thresholds.

The most obvious exception was seen when there had been a transfer from the ADM region to the AVM region: there was a decrement in threshold in four cases, an increment in one, and no change in another.

As in the case of the analyses of the different regions of the CPU in order to compare the relative effectiveness of the NAS, AMY, and the CPU for inducing ICSS, an analysis was made of all self-stimulation sites with respect to the dependent variables that had been studied. For each of these comparisons, the NAS and AMY averages were compared against each other and against the best two and the worst two regions of the CPU.

The NAS had the best scores for percentage of self-stimulation sites, pressing rates (Fig 6 B), number of days to criterion of stability (Fig 6 C), and for threshold scores (Fig 6 A), while the AVM was the best region when days to start self-stimulation were considered (Fig 6 D). The AMY

Fig 6. Mean scores for self-stimulation threshold (A), highest pressing rate/min (B), number of sessions to reach behavioral stability (C), and number of sessions to initiate self-stimulating (D) obtained from the nucleus accumbens (NAS) and the amygdaloid complex (AMY). Also shown are the scores of the best two and the worst two regions of the striatum. Other abbreviations are the same as in Fig 5.


A
THRESHOLD (μA)
60
40
20
0
NAS
AVM
ADM
AMY
ADL
PDL
C
DAYS TO CRITERION
8
6
4
2
0
NAS
AVM
AVL
AMY
ADL
PDL
B
PRESSING RATE/min
50
40
30
20
0
NAS
AVM
ADM
AMY
PDM
PDL
D
DAYS TO ICSS
4
3
2
1
0
AVM
NAS
ADM
AMY
ADL
ADL

ranked fourth in all variables, except for percentage of positive sites where it ranked in second place. After adding the ranks that had been assigned to each region, the NAS resulted with the lowest score (best self-stimulation), followed by the AVM and the AMY. Even when the measure of days to reach behavioral stability had been omitted from the analysis, the same ordering of regions was maintained. Finally, by only taking account of the thresholds and pressing rates, the NAS and the AVM had the same (best) scores, followed by the ADM and then by the AMY.

Septum.

The next dopaminergic projection field to be analyzed is the septal area. Represented in Figs 1 and 2 are the 127 sites that were stimulated; 114 of them produced self-stimulation behavior (89.8%). Taking all the positive sites into account, the following mean values were obtained: threshold, 38.7 uA; highest pressing rate, 14.8/min; number of sessions needed to achieve the criterion of behavioral stability, 5.5; number of sessions to start self-stimulating, 1.2.

There were several significant correlations in which the anterior-posterior dimension was involved. The further anterior the electrode, the higher the thresholds, the higher the number of days to start self-stimulating, and the lower

the density of CA's. This latter finding was expected since it is in the caudal aspect of the septal area that the dense band of DA terminals is found. The only other significant correlation involving the stereotaxic coordinates was a positive one in which the more dorsal placements produced lower thresholds.

A surprising finding was that in contrast with the CPU, AMY and NAS, no significant correlations were found among the rest of the variables, except for the one which indicated that as more sessions of training were needed to start self-stimulating a greater number of sessions to reach stability were also needed.

The findings that lower thresholds were associated with the more anterior and dorsal placements and that no significant correlations were found between the medial-lateral dimension and any of the other variables led to a finer analysis by dividing the septal area into four regions: anterior-dorsal (SAD, n = 30 sites), anterior-ventral (SAV, n = 21), posterior-dorsal (SPD, n = 46), and posterior-ventral (SPV, n = 30).

As in the case of the CPU, the limit between the anterior and posterior regions of the septum was determined by the coronal plane at the A-P = 2.0 level of the Pellegrino

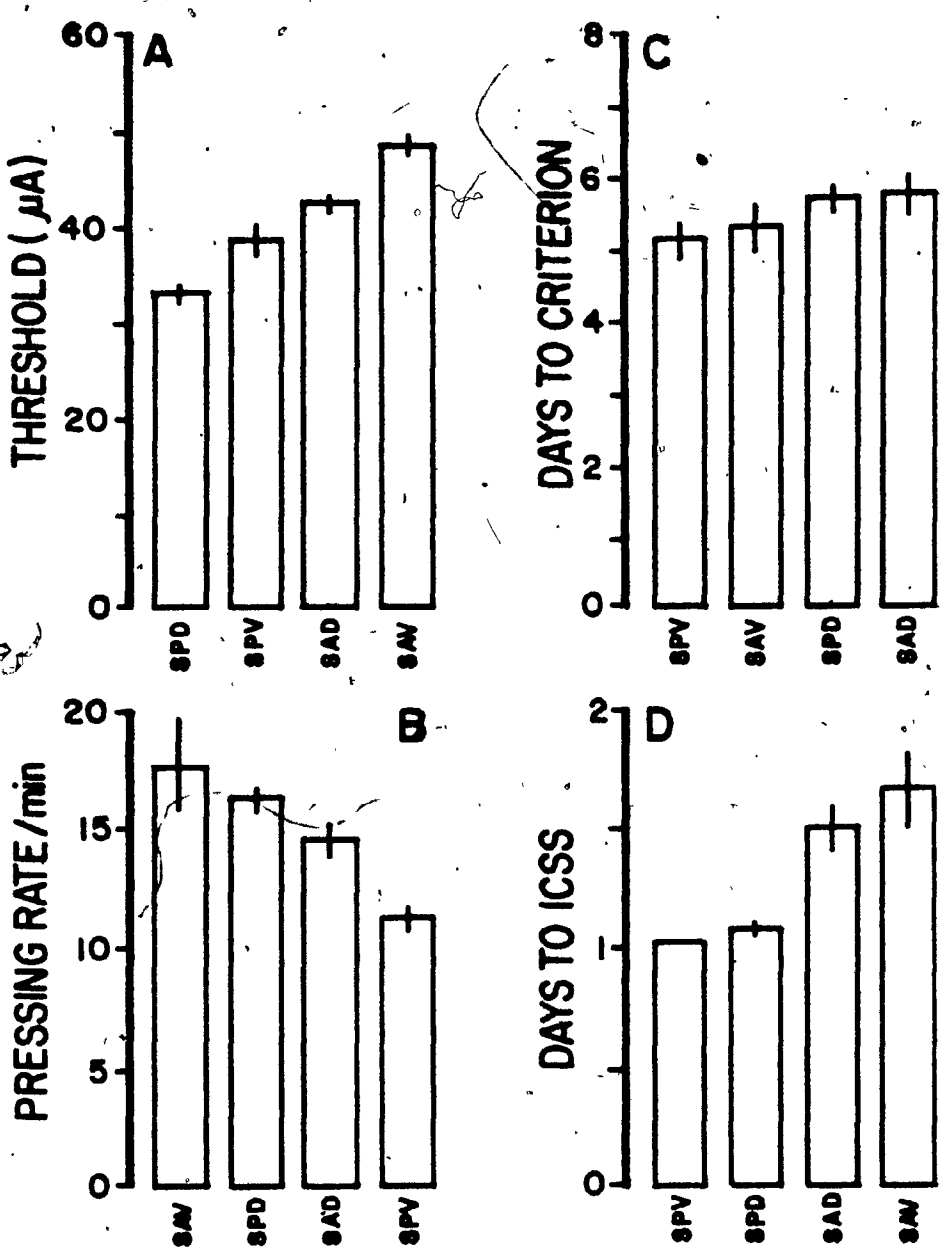
and Cushman atlas (1967), which corresponds to the middle of the A-P extent of the septal area. The dorsal-ventral boundary, at any given A-P level, was defined as half the distance between the most dorsal and most ventral borders of the septum.

ICSS was obtained from all regions with the highest percentage of positive sites in the SPD (95.7%), followed by the SPV (93.3%), the SAD (83.3%), and the SAV (71.4%). The best (lowest) threshold scores were also obtained from the SPD with the rest of the regions ordered (Fig 7 A) like the case of percentage of positive sites.

Another unexpected finding was that the region with the highest thresholds, the lowest percentage of positive sites, and the highest number of sessions to start self-stimulating (see below) produced the highest pressing rates, namely, the SAV region (Fig 7 B). Likewise, the region with the lowest rates of self-stimulation, the SPV, yielded the best measure of the number of days to start lever-pressing (Fig 7 D) and to reach the criterion of stability (Fig 7 C).

When each region was ranked on each of the five dependent variables, from best to worst, and their scores added, the following ordering emerged: SPD, SPV, and the SAD

Fig 7. Self-stimulation characteristics of the four septal regions that were mapped. SPD, postero-dorsal; SPV, postero-ventral; SAD, antero-dorsal; SAV, antero-ventral.


A
THRESHOLD (μA)
60
40
20
0
SPD
SPV
SAD
SAV
C
DAYS TO CRITERION
8
6
4
2
0
SPV
SAV
SPD
SAD
B
PRESSING RATE/min
20
15
10
5
0
SAV
SPD
SAD
SPV
D
DAYS TO ICSS
2
1
0
SPV
SPD
SAD
SAV

and SAV regions with the same (and worst) scores. The same analysis was carried out taking into account various combinations of two, three or four of the variables. In general, the same ordering was maintained, i.e., the posterior regions ranked better than the anterior regions except when a combined ranking was made for thresholds and pressing rates. In this case, the SPD continued to be the "best" ICSS region, followed by the SAV, and then by the SPV and the SAD which had equal scores.

Only five of the 100 correlations that had been computed for the four regions of the septal area were significant. These correlations involved the five dependent variables, each of the coordinates that defined the stimulation sites, and the relative density of catecholamines around those stimulation sites. In the SAV and the SAD regions, the number of sessions to behavioral stability correlated positively with number of sessions to start self-stimulating; thresholds correlated negatively with pressing rates in the SAV and positively with depth of stimulation in the SPD; in the SAV region there was a positive correlation between CA density and pressing rates. None of the correlations were significant in the posterior-ventral area.

The posterior regions provided an excellent opportunity for comparing, in individual animals, the characteristics for

ICSS between sites above, within, and below the conspicuous band of dopamine terminals found in those regions. In seven rats the three zones were stimulated, each with two sites within the DA band; in two additional animals the tissue above the band and one or two sites, respectively, within the band (but none below it) were stimulted . Two-tailed correlated t tests were performed, and no significant differences in thresholds or pressing rates were found as the electrodes had been lowered from one region to the next. Table III shows the raw data collected from each of the nine rats, and as can be seen, the probability of obtaining increments or decrements in thresholds or pressing rates was about 50%. Fig 8, a photograph of a glyoxylic acid-treated brain, shows an electrode track crossing the DA band.

Changes in threshold values found when lowering the electrode are shown in Figures 9 and 10. These changes can be summarized as follows: there was a decrease in threshold when the electrode was moved from the corpus callosum to the SAD region (5 cases) or to the SPD (6 cases); in one case the threshold remained unchanged. When moving from the SAD to the SAV, there was always an increase in threshold (4 cases), while increases (2 cases), decreases (3 cases) or no changes (3 cases) were detected when the SPV was stimulated after the SPD.

TABLE III

THRESHOLDS AND PRESSING RATES OBTAINED FROM SITES ABOVE, WITHIN, AND BELOW THE SEPTAL DOPAMINE BAND

	THRESHOLDS				PRESSING RATES			
S	ABOVE	WITHIN 1	WITHIN 2	BELOW	ABOVE	WITHIN 1	WITHIN 2	BELOW
6	36	30	26	32	22.3	23.3	18.3	16.7
7	52	60	60	60	14.3	9.0	4.7	5.0
2	38	28	18	28	14.3	14.3	11.7	10.0
29	52	44	26	24	12.3	10.0	12.3	13.7
30	30	44	44	48	14.7	14.0	12.0	9.0
31	46	50	50	52	14.3	33.0	14.0	9.3
47	18	22	22	32	13.7	14.0	10.7	14.3
46	24	36	40		14.3	11.3	16.0	
24	42	16			14.3	15.0		
X	37.6	36.7	35.8	39.4	14.9	16.0	12.5	11.1
SEM	4.0	4.7	5.3	5.2	0.9	2.4	1.3	1.4

No significant changes in thresholds or pressing rates were found when the moveable electrodes had been lowered through the septal tissue located above, within, or below the band of dopamine terminals. The width of the dopamine band allowed for stimulation of two sites (within 1 and within 2) in each of eight vertical penetrations and for only one site in an additional animal (rat No. 24). Abbreviations are as follows: S, subject; X, mean score; SEM, standard error of the mean.

Fig 8. Microphotograph showing a coronal section of the posterior region of the septal area, where a well defined diagonal band of dopaminergic terminals is found; also shown is the track left by a moveable electrode which stimulated the tissue above, within, and below the dopamine band.

Fig 9. Self-stimulation current threshold data from consecutive stimulation sites of representative animals (A-H). See legend of Fig 6 for datails.

