Brain Self-Stimulation: Direct Evidence for the Involvement of Dopamine in the Prefrontal Cortex

Abstract. *Rats were trained to self-stimulate the medial prefrontal cortex, a region rich in dopaminergic terminals. After the region adjacent to the electrode site was labeled with [^{14}C]dopamine, it was perfused repeatedly by means of push-pull cannulas. Electrical stimulation of this cortical area in six animals enhanced the release of dopamine and its associated metabolites in nine of 16 experiments. Thus in vivo evidence is provided that dopamine is involved in the brain self-stimulation mechanism within the frontal cortex.*

An intriguing assumption underlying the neurochemical substrates of brain self-stimulation is that a specific neurotransmitter is released as the behavior is initiated and sustained (*1*). Recently, dopamine (DA) has been implicated on the basis of pharmacological and other experiments in self-stimulation behavior (*1*). In addition to the nigrostriatal and mesolimbic dopaminergic pathways (*2*), nerve terminals containing this monoamine have been identified within the medial and sulcal prefrontal cortex by Thierry and co-workers (*3*). In these cortical regions, electrical stimulation is positively reinforcing (*4*) and DA has been proposed as the possible neurotransmitter mediating it.

We now present direct physiological evidence that DA activity increases in vivo in the cortex during electrical self-stimulation behavior. In fact, the endogenous release and turnover of this monoamine is specifically enhanced within the medial prefrontal cortex, at the same anatomical sites in which self-stimulation behavior is maintained.

A monopolar electrode was implanted stereotaxically in adult male Sprague-Dawley rats with its tip resting in the medial prefrontal cortex. At the same time, a 20-gauge guide tube that accommodated a push-pull cannula assembly was positioned within the same stereotaxic plane, but 1.0 mm caudal to the tip of the electrode (*6*). Upon postoperative recovery, each rat was placed in a self-stimulation chamber for 1 hour and trained to depress a lever in order to obtain electrical pulses (*7*) delivered to the cortex. As soon as the animal responded for stimulation at a steady rate for 30 minutes, the DA release experiments were begun.

The region immediately adjacent to the electrode was labeled with 0.5 μc of [^{14}C]dopamine ([^{14}C]DA) (Amersham/Searle) microinjected over a 10-second period in a volume of 1.0 μl according to standard procedures (*8*). After 20 to 30 minutes had elapsed, the cortical site was perfused for 5 minutes with an artificial cerebrospinal fluid by means of push-pull cannulas at a rate of 25 μl per minute (*9*). Successive perfusions were carried out at 15-minute intervals for up to 2 hours. As the declining curve of the washout in radioactivity approached an asymptote, the cortical site was primed by electrical stimulation (*7*), usually 10 minutes before the third or fourth push-pull perfusion. If an animal did not then self-stimulate spontaneously, imposed stimulation of the site was randomly delivered according to the same parameters at which the rat normally self-stimulated.

In addition to the 10-minute period, stimulation of the site continued for the 5-minute interval during the third or fourth perfusion. The amount of radioactivity in each sample of perfusate was determined in a liquid scintillation spectrometer (*10*). Representative experimental and control samples of perfusate collected prior to, during, and after this interval of stimulation were dried under a stream of nitrogen. Subsequently, in a selected sample of experiments, thin-layer chromatographic (TLC) analyses (*11*) were undertaken to determine the proportional values of metabolites of DA as well as newly synthesized norepinephrine. At the end of the experiments, the anatomical position of each electrode tip and perfusion site in the cortex was verified according to standard histological procedures (*12*).