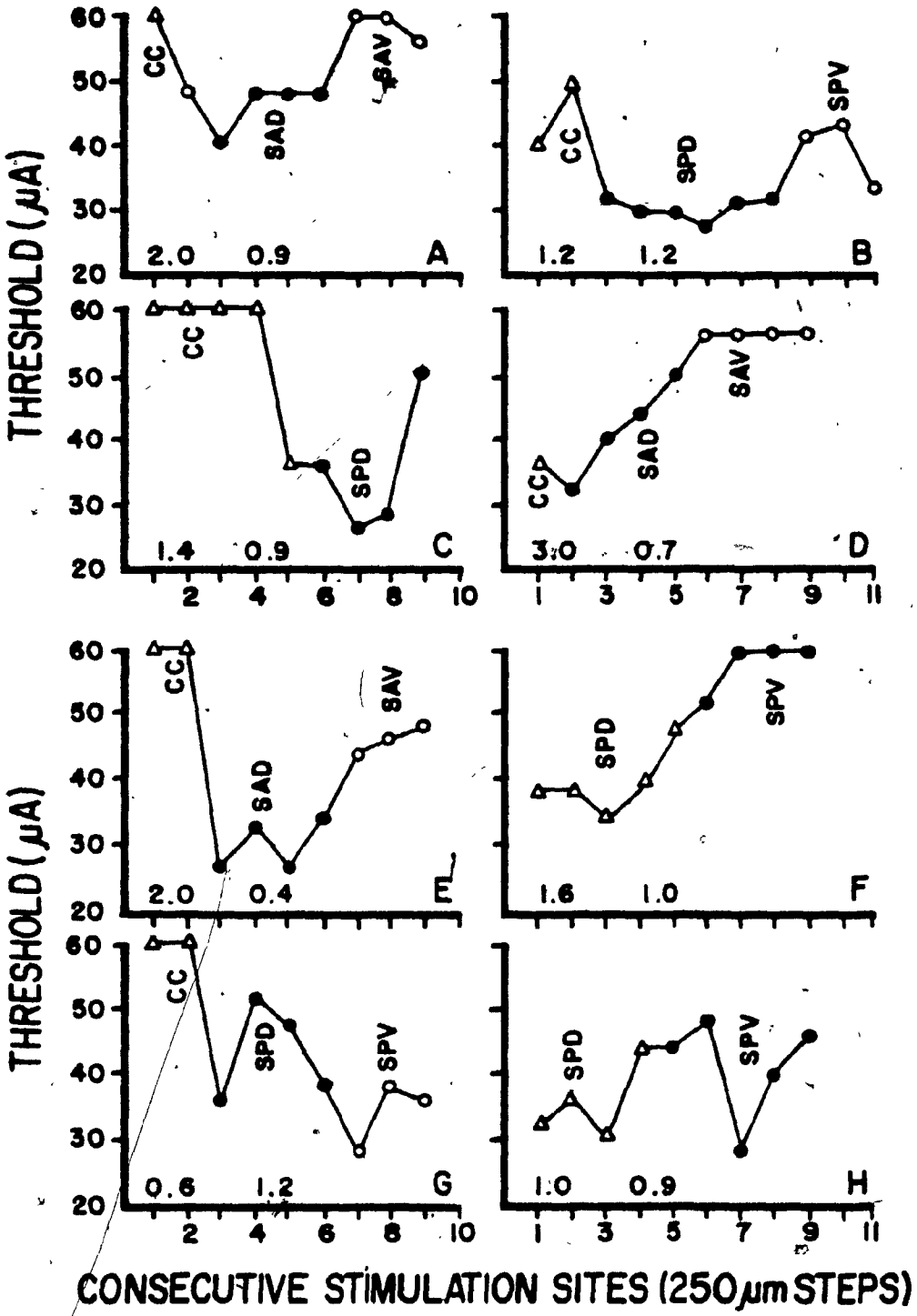
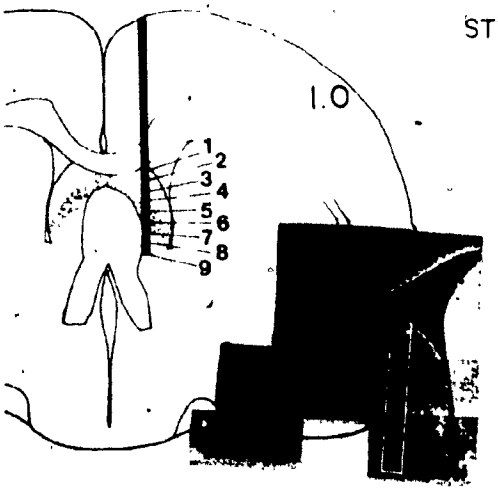
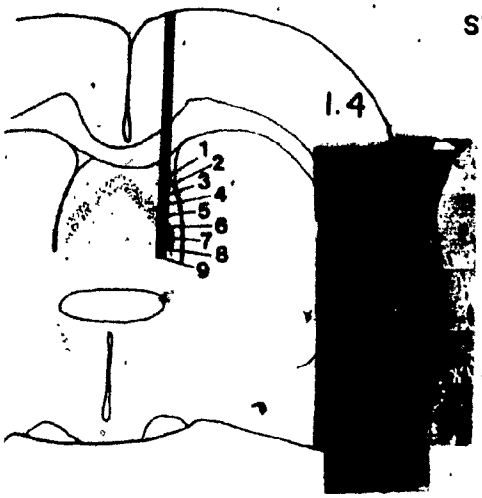

THRESHOLD (μA)
60
50
40
30
20
CC
SAD
SAV
2.0
0.9
A
CC
SPD
SPV
1.2
1.2
B
CC
SPD
1.4
0.9
C
CC
SAD
SAV
3.0
0.7
D
2
4
6
8
10
1
3
5
7
9
11
CC
SAD
SAV
2.0
0.4
E
SPD
SPV
1.6
1.0
F
CC
SPD
SPV
0.6
1.2
G
SPD
SPV
1.0
0.9
H
CONSECUTIVE STIMULATION SITES (250 μm STEPS)

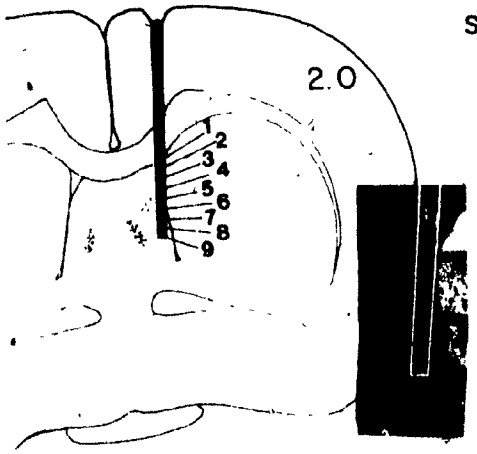
Fig 10. Schematic representation of stimulation sites in three animals implanted with moveable electrodes in the posterior regions of the septal area; insets show photomontages of corresponding glyoxylic acid-treated tissue. Numbers 1 to 9 represent the consecutive stimulation sites with their associated thresholds for self-stimulation and highest pressing rates/min.



STIMULATION SITE	THRESHOLD µA	HIGHEST PRESSING RATE/min
1	32	13.4
2	36	12.6
3	30	12.9
4	44	12.4
5	44	10.9
6	48	7.2
7	28	7.2
8	40	7.6
9	46	7.1



STIMULATION SITE	THRESHOLD µA	HIGHEST PRESSING RATE/min
1	24	12.3
2	50	11.7
3	50	11.8
4	52	11.3
5	44	8.1
6	26	11.6
7	24	12.3
8	30	10.6
9	NOT TESTED	



STIMULATION SITE	THRESHOLD µA	HIGHEST PRESSING RATE/min
1	NO ICSS	
2	48	12.8
3	40	11.8
4	48	9.1
5	48	9.3
6	48	7.4
7	60	4.2
8	60	4.8
9	56	6.0

Olfactory Tubercle and Pyriform Cortex.

Within the OT region and the pyriform cortex (PYR), 18 and 10 sites were explored, respectively (PYR will be analysed separately form the medial, sulcal and entorhinal cortices because the former is not considered to be part of the mesocortical terminal fields, as defined above). In the OT region significantly high negative correlations were found between thresholds and the anterior-posterior and dorso-ventral electrode placements; threshold values were also correlated, positively, with the medial-lateral dimension. In other words, lower thresholds were associated with the more anterior, ventral, and medial stimulation sites (as was the case with the CPU). The only other significant correlation was a negative one between thresholds and DA density. It is interesting to note, however, that no self-stimulation was seen in the four cases where the highest DA density was found. It should be kept in mind that, as explained in the Methods section, all correlations were run with consideration only to self-stimulating animals; thus, if the non-self-stimulators had been taken into account, the correlation between DA density and thresholds would become non-significant.

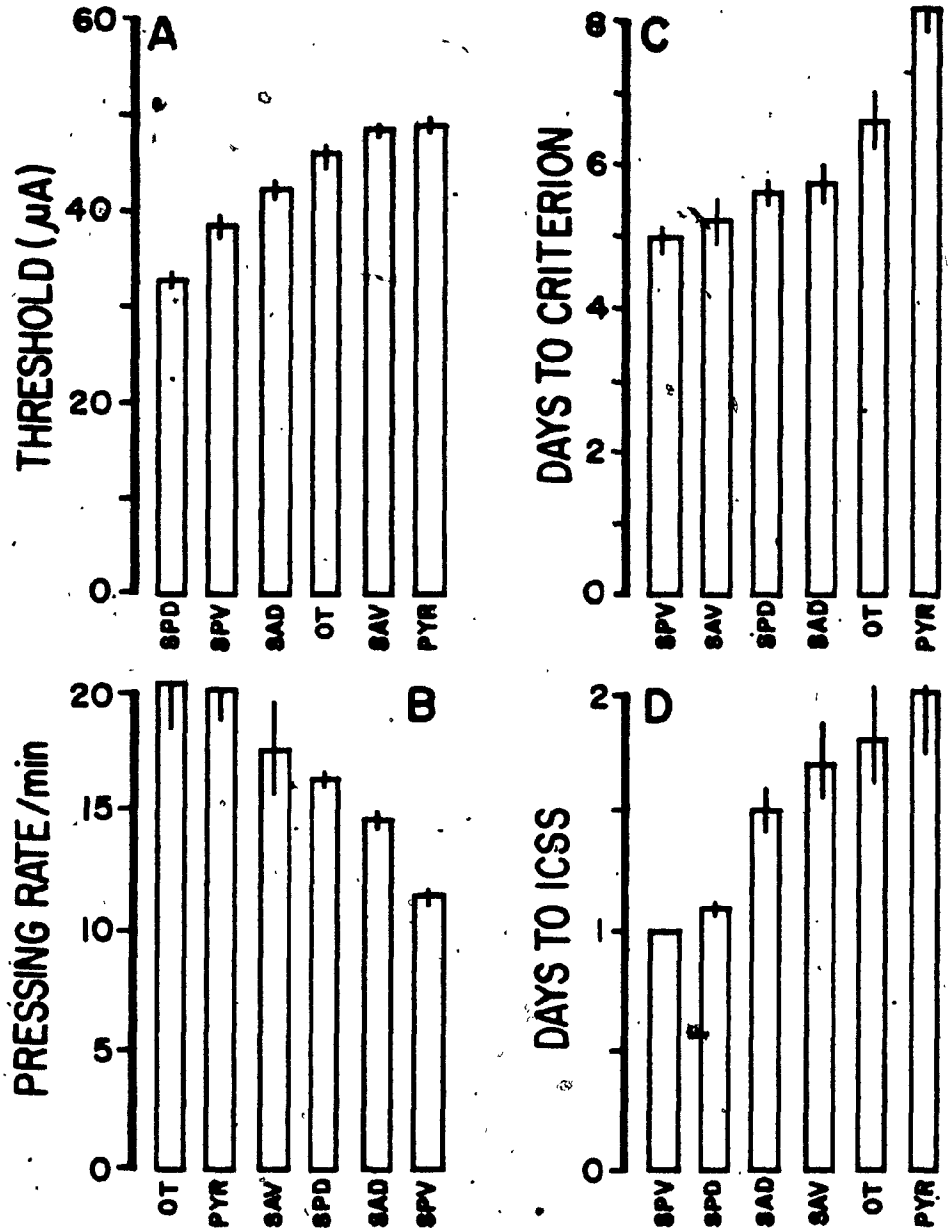
In the case of the PYR, only three of the correlations that were computed were significant. The A-P and the M-L

dimensions were positively correlated with the number of days needed to start self-stimulating, while thresholds were negatively correlated with pressing rates.

Except for the percentage of positive sites (OT = 66.7%; PYR = 100%), the OT region yielded better scores than the PYR for the rest of the dependent variables. Fig 1 shows a schematic representation of the stimulation sites in these two areas.

To determine the relative efficacy of the OT and the PYR for producing BSR, their respective mean scores on each of the dependent variables were rank-ordered, from best to worst, as were those of the four septal regions, and their overall sum of ranks was taken as an index of "goodness" for eliciting self-stimulation behavior. This resulted in the following order: SPV, SPD, OT, SAD and SAV, and PYR. When different combinations of only two, three or four of the variables had been considered, the posterior regions of the septal area (SPD and SPV) always ranked in the first two places and were followed by the OT, the anterior septal regions (SAD and SAV), and PYR (always ranking last). The only exception appeared when thresholds and pressing rates were combined: the SPD and the OT showed the best scores and the rest of the regions shared the same lowest score. Fig 11

Fig 11. Self-stimulation characteristics of the olfactory tubercle (OT), of the pyriform cortex (PYR), and of the four septal regions that were mapped.


A
THRESHOLD (μA)
60
40
20
0
SPD
SPV
SAD
OT
SAV
PYR
C
DAYS TO CRITERION
8
6
4
2
0
SPV
SAV
SPD
SAD
OT
PYR
B
PRESSING RATE/min
20
15
10
5
0
OT
PYR
SAV
SPD
SAD
SPV
D
DAYS TO ICSS
2
1
0
SPV
SPD
SAD
SAV
OT
PYR

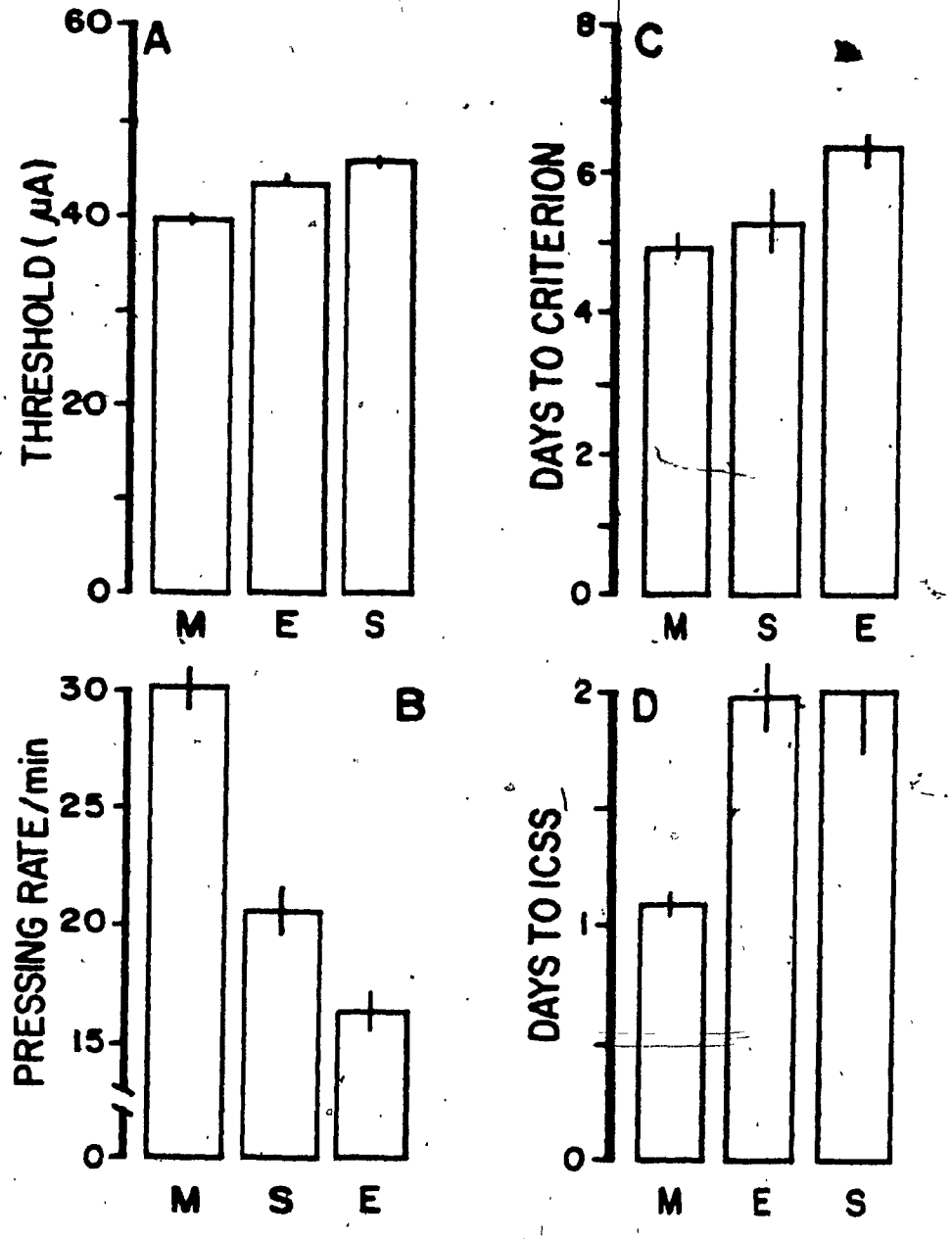
shows the mean scores on each of the dependent variables that were compared.

Cortex.

A total of 125 mesocortical projection sites was tested. A summary diagram showing these sites is presented in Figs 1 and 2. In the medial prefrontal cortex, 53 (76.8%) of the 69 sites were positive for self-stimulation, but no significant correlations were found in this region. In the sulcal cortex 60% (12 out of 20) sites yielded ICSS and, again, no significant correlations between any of the variables were found. Of the 36 entorhinal stimulation sites, which were located outside the field of dopamine islands, 29 (80.6%) were positive. The correlation analysis indicated that, in this region, a higher DA density had been associated with electrode placements in the more anterior, ventral and lateral regions that were explored. A positive correlation was found between number of sessions to behavioral stability and number of sessions to start self-stimulating, and a negative one between pressing rates and number of sessions to start self-stimulating.

When the self-stimulation characteristics of these cortical areas were compared against each other, the medial PFC always ranked first, with the best scores in all of the dependent variables; both sulcal and entorhinal cortices had

Fig 12. Self-stimulation characteristics of the mesocortical projection fields. M, medial prefrontal cortex; S, sulcal prefrontal cortex; E, entorhinal cortex.


A
THRESHOLD (μA)
60
40
20
0
M
E
S
B
PRESSING RATE/min
30
25
20
15
0
M
S
E
C
DAYS TO CRITERION
8
6
4
2
0
M
S
E
D
DAYS TO ICSS
2
1
0
M
E
S

an equal lower score, as can be seen in Fig 12.

Consistent with the results showing a lack of correlations between DA density and any of the dependent variables are the observations that the highest pressing rate (35.3/min) and lowest threshold (28.0 uA) in the medial PFC were obtained from sites that had been given scores on DA density of 2; in the entorhinal cortex, the highest response rate (18.3/min) and lowest threshold (30.0 uA) were associated with a relative density of DA of 1. More interesting was the finding that, in the sulcal cortex, the best rate (31.7/min) and threshold (26.0 uA) were obtained in a zone with no detectable levels of DA.

Seven sites were tested in the parietal cortex, outside the mesocortical projection fields (A-P = 0.2 to 0.6, M-L = 3.8 to 3.7?, D-V = 3.7 to 2.2). The score for DA density was 0 for each of these sites, and no self-stimulation could be elicited from any of them.

Non-Dopaminergic Fiber Tracts.

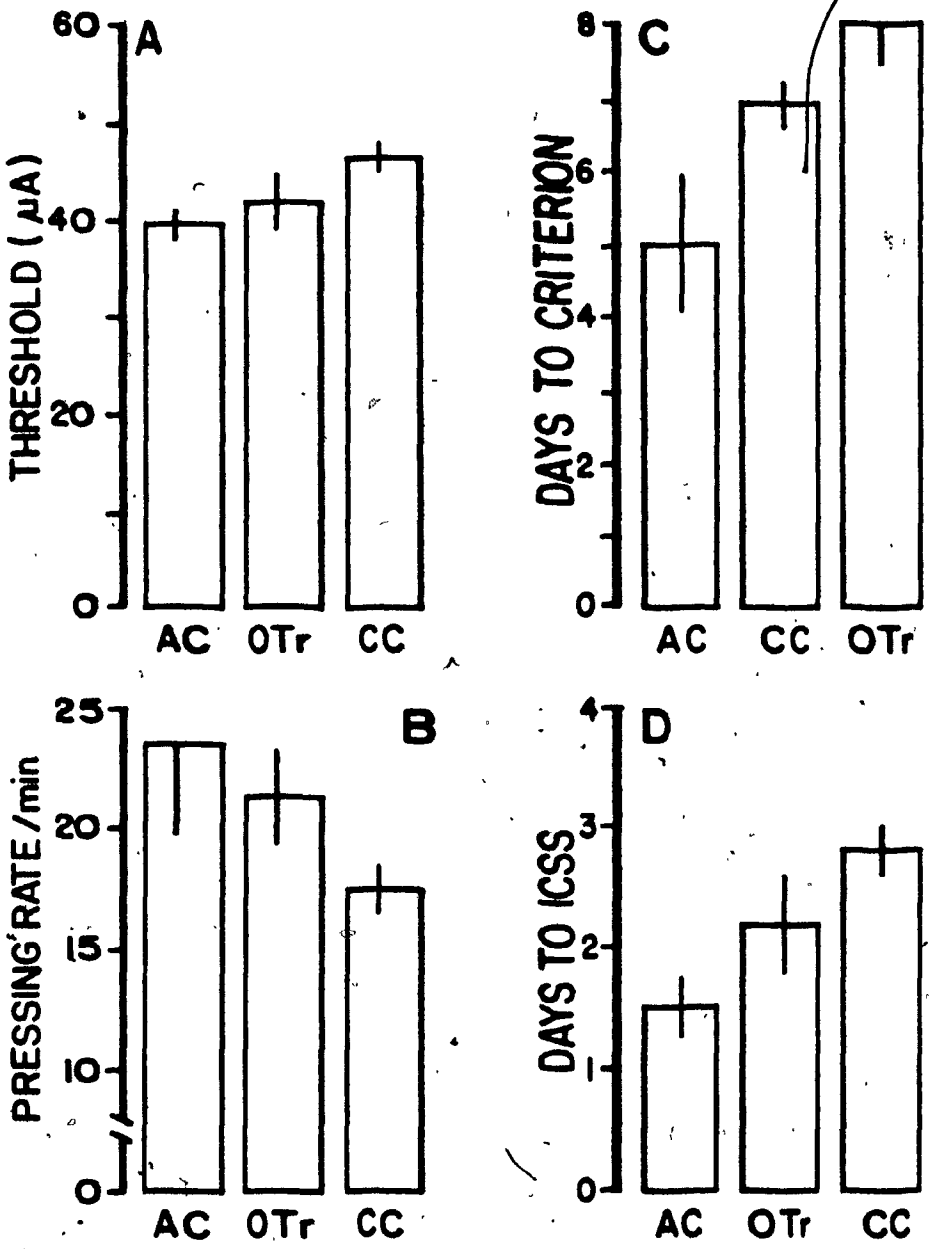
Forty-four stimulation sites were located within the corpus callosum (CC); 50% were positive for BSR. Eight sites were within the olfactory tract (OTr), with five of them yielding self-stimulation (62.5%). Only four placements were

within the anterior commissure (AC); all produced ICSS. Schematic representations of these sites are shown in Figs 1, 2, and 3. The AC yielded the best values for self-stimulation--lowest thresholds, highest pressing rates, fewer sessions to start self-stimulating and to achieve behavioral stability. The OTr ranked second in all of these variables, except for the number of sessions to achieve stability where the CC ranked second (Fig 13).

A few significant correlations were found in these regions. The number of sessions to start lever pressing correlated positively with the number of days to achieve behavioral stability in both the AC and the CC and negatively with pressing rates in the latter structure. In the OTr thresholds correlated positively with sessions to start self-stimulating.

The t test for correlated samples was used to determine whether or not there had been significant differences in thresholds and pressing rates between CC positive sites and positive sites in the tissue beneath it (within 250 um of the CC sites). In nine rats, the dorsal aspect of the septal area was tested after testing the callosum, and in 6 animals testing of the callosum was followed by stimulation of the dorsal CPU. When considering all 15 pairs of threshold values, no significant differences became evident (mean

Fig 13. Self-stimulation characteristics of the non-dopaminergic fiber tracts that were studied. AC, anterior commissure, OTr, olfactory tract; CC, corpus callosum.


A
THRESHOLD (μA)
60
40
20
0
AC
OTr
CC
C
DAYS TO CRITERION
8
6
4
2
0
AC
CC
OTr
B
PRESSING RATE/min
25
20
15
10
0
AC
OTr
CC
D
DAYS TO ICSS
4
3
2
1
0
AC
OTr
CC

values for the CC and the dorsal aspects of the septum-striatum were, respectively, 45.5 and 42.3 uA; $t = 1.50$, d.f. = 14, $P > 0.10$). By the same token, no significant differences appeared in pressing rates (CC = 13.9/min, septum-striatum = 15.7/min; $t = 0.70$, d.f. = 14, $P > 0.20$).

A finer analysis was made by comparing, separately, the CC against the dorsal CPU and against the dorsal regions of the septum. The same picture emerged when the mean threshold value for the six CC sites (48.7 uA) above the CPU was compared against the corresponding six striatal sites (49.0 uA; $t = 0.08$, d.f. = 5, $P > 0.10$) and also when the mean pressing rates were compared (CC = 15.4/min, CPU = 19.3/min; $t = 0.61$, d.f. = 5, $P > 0.20$). In agreement with the above findings, mean pressing rates of the dorsal regions of the septum (13.3/min) did not differ significantly from the rates of the nine CC sites immediately above it (12.9/min; $t = 0.70$, d.f. = 8, $P > 0.05$). There was, however, a significant difference in threshold values between these two regions of the brain (CC = 43.3 uA, dorsal septum = 37.8 uA; $t = 2.57$, d.f. = 8, $P < 0.05$).