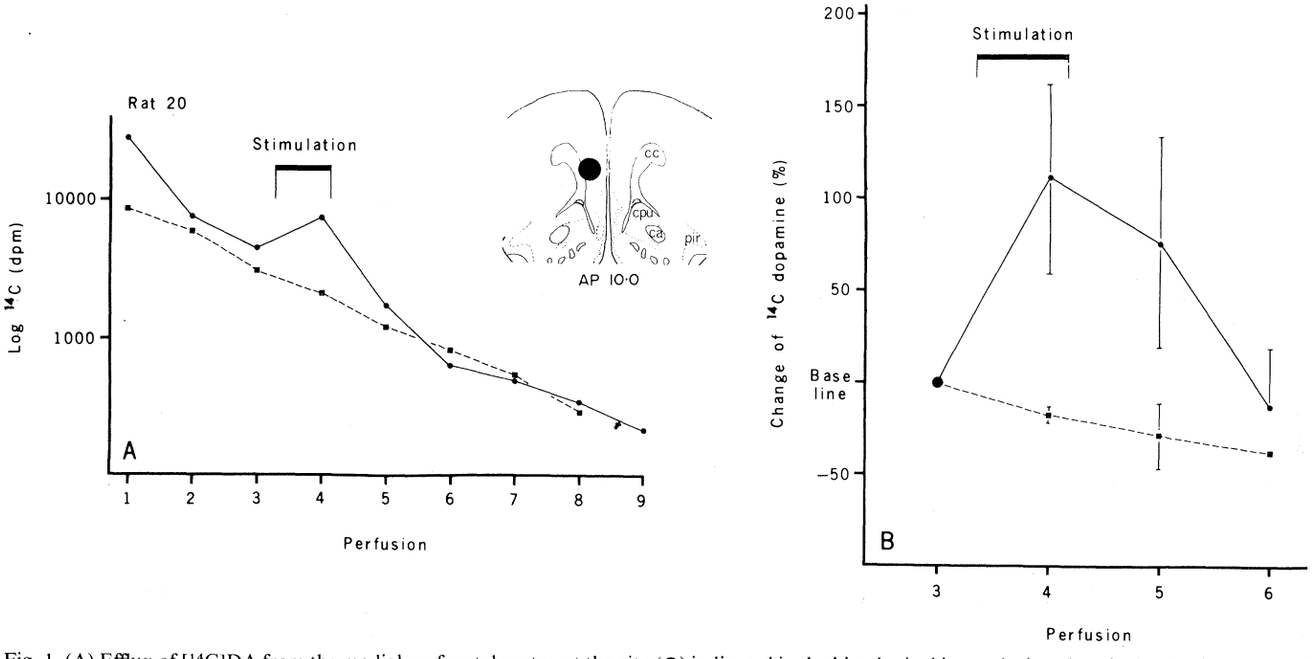

Fig. 1. (A) Efflux of [^{14}C]DA from the medial prefrontal cortex at the site (●) indicated in the histological inset, during electrical self-stimulation of the adjacent tissue. A washout curve of [^{14}C]DA activity occurs when no stimulation is delivered. (B) Average percentage of change in [^{14}C]DA as verified by TLC analysis in samples of perfusate collected from the medial prefrontal cortex during electrical stimulation of this region (N = 6), and the control condition (N = 4) when stimulation was not given (N = 2), or when self-stimulation did not occur (N = 2). Abbreviations: *ca*, anterior commissure; *cc*, corpus callosum; *cpu*, caudate-putamen complex; and *pir*, pyriform. Control, ——; stimulation, ____.

Table 1. Levels of dopamine and its metabolites expressed as the percentage of that radioactivity recovered on the TLC plates in samples collected before (third perfusion), during (electrical stimulation of the frontal cortex) (fourth perfusion), or after (fifth perfusion) electrical stimulation of the frontal cortex. Abbreviations: DOPAC, 3,4-dihydroxyphenylacetic acid; HVA, homovanillic acid; 3-MT, 3-methoxytyramine; NMN, normetanephrine; DA, dopamine; NE, norepinephrine; and ORIG, origin. The range of activity at the origin varied from background level to 492 dpm with a mean and standard error of 121.0 ± 19.9 dpm.

Amine and metabolite	Control perfusions (N = 4)			Experimental perfusions (N = 6)		
	Third	Fourth	Fifth	Third	Fourth	Fifth
DOPAC	21.44 ± 5	15.13 ± 1	12.85 ± 1	7.99 ± 2	5.07 ± 1	13.41 ± 3
HVA	30.68 ± 6	35.62 ± 8	44.58 ± 1	32.88 ± 8	34.98 ± 8	29.31 ± 4
3-MT	2.21 ± 0.3	2.87 ± 0.5	4.34 ± 0.7	4.70 ± 1	4.14 ± 1	4.48 ± 1
NMN	0.53 ± 0.1	1.90 ± 0.6	1.67 ± 0.7	7.40 ± 0.2	2.04 ± 0.6	1.18 ± 0.2
DA	29.13 ± 11	23.22 ± 8	19.29 ± 5	12.23 ± 5	20.54 ± 6	19.17 ± 4
NE	2.02 ± 0.4	2.67 ± 0.6	4.66 ± 0.7	6.29 ± 1	6.49 ± 1	4.63 ± 0.9
ORIG	13.86 ± 3.9	15.95 ± 1.4	17.48 ± 2.5	34.39 ± 8	27.33 ± 7	27.65 ± 9

In nine out of 16 experiments with six rats in which the electrode-perfusion sites were in the medial prefrontal cortex, electrical self-stimulation significantly enhanced the localized release of [^{14}C]DA. Figure 1A illustrates the results of an individual experiment in which electrical stimulation was delivered to the frontal cortex of the rat during the fourth consecutive perfusion. The activity of [^{14}C]DA increased substantially from 4651 to 7361 disintegrations per minute (dpm) during the course of electrical stimulation. During the next perfusion, 15 minutes later, the [^{14}C]DA activity declined when no stimulation was delivered and continued to do so on subsequent perfusions thereafter. On a different day, the control washout curve (Fig. 1A) was obtained similarly by repeated perfusions of the same site but with no electrical stimulation given.

The corresponding results from the TLC analyses of the samples collected from the medial prefrontal cortex of the entire group of animals are shown in Fig. 1B. During electrical self-stimulation the [^{14}C]DA activity was significantly increased by more than 100 percent and, even though stimulation had been terminated, the level of DA in the next perfusion was elevated by more than 75 percent (*13*).

The percentage values of radioactivity determined by TLC analysis for the third through fifth perfusions under stimulation and control conditions for selected experiments are given in Table 1. Since the metabolite values of the two nonstimulated and two of the five electrically stimulated control rats (*14*) were not significantly different, all control TLC data were pooled. As would be expected, the levels of homovanillic acid (HVA), a principal by-product of DA metabolism, were far higher than those of the other catecholamine metabolites (*11*). The level of 3,4-dihydroxyphenylacetic acid in the cortical perfusates declined significantly ($P < .01$) during self-stimulation but the other metabolites, including norepinephrine, were relatively stable throughout repeated perfusions. According to Braestrup *et al.* (*15*), the half-life of HVA and other DA metabolites is less than 15 minutes. Thus, relatively small changes in the metabolic profile would be detectable after 65 to 105 minutes elapsed following the intracortical injection of the DA label (*15*).

These results provide direct in vivo evidence for the involvement of DA in self-stimulation of the prefrontal cortex. They support an earlier suggestion that this putative neurotransmitter is a mediator of self-stimulation in this frontal area (*5*). We failed to observe any enhanced release of [^{14}C]DA from the medial prefrontal cortex during self-stimulation of the ventral tegmental area (*5, 16*).

That the release of DA could be produced simply by the electrical stimulation of cortical tissue is conceivable. If so, the rat's behavior might then be due to localized stimulation of fibers rather than to a functionally specific presynaptic release of DA. However, pharmacological experiments with DA agonists and antagonists strongly support the idea that dopaminergic synapses do play a direct role in mediating self-stimulation of the frontal cortex. For example, spiroperidol, a DA receptor blocking agent, or apomorphine, a DA agonist, attenuates self-stimulation of the orbitofrontal cortex of the monkey as well as the rat (*5*). When injected directly into the cortex, spiroperidol blocks self-stimulation delivered to the hypothalamus and amygdala (*5*). These latter experiments thus correspond with our present results showing a functionally induced release of DA from a homologous site in the rat. The coexistence of noradrenergic terminals in the same cortical area leaves open the possibility that this catecholamine may also play a role in self-stimulation of the prefrontal cortex. At the present time, evidence from the TLC analyses of an increase in norepinephrine either during or after the interval of electrical stimulation is not firmly established (*17*).

The neurochemical properties of the prefrontal cortex are currently of great interest because of its functional involvement in a variety of behaviors (*18*). At present, DA in this area of the brain has been implicated not only in reward and motivation generally (*5*) but also in the manifestation of emotional states (*19*). In addition, the results reported here could also be of relevance to the DA hypothesis of schizophrenia (*20*). That is, a dysfunction to the cortical dopaminergic mechanism may cause an impairment in emotional and goal-directed behavior as frequently witnessed in the schizophrenic patient (*21*).

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6. Using the atlas of L. Pellegrino and A. J. Cushman, the coordinates were: anterior-posterior, 10.4; lateral, 0.75; and horizontal, 3.0. The guide tube was 20-gauge thin-wall (TW) tubing placed 2 mm above and 1 mm behind the tip of the electrode. General procedures described elsewhere were followed [R. D. Myers, Ed., *Methods in*