Two of the five positive sites in the OTr were the only two sites tested in a particular rat; in a second rat there

were three succesive OTr points tested after testing the tissue immediately above it. There was a 41.6% drop in threshold upon entering the OTr (from 48 to 28 uA), as well as a small increment in pressing rates (from 71 to 88). Of the four AC sites, two were tested in each of two rats. When lowering the electrode from the AC into the NAS, there was a small decrement in threshold in one rat (from 38 to 34 uA) and no change in the other. In one of the animals, it was possible to compare threshold values as the electrode was moved from the NAS into the AC, and an increment was found (from 32 to 44 uA). In all cases where there had been a change in threshold, the opposite change was found for pressing rates. Percentage of positive sites and mean values for each of the dependent variables that were measured are presented in Table IV . The ranking of the 21 brain regions refers to the relative efficiency in eliciting ICSS, as described in the Methods section.

TABLE IV

SUMMARY OF THE DATA

Abbreviations are as follows: N, number of sites; DAD, density of dopamine; % percentage of positive sites; TH, threshold; PR, highest pressing rate/min; DC, number of sessions to achieve behavioral stability; DPR, number of sessions to start self-stimulating; *, dopamine density in 6-OHDA lesioned rats. Abbreviations for brain structures are given in Appendix I.

TABLE IV

SUMMARY OF THE DATA

SITE	RANK	N	DAD	%	TH	PR	DC	DPR
NAS	1	30	3.00	96.7	36.1	47.0	4.0	1.06
AVM	2	30	3.00	86.7	36.4	32.0	4.3	1.00
AC	3	4	0.00	100.0	39.5	23.4	5.0	1.50
MCtx	4	69	2.00*	76.8	39.6	30.0	4.9	1.09
SPV	5	30	2.04	93.3	38.4	11.3	5.0	1.00
SPD	6	46	1.34	95.7	32.9	16.2	5.6	1.10
AMY	7	24	1.96	91.7	44.6	21.7	5.1	1.95
ADM	8	39	3.00	71.2	41.9	29.3	5.8	1.38
SAD	9	30	1.20	83.3	42.5	14.5	5.7	1.50
AVL	10	26	3.00	76.9	49.6	20.7	5.1	1.68
PVL	11	25	3.00	80.0	42.9	18.5	6.1	1.52
SAV	12	21	1.12	71.4	48.7	17.6	5.2	1.70
SCtx	13	20	0.83*	60.0	45.7	20.5	5.3	2.00
ECtx	14	36	1.39*	80.6	43.5	16.4	6.3	1.97
PYR	15	18	1.00*	100.0	49.2	20.0	8.2	2.00
OT	16	10	1.57*	66.7	45.8	20.4	6.6	1.80
OTr	17	8	0.00	62.5	42.0	21.2	8.0	2.20
PDM	18	35	3.00	28.6	46.8	17.8	6.0	2.00
ADL	19	26	3.00	46.2	49.7	19.7	6.7	2.60
CC	20	44	0.00	50.0	46.4	17.3	7.00	2.80
PDL	21	26	3.00	19.2	51.1	14.3	6.9	3.22

DISCUSSION

In agreement with earlier experiments, the present study has shown that self-stimulation can be obtained from each of the major dopaminergic projection fields. However, self-stimulation did not bear a special anatomical relation to the location or density of dopamine terminals. First, the boundaries of the terminal fluorescence did not correspond to the boundaries of BSR; self-stimulation was seen regardless of whether the stimulating electrodes were placed within or outside DA terminal fields. This was seen, for example, when the electrode stimulated the corpus callosum first and then the CPU or the septum and when the accumbens was tested before or after testing the anterior commissure. This finding rules out the possibility that BSR in these regions is generally mediated by direct activation of DA terminals or their efferents. Second, except for one case, neither positive nor negative correlations between DA density and any of the parameters that defined the characteristics of self-stimulation were found in any of the regions that had been mapped. Hence, it seems that BRS derived from stimulation of the dopaminergic terminal fields does not involve the direct activation of DA terminals or their

efferents. The possibility that DA terminals and DA efferents are involved in a subsequent step in the reward process remains an open question.

Methodological Considerations

The present study involved the use of a combination of training techniques in an attempt to elicit self-stimulation behavior. As in the case of Routtenberg's (1971) study, training of the rats involved daily 15 min periods of exposure to the experimental box with no response shaping and with delivery of electrical stimulation of fixed intensity every time a lever press response had been made. In some cases this sufficed to produce self-stimulation. When no spontaneous self-stimulation was seen after the 15 min period, the rats were then shaped by the experimenter to approach and press the lever, using an intensity that seemed rewarding, as described by Phillips et al. (1976b); in the present study shaping with a given intensity only lasted for about 15 min rather than 30 min. During shaping, not only the pre-selected current that seemed rewarding was used; other intensity levels were tested as well, as in the case of the Prado-Alcala et al. (1975) study.

This combination of training techniques proved to be

more effective than any of the individual techniques alone. Thus, even when only a maximum of five sessions of training were given at each stimulation site, as opposed to a maximum of 14 or to a total of 28 sessions, respectively, by Phillips et al. (1976b) or by Routtenberg (1971), self-stimulation could be observed in 64.9% of the striatal sites that were tested. In one of those experiments, where self-stimulation was obtained from all regions of the CPU, only 25% of the animals acquired this behavior within the first five sessions of testing (Phillips et al., 1976b, Table I); in the case of Routtenberg's study where 12 stimulation sites in the medial caudate were tested, only 66.7% were positive for self-stimulation (Routtenberg, 1971, Figs 1 and 2), as opposed to 88.5% found in the equivalent region in the present study (ADM + AVM). Finally, in the experiment reported by Prado-Alcala et al. (1975), none of the 37 placements, scattered throughout all of the CPU, produced self-stimulation.

Since in the present mapping study several brain sites were tested in every animal, a general comment about the use of the moveable electrode is warranted. One of the advantages of using the moveable electrode (Wise, 1976) is that multiple brain sites can be tested in the same animal, thus allowing for intra-subject comparisons. There is, however, a potential disadvantage when using this technique

in studies of the kind reported here where comparisons among different sites within a vertical penetration are made. This problem stems from the possibility of a carry-over effect from the stimulation of one site to the next. This effect could produce a biased tendency in responding after the rats have been tested for several days or weeks at more dorsal sites.

Thus, increased response rates obtained from a ventral site could simply reflect an improvement in performance after this response was learned during trials where a more dorsal site had been tested; similarly, if a decrement in threshold occurs after lowering the electrode, it could be due to the development of some sort of sensitization of the newly stimulated tissue resulting from stimulation above it.

To determine the relative importance of multiple testing with a single electrode per se, correlation analyses were run between the depth of stimulated sites and each of the dependent variables for each of the regions that were studied (CPU, septum, NAS, AMY, entorhinal and pyriform cortices, sulcal and medial PFC, CC, AC, and OTr). Out of the 60 correlation coefficients obtained, only 13 were statistically reliable. Depth correlated negatively with thresholds in the CPU, AMY, OT, and medial PFC and positively in the septal

area and OTr. Depth of stimulated tissue also correlated negatively with pressing rates in the AMY and positively in the medial PFC; days to criterion and days to start lever pressing correlated positively with depth in the AMY and OTr, respectively, and negatively in the CPU.

Given these results, it seems reasonable to conclude that whatever changes in values of the dependent variables were found as the electrodes had been lowered, they were due to differences in the normal functional properties of the tissue that had been stimulated and not to any simple form of carry-over effect. If the latter had been the case, consistent (negative or positive) correlations should have been found in all or most of the regions between the depth of the stimulating electrode and all (or most) of the dependent variables. Inspection of Figs 5 and 9, where changes in threshold as a function of depth of stimulation are depicted, will help clarify this point. As can be seen, there were instances where definite decrements (Fig 5, F) or increments (Figs 5, E and H; 9, D and F) in threshold had been found, but in most cases both types of change were apparent in individual rats (Figs 5, A-D; 9, A-C, G and H).

Regional Findings

Nigro-Neostriatal System

In agreement with the report of Phillips et al. (1976b), the present study revealed that good self-stimulation can be obtained with electrode sites throughout the caudate. The ventro-medial quadrant of the anterior aspect of the neostriatum yields the highest pressing rates. This region also yielded the highest percentage of positive sites, lowest thresholds, and fewest sessions to start self-stimulating and to achieve behavioral stability. The worst scores on each of these variables were obtained from the postero-dorso-lateral region of the CPU.

As pointed out in the Introduction, several experiments in the literature are in conflict on the question of whether electrical stimulation of the caudate nucleus produces positive reinforcement (O'Donohue and Hagamen, 1967; Phillips et al., 1976b; Routtenberg, 1971), aversive consequences (Olds and Olds, 1963; Wurtz and Olds, 1963), increased operant responding due to a generalized motor activation (Justesen et al., 1963), or no rewarding consequences at all (Prado-Alcala et al., 1975). This wide range of interpretations represents a good example of how different methodologies can produce confusing results. The results of the present experiment are offered as a definitive answer to the question: almost all regions of the caudate nucleus are capable of supporting self-stimulation.

It was not until recently that estimates were made of conduction velocities and refractory periods of the reward-relevant fibers that are directly activated by a self-stimulation electrode. These estimates strongly suggest that the reward-relevant fibers of the MFB are myelinated and have response characteristics incompatible with those of the non-myelinated, slow-conducting, catecholamine axons (Gallistel et al., 1981; Shizgal et al., 1980; Yeomans, 1979). It is assumed that MFB stimulation fails to activate DA fibers directly because they have a high threshold for direct excitation (Shizgal et al., 1980).

The inferences that follow are based on the assumption that, even in the dopaminergic projection fields, electrical stimulation in regions of high dopamine terminal density is likely to directly activate the dopaminergic post-synaptic efferents, as well as non-dopaminergic afferents and efferents, but not the dopaminergic terminals themselves (since self-stimulation thresholds in this region are of the same order of magnitude as those in the MFB).

As judged through the glyoxylic acid-induced histofluorescence, the CPU has a relatively homogeneous, high, DA density and thus a reasonably uniform density of DA efferents. This fact notwithstanding, a wide range of

self-stimulation scores was obtained from various striatal regions, presumably reflecting regional differences in the rewarding effects of CPU electrical stimulation. It was found that in individual animals, and even within particular regions of the CPU, there had been high differential values in threshold; for instance, in the ADL there was a differential value of 114% between the lowest and highest thresholds. As well, this heterogeneity of values was found in the rest of the measures of BSR (Fig 4). Thus, regional variations in ICSS were not an obvious function of corresponding variations in DA fluorescence.

The case could be made, however, that the differences in BSR seen among the various regions of the caudate (the anterior regions yielding better ICSS characteristics than the posterior regions) could be related to topographical variations in DA parameters that are not seen with the relatively insensitive methods of fluorescence histochemistry. DA content and [3-H]DA uptake have been found to decrease regularly from the rostral to the caudal regions of the striatum (Tassin, Cheramy, Blanc, Thierry, and Glowinski, 1978). Also, when the degree of DA receptor binding was measured, a similar rostro-caudal gradient was found, as well as a lower dopaminergic receptor activity in the ventral than in the dorsal striatum (Bockaert, Premont, Glowinski,

Thierry, and Tassin, 1976). In neither case is there a difference between the medio-lateral and dorsal aspects of the CPU. In the self-stimulation mapping study the ventral and medial regions of the CPU yielded better behavioral scores than the dorsal and lateral regions. Thus it appears that there is no special relationship between striatal DA density, uptake, content or receptor activity and striatal self-stimulation, even when sensitive assay measures are considered. CPU self-stimulation would thus appear to result from direct activation of neither DA terminals nor DA efferent cell somata.

Taken together, several studies may provide a basis for a hypothesis as to the neuroanatomical substrate for BSR derived from the nigro-striatal system. The ventral regions of the striatum, which produce the best measures of ICSS (Phillips et al., 1976b; this study) and where manipulations that alter dopaminergic transmission produce marked changes in self-stimulation behavior (Neill et al., 1975, 1978), project primarily to the dorsal SN compacta and to the A10 and A8 cell groups (Bunney and Aghajanian, 1976a; Domesick, 1977; Nauta, Smith, Faul, and Domesick, 1978). The medial and ventral aspects of the CPU are, in turn, innervated by the medial SN, lateral A10, and A8 efferents (Fallon and Moore, 1978). It was in the region of the SN compacta and of the A10 cell group that high rates of self-stimulation and

low thresholds were found by Corbett and Wise (1980); no self-stimulation was seen with electrode placements in the most caudal aspects of these areas containing DA cells nor outside this high DA-containing region, such as the SN reticulata. The zona reticulata is innervated, mainly, by the dorsal regions of the CPU (Domesick, 1977; Nauta et al., 1978), within which the worst self-stimulation was found in the present study. Consequently, the best self-stimulation sites within the striatum are anatomically linked to the best SN-VTA self-stimulation sites, and the worst self-stimulation region within the caudate is most strongly connected with the SN reticulata, where BSR is not apparent. It is also evident that the CPU has a parallel rostro-caudal distribution of positive self-stimulation sites to that seen in the region of the VTA where, again, good self-stimulation effects are seen in the rostral regions of this DA cell conglomerate while poor rewarding effect of electrical stimulation is seen in its caudal regions.

In the case of the anterior-ventral-medial CPU self-stimulation, the electrical stimulation could have directly activated DA efferents or some striatal output fibers that feedback, directly or indirectly, to DA cells in the region of the VTA. This circuit could be the same circuit (described in the Introduction) that seems to be

involved in passive avoidance learning, which is composed of the nigro-neostriatal dopaminergic projection that makes synaptic contact with caudate cholinergic interneurons; these interneurons, in turn, activate striato-nigral GABAergic neurons that close the circuit by synapsing on SN-VTA dopaminergic neurons. There are also some indications that this GABAergic link may be involved in BSR. For example, Kent and Fedinets (1976) found that GABA blocking agents (picrotoxin and bicuculline) depressed LH self-stimulation rates without affecting lever-press escape responses. On the other hand, a GABA-mimetic agent (muscimol) produced dose-related increments in the rewarding value of brain stimulation (Zarevics and Setler, 1979b). Later it was reported that picrotoxin produced inconsistent effects on CPU self-stimulation; in some animals there was a reduction in stimulation rates which was accompanied by an increase in "reward value", while no changes in these parameters had been seen in other animals (Kent, 1978). This latter work was reported in abstract form and no histological material was presented, and it can only be speculated that the different effects of picrotoxin could have been due to differences in electrode positions within the CPU. Whatever the case may be, the point is that the GABA link in the nigro-neostriatal feedback loop may prove to be an important link in BSR processes.

There is also the possibility that self-stimulation from the ventro-medial regions of the caudate may be associated with other fiber bundles, such as the one described by Routtenberg (1971) which is a descending pathway that originates in the prefrontal cortex and runs along the medial aspect of the CPU and then through the internal capsule and descends to at least the level of the mesodiencephalic junction. The involvement of this fiber system in BSR has been suggested further by Clavier and Corcoran (1976); they found that electrolytic lesions of self-stimulation regions of the sulcal PFC produced a marked reduction of ICSS derived from the SN. Their associated histological work demonstrated that there was a fiber pathway from the sulcal cortex to the regions of the SN self-stimulation sites.

There are no published lesion or pharmacological studies specifically dealing with the involvement of dopaminergic activity in self-stimulation of the AVM region and, therefore, no conclusive statements can be made about the involvement of DA in self-stimulation of this region.

In summary, the mapping of the CPU showed no obvious relationships between dopamine density and self-stimulation behavior. This finding leads, in turn, to the conclusion that if striatal dopamine should play a role in striatal BSR,

as seems to be the case (Mora et al., 1976; Phillips et al., 1976b, 1979), it should do so in a complicated manner which is independent of the absolute values of its distribution in this region of the brain and which possibly involves afferents to the DA cells, rather than efferents from them, as the directly activated substrate.

Mesolimbic System

Accumbens. When a comparison of the overall rankings of effectiveness for producing self-stimulation was made among all the regions that were mapped (Table III), the accumbens obtained the best score. As in the case of the CPU, the best BSR within the NAS was associated with the medial and ventral electrode placements. The AVM region of the caudate ranked second in effectiveness to support self-stimulation. These two regions had a high percentage of positive self-stimulation sites and ranked within the first three places on all of the dependent variables; furthermore, both regions, which had equally high DA densities, showed relatively low and homogeneous thresholds. These close anatomical and functional relationships, coupled with the fact that the dopaminergic innervation of these regions shares the same primary source of origin (the A10 cell group), suggest that these regions could share a common neurochemical substrate for self-stimulation. These facts also fit with the notion that the ventral caudate and the NAS

are part of a single anatomical entity (the "ventral striatum") which has been proposed by several authors (e.g., Heimer and van Hoesen, 1979; Newman and Winans, 1980).

Olfactory tubercle. The concept of a ventral striatum not only involves the conceptual unification of the ventral caudate with the NAS but also with the olfacory tubercle (Heimer and van Hoesen, 1979; Newman and Winans, 1980). As stated earlier, the AVM region of the CPU and the NAS showed a striking functional similarity regarding BSR (with the exception that pressing rates correlated positively in the NAS and negatively in the AVM with thresholds and number of sessions to reach behavioral stability). As discussed below, the OT does not share these similarities with the NAS and the AVM region of the striatum and, therefore, does not seem to be functionally related to them.

The mapping of the region of the OT revealed no similarities with the ventral CPU or the NAS regarding self-stimulation, apart from the finding that lower thresholds were associated with the more anterior, ventral and medial stimulation sites. While the NAS and the AVM ranked first and second, respectively, in the overall ranking of effectiveness to produce ICSS, the OT ranked sixteenth, whereas relatively low thresholds for self-stimulation

were found in the AVM and NAS, high thresholds were obtained from the OT. In the former two regions a number of correlations between several variables were found to be significant but none were found so in the OT.

Five of the OT stimulation sites had a relative density of DA that was as high as that found in the NAS and in the CPU. In four of these sites no self-stimulation behavior could be elicited, and in the fifth only low rates of responding were seen (11.3/min). Similar results regarding the relationship between CA density and BSR were obtained from stimulation of the amygdaloid complex.

Amygdala. In the AMY the correlation analysis indicated that the more lateral placements and the regions with higher DA density corresponded with higher stimulation thresholds. These correlations are a reflection of the finding that the worst self-stimulation characteristics within the AMY are yielded by a laterally situated region which contains a relatively high density of dopamine--the lateral nucleus.

Thus in the case of amygdala BSR, as well as in the case of olfactory tubercle BSR, there was a tendency for BSR to be weak where electrodes were best situated to activate DA efferents. In these two cases where the highest dopamine density was correlated with the poorest self-stimulation, it

is clear that activation of dopamine efferents is not the mechanism for brain stimulation reward. It remains possible that it is dopamine terminals themselves which are activated by rewarding stimulation in these regions, but this seem unlikely. For this to be possible, it would have to be the case that the threshold for activation of dopamine terminals in these regions was well below that which seems required to activate dopaminergic fibers in other regions (Gallistel et al., 1981). If the thresholds were lower in these regions than it appears to be in others and if activation of DA terminals in this region is responsible for the rewarding effects of stimulation, then the fact that stimulation in the densest region of terminals was less rewarding than in surrounding regions could be interpreted as reflecting the fact that stimulation of the dopaminergic efferents would counteract the rewarding effects of stimulating dopaminergic terminals themselves. It seems more likely, however, that brain stimulation in these areas is rewarding because of activation of either intrinsic neurons or fibers of passage that are neither dopaminergic nor direct dopaminergic efferents.

Septum. The mapping of the septal area produced some surprising results. First, when taking into account all self-stimulation sites, only one correlation coefficient

between the various dependent variables turned out to be significant (a positive relationship between the number of sessions to start self-stimulating and the number of sessions to behavioral stability). Second, even though it was the anterior-ventral region which showed the lowest percentage of positive stimulation sites and the worst scores on threshold and number of sessions to start self-stimulating, it was this region that yielded the highest septal response rates. Third, it was in the anterior-ventral region of the septal area, out of all the brain regions studied, where the only positive correlation between CA density and a behavioral variable (pressing rate) had been found. This was true in spite of the fact that it was in the posterior regions of the septum where the highest CA density had been found. If the stimulation of the anterior-ventral region activated neurons that were post-synaptic to DA terminals and if dopamine inhibits its efferents in this region, then we would be facing a case where dopamine could have an inhibitory effect on self-stimulation, i.e., dopamine would have an effect opposite to that of electrical stimulation. However, since CA density did not correlate significantly with thresholds or with any other of the dependent variables, it is very probable that catecholaminergic activity in this region is involved in BSR in a more complicated way, if at all.

The significant correlation between CA density and

pressing rate in this region must be interpreted cautiously in any case, since in the anterior regions of the septum there is an important noradrenergic innervation. The histofluorescence method, as used in this part of the study, does not allow differentiation between the two primary catecholamines.

Perhaps the most interesting aspect of the septal mapping is that, in comparison with the rest of the dopaminergic terminal fields, the posterior septal region lent itself better to a direct test of the hypothesis that BSR results from direct activation of DA terminals or their efferents. This was because it is in this region that the major dopaminergic innervation is found in a conspicuous, precisely defined, diagonal band.

If the mesolimbic dopaminergic projection or its efferents play a critical role in septal self-stimulation, either by inhibiting or by activating its synaptic contacts, then one would expect to find a change in thresholds or pressing rates as the self-stimulation electrode was moved from the tissue above the DA band into the band itself. A further change in self-stimulation when the electrode was subsequently lowered out of the band would also be expected.

Whatever effect its release has on posterior septal neurons, dopamine seems not to bear a special relation to self-stimulation involving electrodes in this region. This statement is based on the fact that there were no significant differences in thresholds or pressing rates when the tissue above, within, and below the DA band was succesively stimulated (Table III). Hence, it can be concluded that activation of neither dopamine terminals nor dopamine efferents is critically involved in septal self-stimulation. The activation of some different chemically-coded system, widely distributed throughout the septal area, must be postulated as the directly-activated substrate for self-stimulation of this mesolimbic projection field.

Pyriform cortex. The pyriform cortex, where dopamine-containing axons are found (Lindvall, Bjorklund, Moore, and Stenevi, 1974), ranked poorly on efficacy to elicit self-stimulation (fifteenth place) in spite of the fact that 100% of the stimulation sites had supported this behavior. No correlation coefficients between DA density and any of the rest of the variables could be computed, because the same minimal fluorescence intensity (score = 1) was detected around each of the stimulation sites.

In this region of evenly distributed DA density, high differential scores in thresholds and pressing rates were

found (ranging between 36 and 69 uA and 7.0 and 37.7 responses/min, respectively). This outcome resembles that found in the case of the CPU, except that in the striatum there is a high dopamine density. Thus, it can be said that if in the region of the pyriform cortex dopaminergic activity plays some role in BSR, it should be a complicated one, which is independent of the absolute levels of this catecholamine. This interpretation should be taken cautiously since only 10 sites were studied, involving three animals. It is likely that these results are only expressing a fragmented picture of the functional relationships between DA and self-stimulation of this area. There are no other published studies relating dopamine activity and self-stimulation of the pyriform cortex and, therefore, there are no empirical sources upon which to base and to expand this discussion.

Mesocortical System

Prefrontal cortex. Routtenberg and Sloan (1972) mapped the frontal cortex for self-stimulation effects and found somewhat higher pressing rates in the medial than in the sulcal PFC and no self-stimulation in the other cortical regions, which are outside the mesocortical dopamine projection fields. The present study confirms their findings since it was found that the highest average pressing rate in the medial PFC was 30.0/min while in the sulcal PFC it was

20.5/min, and no self-stimulation could be elicited from the parietal sites that were tested. Furthermore, it was found that there was a higher percentage of positive self-stimulation sites (76.9%) in the medial than in the sulcal (60.0%) PFC, and the former had better scores on each of the dependent variables (Fig 12). The relative magnitude of these differences was reflected in the overall ranking for effectiveness in eliciting self-stimulation; the medial PFC ranked in fourth place while the sulcal cortex ranked in thirteenth place. Hence, the conclusion can be made that these two cortical regions have different functional characteristics regarding BSR.

These differences could be explained by the finding that CA density around the stimulation sites was higher in the medial (1.49) than in the sulcal (0.83) cortex and could be taken as evidence to support the DA hypothesis of BSR. Two additional findings, however, are not consistent with this hypothesis. First, neither of these cortical regions produced significant correlations between DA density and any of the dependent variables that were measured. Second, in the sulcal cortex the highest pressing rate and lowest threshold were obtained from a stimulation site which had no detectable levels of dopaminergic fluorescence. Thus, it seems clear that PFC self-stimulation is not mediated by direct activation of the mesocortical dopaminergic terminals

or of their efferents. Moreover, the lack of correlations between DA density and self-stimulation characteristics support the idea that BSR derived from the prefrontal cortex is independent of mesocortical dopaminergic activity (see, for example, Robertson, Laferriere, and Franklin, 1981; Simon et al., 1979).

Entorhinal cortex. It was found in the present study that those stimulation sites at the more ventral, anterior, and lateral region that were mapped in the entorhinal cortex were associated with a higher DA density. Dopamine density, on the other hand, did not significantly correlate with any of the variables that defined the characteristics of self-stimulation. These results would seem inconsistent with those of Collier et al. (1977); they reported that there was a trend toward higher pressing rates in a region where a high density of dopamine is found, namely, the ventro-lateral aspect of this cortical region where dopamine islands are found. This inconsistency may stem from the circumstance that the most ventral regions of the entorhinal cortex, explored by Collier et al. (1977), were not mapped in the present study.

In relation to the other neocortical areas that were explored, the entorhinal cortex appears to have a closer

functional similarity, regarding self-stimulation, to the sulcal than to the medial PFC (Fig 12). When these three areas were compared with each other, the medial cortex ranked first on all of the dependent variables, and the other two regions shared the same lower ranking; when compared with the rest of the regions, the medial cortex ranked fourth while the sulcal and the entorhinal cortices ranked thirteenth and fourteenth, respectively (Table IV). At present there are no sufficient data available to explain these functional relationships.

In summary, the data obtained from the mapping of the prefrontal and entorhinal cortices fail to give support to the hypothesis that direct activation of mesocortical dopaminergic terminals or efferents or indirect inhibition of DA efferents is critical for self-stimulation elicited from these cortical regions. The data would favor the notion that a non-catecholaminergic system is directly activated in the case of cortical BSR.

Myelinated Fiber Tracts

The finding that self-stimulation can be elicited from non-dopaminergic fiber tracts was not unexpected. There are instances of BSR derived from stimulation of regions of the brain devoid of dopamine, such as the posterior part of the medial entorhinal cortex (Ott et al., 1980) and the ventral

fornix columns (Brown and Winocur, 1973). Early experiments (Lilly, 1958; Olds, 1960; Olds and Olds, 1963) showed that positive reinforcement could be obtained from stimulation through electrodes implanted in the anterior commissure; this finding was later confirmed and extended. All electrodes implanted within, or in contact with, the AC yielded self-stimulation, as did five out of thirteen electrodes in the corpus callosum (Routtenberg, 1971, Figs 1 and 2). In the present study self-stimulation was obtained from the four AC placements, from 22 of the 44 CC sites, and from five of the eight OTr placements.

The data obtained from the two commissures (AC and CC) and from the OTr should be taken cautiously, however. These fiber systems are surrounded by, or in close proximity to, adjacent tissue that supports self-stimulation. Hence, self-stimulation obtained with electrodes within the CC could have been observed as a consequence of the direct activation, due to spread of current, of the adjacent striatal or septal tissue; likewise, self-stimulation of the AC and the OTr could have been due to activation of the accumbens and the pyriform cortex or the olfactory tubercle, respectively. Although these possibilities could not be ruled out in the present experiment (nor in those mentioned above), two findings indicate that in some cases BSR was probably

mediated by the direct activation of the fiber tracts.

First, there were no significant differences in pressing rates between the callosal placements that supported self-stimulation and the caudate or septal sites beneath the CC. The same result was obtained when thresholds were compared between the CC and the same CPU sites; as depicted in Figs 5 and 9, there were some instances of moderate increments in thresholds when the electrode had been lowered from the CC into the CPU. On the other hand, a significant difference was found in threshold values between the CC and the corresponding septal sites; as shown in Fig 9 A-E, large decreases in threshold were obtained when passing from the CC to the dorsal region of the septum. Spread of current to the septal area might thus explain the latter cases, but spread to the caudate seems inadequate to explain the former.

Second, in the only case where comparisons could be made between the OTr and the tissue above it, a 41.6% drop in threshold and an increment in pressing rate were found upon entering the OTr. An increment in threshold was measured when lowering the electrode from the accumbens to the AC, while no change in one case and a small decrement in another were measured when further lowering the electrode from the AC into the accumbens.

If self-stimulation from electrode sites within the fiber tracts were due to spread of current to neighbouring tissue, then higher thresholds and lower pressing rates should have been consistently found in the AC, CC and OTr relative to those sites immediately (within 250 um) outside these regions. It was only in the case of the callosal stimulation above the septal area that thresholds were significantly higher and, because of this, it was the only instance where CC self-stimulation was likely to be due to current spread. Taken together, the data from this mapping of the myelinated fiber tracts reveal a clear example where the direct activation of dopaminergic fibers or their efferents is not a necessary condition for the production of BSR.

SUMMARY AND CONCLUSIONS

Self-stimulation was obtained with electrodes placed in all terminal fields of the ascending dopaminergic systems, but in only one of the twenty-one regions that were mapped was there a correlation between dopamine fluorescence density and self-stimulation. The results obtained from the mapping of the non-catecholaminergic fiber systems suggest that the directly activated reward-relevant neural substrate is most probably non-dopaminergic; this conclusion fits well with the conclusion of electrophysiological studies based on MFB electrodes (Shizgal et al., 1980; Yeomans, 1979). The present results are thus inconsistent with the widely, until recently, held idea (Gallistel et al., 1981) that direct DA activation is critically involved in brain stimulation reward. This idea has been supported, mainly, by results of experiments involving systemic application of DA agonists and antagonists, which produce their effects in all dopamine-sensitive areas of the brain. Such studies do not, of course, pinpoint the mechanisms at the electrode tip itself.

A parsimonious explanation of some experimental results related to the hypothesis that dopamine is, nevertheless, critically involved in brain stimulation reward will now be suggested.

Electrical stimulation of any of the DA terminal fields, and of any other regions of the brain, could produce the activation of afferent fibers to mesencephalic DA neurons. Activation of these neurons could produce a generalized release of DA in several terminal fields; depending on the degree of activation of the DA afferents and mesencephalic DA cells, a greater or lesser amount of DA would be released and, as a consequence, a greater or lesser rewarding state could be produced. This rewarding state could be mediated by either the combined dopaminergic synaptic activity in the various terminal fields where DA release occurs or by dopaminergic activity in a few (perhaps only one) critical regions of these dopaminergic terminal fields. The suggestion is that while rewarding stimulation seems not to directly activate DA neurons, it may, nevertheless, activate them indirectly (trans-syanptically).

This hypothetical series of events would explain, for example, the lack of correlations between BSR produced by the stimulation of specific DA projection fields and their associated DA density at the electrode tip. Because the

rewarding state is dependent upon the indirect release of dopamine in one or more distal terminal fields, and not necessarily upon the direct release of dopamine in the region of the electrode, drugs that modify DA synaptic activity would modify self-stimulation, with electrodes in non-dopaminergic as well as dopaminergic regions of the brain. If more than one dopamine field is involved, this would also explain why no dramatic effects on BSR have been produced by pharmacological interference with synaptic activity of specific structures of the forebrain.

To conclude, it is postulated that direct activation of dopamine terminals or dopamine efferents is not usually involved in the production of brain stimulation reward in the various dopaminergic terminal fields. The hypothesis is advanced that brain stimulation reward may, nevertheless, be ultimately dependent on indirect (trans-synaptic) release of dopamine in one or more areas of the brain, not necessarily at the electrode tip.

Further research is needed to define anatomical systems which have boundaries and relative densities corresponding to the reward circuitry of the brain as mapped with the brain stimulation reward paradigm.

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APPENDIX I

ABBREVIATIONS OF BRAIN STRUCTURES THAT WERE STUDIED.

AC	Anterior commissure
ADL	Antero-dorso-lateral region of the caudate putamen
ADM	Antero-ventro-medial region of the caudate putamen
AMY	Amygdaloid complex
AVL	Antero-ventro-lateral region of the caudate putamen
AVM	Antero-ventro-medial region of the caudate putamen
CC	Corpus callosum
CPU	Caudate putamen
ENT	Entorhinal cortex
MED PFC	Medial prefrontal cortex
NAS	Nucleus accumbens septi
OLF TRACT	Olfactory tract
OT	Olfactory tubercle
PDL	Postero-dorso-lateral region of the caudate putamen
PDM	Postero-dorso-medial region of the caudate putamen
PVL	Postero-ventro-lateral region of the caudate putamen
PVM	Postero-ventro-medial region of the caudate putamen
PYR	Pyriform cortex
SAD	Antero-dorsal region of the septum
SPD	Postero-dorsal region of the septum
SPV	Postero-ventral region of the septum

APPENDIX II

Threshold and highest pressing rate/min (first and second rows after small letters, respectively) obtained from each stimulation site, which are represented in Figures 2, 3, and 4. Numbers 1 to 11 represent successive stimulation points (250 um steps), numbers in parenthesis correspond to the A-P dimension of the Pellegrino and Cushman stereotaxic atlas (1967), and small letters refer to each moveable electrode that was used. **, pressing rate of less than 5/min (no self-stimulation).

APPENDIX II

FIGURE 2

	1	2	3	4	5	6	7	8	9	10	11
					(5.2)						
a	56 15.7	34 16.7	30 16.7	42 20.3	40 25.0	36 28.0	52 29.3	48 24.3	50 29.3	52 30.0	
b	60 **	60 **	60 **	60 **	60 **	60 **	60 **				
					(4.8)						
c	44 22.3	48 20.0									
					(4.6)						
d	48 35.5	56 32.3									
e	42 21.0	50 18.7	28 30.1	38 31.0	36 33.3	44 32.7					
f	60 **	60 **	60 **	60 **							
					(4.4)						
g	60 **	52 27.3	56 24.0								
					(4.2)						
h	60 **	60 **	60 **	60 **	60 **						
i	60 **	60 **	60 **	60 **							
j	36 12.0	40 13.3	46 15.0	50 15.0	56 14.7	60 **	60 **	60 **			
					(4.0)						
k	60 **	60 **	60 **	60 **	60 **	60 **	60 **	60 **			

l	38	30	32	36	38	36	32	18	24		
	27.3	34.0	31.7	37.7	35.7	42.3	40.7	43.0	22.0		
m	60	52	56	50	56	48	40	38	36		
	**	18.0	13.7	23.0	26.7	24.0	30.3	39.0	41.0		
n	42	38	38	34	34	38	36	38	32		
	56.3	31.3	34.7	32.7	42.3	43.0	59.0	100.0	98.3		
o	60	60	32	60	28	60	60	60	60		
	**	**	19.7	**	15.0	**	**	**	**		
p	50	26	44								
	30.0	34.3	19.0								
q	52	36									
	24.0	37.3									
					(3.8)						
r	26	34	34	30	34	40	34	34	38		
	24.7	35.7	29.0	41.0	34.0	41.3	57.0	49.3	57.0		
s	36	38	34	36	36	34	44	30	40		
	11.0	11.3	13.3	14.7	10.0	19.3	11.7	12.3	11.7		
t	52	60									
	25.0	7.0									
					(3.6)						
a	40	42	42	40	44	46					
	18.7	20.7	30.3	40.7	34.3	15.0					
b	42	38	40								
	16.3	20.3	24.7								
c	60	56	56	40	36	46	46	46	44		
	**	8.0	9.3	31.0	32.3	26.0	44.3	40.3	44.0		
					(3.4)						
d	46	32	34	36	38	40	38	42	44	40	40
	9.0	15.7	21.3	31.3	42.0	53.0	59.7	57.0	72.3	63.3	59.0
e	34	42	40	52	56	52	56	50	36		
	11.7	17.3	22.0	19.3	16.0	16.3	12.7	18.0	16.3		
f	60	50	60	56	28	36	52				
	**	8.3	12.0	20.3	25.7	22.3	23.3				

(3.2)

g	34	36	30	34	30	32	44	40	40	38	40
	13.3	22.7	31.0	37.0	50.7	45.3	29.7	41.7	63.0	70.0	79.0
h	60	60	60	60	50	48	46	48	52	48	
	**	**	6.0	9.3	19.7	21.7	22.7	23.0	17.7	24.3	

(3.0)

i	36	32	40	44	50	56	56	56	56		
	11.0	14.7	20.3	20.7	21.7	28.0	16.7	15.0	16.7		
j	60	38	44	44	44	46	50	48	52	50	50
	**	29.0	23.3	22.0	17.0	21.7	19.3	21.7	20.0	21.0	31.0
k	56	56	56	52	46	48	46				
	17.7	9.7	19.7	20.7	18.7	21.7	25.7				
l	46	60	46	42	50	48	28	28	42		
	18.0	14.0	22.3	19.3	18.7	23.7	29.3	22.3	22.3		

(2.8)

m	60	52	36	52	60	60	60	60	60
	7.3	10.0	12.3	10.3	9.0	**	**	**	**

(2.6)

n	60	52	60	60	50	56	48	52	42
	**	5.7	7.0	5.3	7.3	7.7	7.0	7.7	9.0
o	38	32	30	32	30	34	34	38	38
	43.3	37.3	36.7	37.7	37.7	45.7	47.3	56.0	63.7
p	60	60	60	60	60	60	60	60	60
	**	**	**	**	**	**	**	**	**
q	60	60	52						
	**	7.7	13.0						

APPENDIX II

FIGURE 3

	1	2	3	4	5	6	7	8	9	10	11
					(2.4)						
a	26	44	30	44	48	60	60	60	60	60	
	19.0	15.3	17.3	11.0	9.0	**	**	**	**	**	
b	60	60	60	60	60	60	60	60	60		
	**	**	**	**	**	**	**	**	**		
c	60	60	60	60	60	60	60	60	60		
	**	9.3	5.7	**	6.0	7.0	**	5.7	7.7		
					(2.2)						
d	60	60	60	60	60	60	60	60	26	44	42
	**	**	5.7	5.3	**	**	**	5.3	15.0	52.0	60.7
					(2.0)						
e	60	60	50	48	56						
	**	8.7	13.3	12.3	14.0						
f	60	60	26	32	26	34	44	46	48		
	**	**	16.7	23.3	21.3	20.0	21.7	18.0	12.7		
g	60	48	40	48	48	48	60	60	56		
	**	14.0	13.3	10.7	10.0	7.7	6.3	6.0	6.7		
h	28	48	30	40	34	32	34	26	34		
	35.7	33.0	43.0	41.0	47.7	43.0	38.7	42.7	41.7		
i	60	50	50	48	46	42	32	34	32	28	34
	**	20.7	15.3	28.3	23.3	16.7	4.0	45.7	42.0	48.3	41.3
					(1.8)						
j	52	44	44	42	56	48	32	36	32		
	17.7	17.7	20.0	21.7	14.3	14.7	17.0	21.7	26.3		
k	60	60	60	60	60	60	60	60	60		
	**	**	**	**	**	**	**	**	**		
l	60	60	60	60	50						
	**	**	**	12.3	21.7						

(1.6)

a	38	38	34	40	48	52	60	60	60		
	17.0	16.3	19.0	14.0	12.3	14.3	9.0	**	**		
b	60	60	60	48	52	52	34	30	32		
	**	6.3	8.0	8.7	9.0	12.7	19.7	26.7	24.3		

(1.4)

c	28	24	50	48	52	44	26	24	30		
	12.3	13.0	13.3	12.3	12.3	9.3	12.3	13.7	11.7		
d	60	36	30	26	32	40	38	48	52		
	**	22.3	23.3	18.2	16.7	15.7	14.3	19.7	8.0		
e	60	60	60	60	26	26	16	18	50		
	**	**	**	**	17.3	14.7	16.3	15.3	12.3		
f	46	50	60	46	56	60	60	60	60		
	35.0	25.7	**	25.7	17.7	6.3	**	**	**		

(1.2)

g	30	40	22	20	20	18	22	22	32	34	24
	22.7	28.3	16.7	15.0	13.7	13.7	14.0	10.7	14.3	9.3	8.7
h	24	22	20	28	32	24	36	40			
	13.3	14.3	12.3	13.0	12.3	14.3	11.3	16.0			
i	60	60	60	44	46	50	50	52	56	60	
	13.0	**	**	15.0	14.3	33.0	14.0	9.3	6.0	8.3	

(1.0)

j	46	56	40	26	14	20	10	16	10	56
	29.0	29.0	23.3	21.0	25.0	17.0	9.3	11.0	9.0	6.3
k	60	60	42	16						
	**	**	14.7	14.7						
l	32	36	30	44	44	48	28	40	46	
	15.3	14.3	14.7	14.0	12.0	9.0	9.0	8.0	7.7	
m	60	60	60	60	60	60	60	60	60	
	**	**	**	**	**	**	**	**	**	

APPENDIX II

FIGURE 4

	1	2	3	4	5	6	7	8	9	10	11
					(0.6)						
a	60	60	60	60	60	60	60	60	60		
	**	**	**	**	**	**	**	**	**		
b	48	60	38	56	56	44	36	44	40		
	8.0	**	17.0	15.3	10.0	19.3	23.3	30.3	23.0		
					(0.2)						
c	60	60	60	60	60	60	60	60	60		
	**	**	**	**	**	**	**	**	**		
d	60	60	60	60	60	60	60	60	60	60	
	**	**	**	**	**	10.7	**	**	**	**	
e	60	60	60	60	60	60	44	60	30	42	
	5.7	**	5.7	9.7	7.0	13.0	26.3	18.0	23.0	11.7	
					(0.0)						
f	32	38	38	48	48	56	42	48	50		
	28.3	24.7	34.7	23.7	34.0	10.3	22.3	23.7	26.0		
					(-0.2)						
g	28	32	36	42	42						
	27.3	30.0	36.3	19.7	30.3						
					(-0.4)						
h	60	60	60	60	52	50	44	50			
	**	**	**	10.0	19.7	17.3	19.0	18.7			
					(-0.6)						
a	34	32	36	24	50	26	32	36	30		
	12.3	16.0	17.0	24.3	24.0	21.0	18.3	27.0	17.3		
					(-2.6)						
b	60	60	32	30	42	36	60	30	46	60	
	**	7.3	16.3	18.3	15.3	13.0	7.3	15.3	15.0	**	
					(-2.8)						

c	60	60	48	52	60	50			
	**	**	11.3	14.0	**	15.7			
					(-3.2)				
d	50	46	44	44	46	42	60	60	60
	28.0	22.0	20.3	20.3	25.7	52.3	**	**	**
					(-3.4)				
e	32	30	46	43	34	48	60		
	12.7	15.3	16.3	18.3	17.0	15.0	**		
f	32	32	32	50					
	16.7	16.7	14.3	11.0					

APPENDIX III

Tables showing the correlation coefficients, and associated P values, between each of the variables that were studied. ::::: means uncomputable.

Abbreviations are as follows: TH, threshold; PR, pressing rate/min; DC, number of sessions needed to achieve the criterion of behavioral stability; DPR, number of sessions needed to start self-stimulating; A, L, and H refer, respectively, to the anterior-posterior, medial-lateral, and dorso-ventral stereotaxic coordinates.

In all cases N refers to the number of sites that were tested in animals that showed self-stimulation behavior.

See Appendix I for abbreviations of brain structures.

ALL CPU POSITIVE SITES, N = 135

	TH	PR	DC	DPR
A	0.020	0.171	-0.182	-0.153
P	0.815	0.048	0.035	0.076
L	0.340	-0.475	0.207	0.305
P	0.001	0.001	0.016	0.001
H	-0.175	0.085	-0.250	-0.235
P	0.042	0.329	0.003	0.006
TH	1.000	-0.656	0.175	0.500
P	0.001	0.001	0.042	0.001
PR	-0.656	1.000	-0.325	-0.458
P	0.001	0.001	0.001	0.001
DC	0.175	-0.325	1.000	0.510
P	0.042	0.001	0.001	0.001
DPR	0.500	-0.458	0.510	1.000
P	0.001	0.001	0.001	0.001

ADM, N = 29.

	TH	PR	DC	DPR
A	0.298	-0.199	-0.247	0.013
P	0.116	0.300	0.197	0.947
L	0.597	-0.762	0.182	0.532
P	0.001	0.001	0.344	0.003
H	0.019	0.309	-0.506	-0.271
P	0.922	0.103	0.005	0.155
TH	1.000	-0.701	-0.019	0.556
P	0.001	0.001	0.921	0.002
PR	-0.701	1.000	-0.053	-0.531
P	0.001	0.001	0.787	0.003
DC	-0.019	-0.052	1.000	0.293
P	0.921	0.787	0.001	0.123
DPR	0.556	-0.531	0.293	1.000
P	0.002	0.003	0.123	0.001

ADL, N = 15.

	TH	PR	DC	DPR
A	-0.650	0.833	-0.264	-0.539
P	0.004	0.001	0.171	0.019
L	0.284	-0.495	0.087	0.414
P	0.153	0.030	0.379	0.063
H	-0.486	0.734	-0.155	-0.356
P	0.033	0.001	0.291	0.097
TH	1.000	-0.802	-0.189	0.073
P	0.001	0.001	0.250	0.398
PR	-0.802	1.000	-0.121	-0.370
P	0.001	0.001	0.333	0.087
DC	-0.189	-0.121	1.000	0.280
P	0.250	0.333	0.001	0.157
DPR	0.073	-0.370	0.280	1.000
P	0.398	0.087	0.157	0.001

AVM, N = 26.

	TH	PR	DC	DPR
A	0.404	-0.584	0.155	:::::
P	0.041	0.002	0.450	:::::
L	0.544	-0.627	0.199	:::::
P	0.004	0.001	0.331	:::::
H	-0.281	0.184	-0.109	:::::
P	0.165	0.369	0.596	:::::
TH	1.000	-0.469	-0.058	:::::
P	0.001	0.016	0.779	:::::
PR	-0.469	1.000	-0.457	:::::
P	0.016	0.001	0.019	:::::
DC	-0.058	-0.457	1.000	:::::
P	0.779	0.019	0.001	:::::
DPR	:::::	:::::	:::::	:::::
P	:::::	:::::	:::::	:::::

AVL, N = 22.

	TH	PR	DC	DPR
A	-0.529	0.674	-0.167	-0.409
P	0.011	0.001	0.459	0.059
L	0.252	-0.734	0.294	0.196
P	0.259	0.001	0.185	0.382
H	0.052	0.330	-0.536	-0.240
P	0.818	0.134	0.010	0.281
TH	1.000	-0.650	0.182	0.755
P	0.001	0.001	0.418	0.001
PR	-0.650	1.000	-0.488	-0.679
P	0.001	0.001	0.021	0.001
DC	0.182	-0.488	1.000	0.592
P	0.418	0.021	0.001	0.004
DPR	0.755	-0.679	0.592	1.000
P	0.001	0.001	0.004	0.001

PDM, N = 13.

	TH	PR	DC	DPR
A	-0.574	0.426	-0.363	-0.600
P	0.040	0.147	0.223	0.030
L	0.631	-0.414	0.302	0.594
P	0.021	0.160	0.316	0.032
H	-0.449	0.071	0.073	-0.251
P	0.124	0.819	0.813	0.409
TH	1.000	-0.718	0.136	0.622
P	0.001	0.006	0.659	0.023
PR	-0.718	1.000	-0.511	-0.577
P	0.006	0.001	0.074	0.039
DC	0.136	-0.511	1.000	0.540
P	0.659	0.074	0.001	0.057
DPR	0.622	-0.577	0.540	1.000
P	0.023	0.039	0.057	0.001

PDL, N = 9.

	TH	PR	DC	DPR
A	0.337	0.198	-0.002	-0.021
P	0.187	0.305	0.498	0.478
L	-0.392	-0.133	-0.024	0.004
P	0.148	0.319	0.476	0.496
H	-0.782	0.266	-0.473	-0.415
P	0.006	0.245	0.099	0.134
TH	1.000	-0.548	0.676	0.552
P	0.001	0.063	0.023	0.062
PR	-0.548	1.000	-0.864	-0.827
P	0.063	0.001	0.001	0.003
DC	0.676	-0.864	1.000	0.983
P	0.023	0.001	0.001	0.001
DPR	0.552	-0.827	0.983	1.000
P	0.062	0.003	0.001	0.001

PVL, N= 21.

	TH	PR	DC	DPR
A	0.641	-0.697	0.102	0.579
P	0.001	0.001	0.330	0.003
L	-0.258	0.111	0.175	-0.359
P	0.129	0.316	0.224	0.055
H	0.122	0.001	-0.230	0.078
P	0.300	0.499	0.158	0.368
TH	1.000	-0.748	0.148	0.402
P	0.001	0.001	0.261	0.035
PR	-0.748	1.000	-0.195	-0.347
P	0.001	0.001	0.199	0.062
DC	0.148	-0.195	1.000	0.540
P	0.261	0.199	0.001	0.006
DPR	0.402	-0.347	0.540	1.000
P	0.035	0.062	0.006	0.001

ALL SEPTAL POSITIVE SITES, N = 114.

	TH	PR	DC	DPR	DOP
A	0.397	0.067	-0.46	0.250	-0.266
P	0.001	0.460	0.626	0.007	0.004
L	-0.088	-0.010	0.111	0.108	0.166
P	0.350	0.920	0.242	0.251	0.077
H	0.479	-0.057	-0.153	0.094	0.148
P	0.001	0.546	0.105	0.319	0.115
TH	1.000	-0.158	-0.154	0.220	-0.049
P	0.001	0.093	0.101	0.019	0.605
PR	-0.158	1.000	-0.127	-0.205	0.089
P	0.093	0.001	0.178	0.029	0.349
DC	-0.154	-0.127	1.000	0.423	-0.117
P	0.101	0.178	0.001	0.001	0.214
DPR	0.220	-0.205	0.423	1.000	-0.188
P	0.019	0.029	0.001	0.001	0.046

SAD, N = 25.

	TH	PR	DC	DPR	DOP
A	0.082	-0.001	-0.134	-0.008	0.190
P	0.697	0.995	0.524	0.971	0.364
L	0.256	-0.313	0.119	0.386	0.291
P	0.217	0.128	0.571	0.057	0.158
H	0.088	-0.016	-0.254	-0.050	0.201
P	0.677	0.939	0.221	0.812	0.335
TH	1.000	-0.627	-0.169	0.044	-0.004
P	0.001	0.001	0.420	0.833	0.984
PR	-0.627	1.000	-0.066	-0.359	0.022
P	0.001	0.001	0.753	0.078	0.918
DC	-0.169	-0.066	1.000	0.544	-0.239
P	0.420	0.753	0.001	0.005	0.250
DPR	0.044	-0.359	0.544	1.000	-0.244
P	0.833	0.078	0.005	0.001	0.240

SAV, N = 29.

	TH	PR	DC	DPR	DOP
A	0.160	-0.046	-0.351	-0.360	-0.154
P	0.540	0.862	0.167	0.156	0.555
L	-0.210	0.294	0.277	0.206	0.501
P	0.419	0.251	0.283	0.428	0.040
H	-0.189	0.321	-0.041	-0.120	0.400
P	0.468	0.209	0.875	0.645	0.111
TH	1.000	-0.303	0.367	0.397	-0.252
P	0.001	0.237	0.147	0.114	0.329
PR	-0.303	1.000	-0.421	-0.351	0.930
P	0.237	0.001	0.092	0.167	0.001
DPR	0.397	-0.351	0.881	1.000	-0.196
P	0.114	0.167	0.001	0.001	0.452

SPD, N = 44.

	TH	PR	DC	DPR	DOP
A	0.151	-0.093	0.008	0.153	-0.160
P	0.329	0.548	0.961	0.321	0.299
L	0.024	-0.180	0.081	0.086	-0.217
P	0.876	0.242	0.601	0.578	0.157
H	0.545	-0.301	-0.012	-0.145	0.325
P	0.001	0.047	0.939	0.348	0.032
TH	1.000	-0.056	-0.039	0.057	0.095
P	0.001	0.718	0.800	0.713	0.541
PR	-0.056	1.000	-0.044	0.011	0.305
P	0.718	0.001	0.778	0.943	0.044
DC	-0.039	-0.044	1.000	0.279	-0.076
P	0.800	0.778	0.001	0.067	0.623
DPR	0.057	0.011	0.279	1.000	-0.096
P	0.713	0.943	0.067	0.001	0.537

SPV, N = 28.

	TH	PR	DC	DPR	DOP
A	0.340	0.311	-0.276	:::::	0.000
P	0.077	0.107	0.156	:::::	1.000
L	-0.234	-0.206	0.174	:::::	0.459
P	0.231	0.294	0.376	:::::	0.014
H	0.371	-0.285	-0.243	:::::	-0.214
P	0.052	0.141	0.214	:::::	0.274
TH	1.000	-0.263	-0.442	:::::	-0.050
P	0.001	0.177	0.019	:::::	0.800
PR	-0.263	1.000	0.023	:::::	0.141
P	0.177	0.001	0.910	:::::	0.475
DC	-0.442	0.023	1.000	:::::	-0.017
P	0.019	0.910	0.001	:::::	0.932
DPR	:::::	:::::	:::::	:::::	:::::
P	:::::	:::::	:::::	:::::	:::::

NAS, N = 29.

	TH	PR	DC	DPR
A	-0.089	-0.001	0.429	0.212
P	0.647	0.998	0.020	0.270
L	-0.296	-0.465	0.288	0.533
P	0.119	0.011	0.130	0.003
H	0.177	0.482	0.516	0.196
P	0.360	0.008	0.004	0.308
TH	1.000	0.388	-0.201	-0.209
P	0.001	0.037	0.296	0.277
PR	0.388	1.000	0.130	-0.201
P	0.037	0.001	0.502	0.296
DC	-0.201	0.130	1.000	0.721
P	0.296	0.502	0.001	0.001
DPR	-0.209	-0.201	0.721	1.000
P	0.277	0.296	0.001	0.001

AMY

	TH	PR	DC	DPR	DOP
A	-0.391	-0.221	0.231	-0.098	-0.428
P	00.072	0.324	0.302	0.663	0.047
L	0.591	-0.337	0.001	-0.040	0.529
P	0.004	0.125	0.998	0.858	0.011
H	-0.717	0.443	0.017	-0.223	-0.674
P	0.001	0.039	0.940	0.318	0.001
TH	1.000	-0.580	-0.167	0.282	0.551
P	0.001	0.005	0.458	0.203	0.008
PR	-0.580	1.000	-0.213	-0.090	-0.283
P	0.005	0.001	0.340	0.691	0.202
DC	-0.167	-0.213	1.000	0.172	-0.272
P	0.458	0.340	0.001	0.445	0.221
DPR	0.282	-0.090	0.172	1.000	0.087
P	0.458	0.340	0.001	0.445	0.221

OT, N = 12, WITHOUT 6-OHDA.

	TH	PR	DC	DPR	DOP
A	-0.795	-0.111	0.234	-0.290	-0.035
P	0.002	0.732	0.463	0.361	0.913
L	0.805	-0.156	-0.136	0.314	-0.022
P	0.002	0.627	0.674	0.320	0.946
H	-0.800	0.427	0.177	-0.303	0.480
P	0.002	0.166	0.582	0.338	0.114
TH	1.000	-0.138	-0.300	0.298	-0.140
P	0.001	0.669	0.344	0.347	0.665
PR	-0.138	1.000	-0.234	-0.066	0.238
P	0.669	0.001	0.464	0.838	0.457
DC	-0.300	-0.234	1.000	0.320	0.046
P	0.344	0.464	0.001	0.311	0.887
DPR	0.300	-0.066	0.320	1.000	0.066
P	0.347	0.838	0.311	0.001	0.838

OT, N = 7, WITH 6-OHDA.

	TH	PR	DC	DPR	DOP
A	:::::	:::::	:::::	:::::	:::::
P	:::::	:::::	:::::	:::::	:::::
L	:::::	:::::	:::::	:::::	:::::
P	:::::	:::::	:::::	:::::	:::::
H	-0.915	0.678	0.533	-0.158	0.866
P	0.004	0.094	0.218	0.735	0.012
TH	1.000	-0.516	-0.753	0.083	-0.906
P	0.001	0.236	0.051	0.860	0.005
PR	-0.516	1.000	0.295	0.130	0.565
P	0.236	0.001	0.521	0.782	0.186
DC	-0.753	0.295	1.000	0.498	0.601
P	0.051	0.521	0.001	0.256	0.154
DPR	0.083	0.130	0.500	1.000	-0.091
P	0.860	0.782	0.256	0.001	0.846

PYR, N = 10.

	TH	PR	DC	DPR	DOP
A	0.155	0.013	0.249	0.777	:::::
P	0.670	0.971	0.487	0.008	:::::
L	0.189	-0.041	0.255	0.783	:::::
P	0.602	0.912	0.478	0.007	:::::
H	0.025	-0.088	-0.176	-0.026	:::::
P	0.945	0.810	0.627	0.944	:::::
TH	1.000	-0.823	0.276	0.528	:::::
P	0.001	0.003	0.440	0.117	:::::
PR	-0.823	1.000	-0.264	-0.368	:::::
P	0.003	0.001	0.460	0.295	:::::
DC	0.276	-0.264	1.000	0.361	:::::
P	0.440	0.460	0.001	0.306	:::::
DPR	0.528	-0.368	0.361	1.000	:::::
P	0.117	0.295	0.306	0.001	:::::

MED PFRC, N = 53, WITHOUT 6-OHDA.

	TH	PR	DC	DPR	DOP
A	0.280	-0.176	-0.081	0.208	0.709
P	0.043	0.203	0.562	0.135	0.001
L	0.256	-0.218	-0.176	0.082	0.610
P	0.065	0.117	0.207	0.559	0.001
H	-0.577	0.441	0.248	-0.241	-0.430
P	0.001	0.001	0.074	0.082	0.001
TH	1.000	-0.315	-0.320	0.260	-0.027
P	0.001	0.022	0.02	0.060	0.849
PR	-0.315	1.000	0.190	-0.087	-0.119
P	0.022	0.001	0.173	0.536	0.395
DC	-0.320	0.190	1.000	0.227	0.110
P	0.020	0.173	0.001	0.102	0.433
DPR	0.260	-0.087	0.227	1.000	0.146
P	0.060	0.536	0.102	0.001	0.298

MED PFC, N = 8, WITH 6-OHDA.

	TH	PR	DC	DPR	DOP
A	:::::	:::::	:::::	:::::	:::::
P	:::::	:::::	:::::	:::::	:::::
L	-0.648	-0.451	-0.383	-0.655	:::::
P	0.041	0.131	0.174	0.039	:::::
H	-0.385	0.411	-0.741	-0.436	:::::
P	0.173	0.156	0.018	0.140	:::::
TH	1.000	-0.124	-0.031	0.241	:::::
P	0.001	0.385	0.471	0.283	:::::
PR	-0.124	1.000	-0.042	0.395	:::::
P	0.385	0.001	0.461	0.167	:::::
DC	-0.031	-0.042	1.000	0.585	:::::
P	0.471	0.461	0.001	0.064	:::::
DPR	0.241	0.395	0.585	1.000	:::::
P	0.283	0.167	0.064	0.001	:::::

SULCAL PFC, N = 12.

	TH	PR	DC	DPR	DOP
A	0.273	-0.084	0.570	0.109	0.438
P	0.390	0.796	0.053	0.735	0.155
L	0.146	-0.686	-0.472	-0.246	0.525
P	0.652	0.014	0.122	0.442	0.080
H	0.468	0.391	0.128	-0.178	0.179
P	0.125	0.209	0.692	0.579	0.578
TH	1.000	-0.115	-0.084	-0.164	0.500
P	0.001	0.723	0.795	0.612	0.098
PR	-0.115	1.000	-0.048	0.081	-0.652
P	0.723	0.001	0.883	0.802	0.022
DC	-0.084	-0.048	1.000	0.630	0.030
P	0.795	0.883	0.001	0.028	0.926
DPR	-0.164	0.081	0.630	1.000	-0.174
P	0.612	0.802	0.028	0.001	0.588

ENT, N = 29, WITHOUT 6-OHDA.

	TH	PR	DC	DPR	DOP
A	0.282	-0.276	0.297	0.156	-0.395
P	0.139	0.148	0.118	0.420	0.034
L	0.255	-0.239	0.291	0.127	-0.383
P	0.183	0.211	0.126	0.512	0.040
H	-0.140	-0.204	-0.041	-0.194	0.796
P	0.469	0.288	0.833	0.314	0.001
TH	1.000	-0.290	0.042	0.384	-0.265
P	0.001	0.128	0.828	0.040	0.165
PR	-0.290	1.000	-0.318	-0.499	0.029
P	0.128	0.001	0.093	0.006	0.883
DC	0.042	-0.318	1.000	0.614	-0.036
P	0.828	0.093	0.001	0.001	0.851
DPR	0.384	-0.499	0.614	1.000	-0.207
P	0.040	0.006	0.001	0.001	0.283

ENT , N = 13, WITH 6-OHDA.

	TH	PR	DC	DPR	DOP
A	-0.347	0.145	-0.051	-0.561	0.527
P	0.123	0.319	0.435	0.023	0.032
L	-0.347	0.145	-0.051	-0.561	0.527
P	0.123	0.319	0.435	0.023	0.032
H	-0.078	-0.118	0.099	-0.343	0.879
P	0.400	0.351	0.374	0.126	0.001
TH	1.000	-0.817	0.215	0.744	-0.015
P	0.001	0.001	0.241	0.002	0.481
PR	-0.817	1.000	-0.364	-0.694	-0.190
P	0.001.	0.001	0.108	0.004	0.267
DC	0.215	-0.367	1.000	0.454	0.029
P	0.241	0.108	0.001	0.060	0.463
DPR	0.744	-0.694	0.454	1.000	-0.336
P	0.002	0.004	0.060	0.001	0.131

AC, N = 4.

	TH	PR	DC	DPR
A	-0.845	-0.945	0.686	0.577
P	0.155	0.055	0.314	0.423
L	0.845	0.945	-0.686	-0.577
P	0.155	0.055	0.314	0.423
H	0.627	0.927	-0.854	-0.796
P	0.373	0.073	0.146	0.204
TH	1.000	0.645	-0.754	-0.683
P	0.001	0.355	0.246	0.317
PR	0.645	1.000	-0.654	-0.552
P	0.355	0.001	0.346	0.448
DC	-0.754	-0.654	1.000	0.990
P	0.246	0.346	0.001	0.010
DPR	-0.683	-0.552	0.990	1.000
P	0.317	0.448	0.010	0.001

CC, N = 21.

	TH	PR	DC	DPR
A	0.018	-0.319	-0.239	0.071
P	0.939	0.158	0.297	0.759
L	0.154	0.008	-0.258	0.061
P	0.505	0.974	0.259	0.794
H	0.187	-0.392	-0.198	0.070
P	0.418	0.079	0.389	0.764
TH	1.000	-0.310	0.221	0.374
P	0.001	0.172	0.336	0.095
PR	-0.310	1.000	-0.361	-0.499
P	0.172	0.001	0.108	0.021
DC	0.221	-0.361	1.000	0.793
P	0.336	0.108	0.001	0.001
DPR	0.374	-0.499	0.793	1.000

OLF TRACT, N = 5.

	TH	PR	DC	DPR
A	0.895	-0.561	0.584	0.919
P	0.040	0.325	0.301	0.028
L	:::::	:::::	:::::	:::::
P	:::::	:::::	:::::	:::::
H	0.968	-0.699	0.404	0.933
P	0.007	0.189	0.500	0.021
TH	1.000	-0.719	0.343	0.900
P	0.001	0.171	0.572	0.037
PR	-0.719	1.000	-0.176	-0.814
P	0.0171	0.001	0.777	0.094
DC	0.343	-0.176	1.000	0.596
P	0.572	0.777	0.001	0.289
DPR	0.900	-0.814	0.596	1.000
P	0.037	0.094	0.289	0.001