Psychobiology (Academic Press, London, 1971), vol. 1, pp. 67–91].
7. A train of 0.3 second of square negative pulses at a frequency of 100 hertz and 0.1-second duration was delivered with each lever press. The mean self-stimulation rate was 150 presses per 10 minutes.
8. The [^{14}C]DA had a specific activity of 60 mc/mmole and the concentration in the stock solution was 3.1 μg per microliter per 0.5 μc expressed as the free base. [^{14}C]Dopamine was obtained from Amersham/Searle Corporation, Arlington Heights, Ill.
9. R. D. Myers, in *Methods in Psychobiology*, R. D. Myers, Ed. (Academic Press, London, 1972), vol. 2, p. 169. The push-pull cannulas were connected by polyethylene tubing to calibrated 1.0-ml B-D tuberculin syringes mounted on a Harvard apparatus infusion-withdrawal pump. The artificial cerebrospinal fluid was always freshly prepared in the following concentrations: Na^+, 127.65 mM; K^+, 2.55 mM; Ca^{2+}, 1.26 mM; Mg^{2+}, 0.93 mM; and Cl^-, 134.58 mM. Ascorbic acid in a concentration of 0.2 mg/ml was added to bring the pH of the solution to 3.8 in order to retard the degradation of the amine. In every experiment, the artificial cerebrospinal fluid was passed through a Swinnex Millipore filter, pore size 0.22 μm.
10. After the effluent was placed immediately on crushed ice, a 50 μl portion of the chilled sample was placed in a scintillation vial containing 3 ml of PCS (Amersham/Searle); it was counted for 10 minutes and the activity was converted to disintegrations per minute.
11. Using the two-way procedure of R. M. Fleming and W. G. Clark [*J. Chromatog.* **52**, 305 (1970)], which is described in detail by G. Martin and R. D. Myers [*Am. J. Physiol.* **229**, 1547 (1975)], the following amines and metabolites were analyzed: (i) 3,4-dihydroxyphenylacetic acid, (ii) 4-hydroxy-3-methoxyphenylacetic acid (homovanillic acid), (iii) 3-methoxytyramine, (iv) normetanephrine, (v) 3-hydroxytyramine, and (vi) norepinephrine (see Table 1). Prior to the TLC analysis of the experimental samples, a control washout curve consisting of six samples was analyzed to quantitate the relation between dopamine, norepinephrine, and their respective metabolites.
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13. The data in Fig. 1B illustrate the average percentage of change in the total amount of [^{14}C]DA recovered from thin-layer chromatograms between the third and fourth perfusion (t = 2.03, P < .05) and the third and fifth perfusion (t = 1.67, P < .10). Elevated DA activity in the sample collected after electrical stimulation was terminated is not readily explained, but could be due either to the distance between the cannula tips and the dopaminergic terminals involved in the behavior or to a recurrence or persistence of functional activity of these neurons beyond the interval of local excitation.
14. Those sites that did not support self-stimulation yet were in close proximity to active sites were used as control sites for the nonspecific activity of electrical stimulation. The dimensions of the regions of stimulated-induced release of DA were demarcated stereotaxically within anterior-posterior, 9.5 to 10.5; lateral, 0.25 to 1.25; and horizontal, +3.0 to +4.5. Inactive sites at which the pattern of DA release followed a washout were, for example, as little as within 0.75 mm lateral to this region.
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17. In one experiment, we did find increases in newly synthesized norepinephrine paralleling the release of dopamine [R. D. Myers and F. Mora, *Brain Res. Bull.* **2**, 105 (1977)].
18. See references in Mora *et al.* (*5*) and S. J. Cooper, *Nature (London)* **254**, 439 (1975); R. M. Santos-Anderson and A. Routtenberg, *Brain Res.* **103**, 243 (1976).
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Immune Response in the Sea Urchin *Lytechinus pictus*

Abstract. *The sea urchin shows an immune response to grafted tissue similar to that found in vertebrates. Unrelated animals rejected allografts in about 30 days. Acceptance of allografts was observed for tissue exchanged between some F_2 and F_3 inbred animals. The percentage of acceptances reflected the degree of inbreeding. Accelerated second set rejection was also found. These grafts were rejected in one-third of the time compared to first sets.*

The rejection of tissue transplanted from one member to another member of the same species, an allogeneic transplant, is one characteristic of an animal's ability to mount an adaptive immune response to foreign tissue. Experimental allogeneic rejection has been demonstrated for all groups of vertebrates tested so far (*1*). Recent interest in the phylogenetic origin of the immune response has led to investigations of various invertebrate classes. Incompatibility responses, which have been described as nonimmunological, are found in the lower invertebrate phyla, such as the Protozoa (*2*), Coelenterata (*3*), and Porifera (*4*). Incompatibility responses in these phyla are more commonly found when xenogeneic grafts (transplants between species) are employed. Allogeneic rejection has been reported for two invertebrate phyla, the annelids (*5*) and the echinoderms (*6*).

We have tested the sea urchin *Lytechinus pictus* for the ability to reject allografts, and have found that a highly sensitive immune system is present. This work was done with the sea urchin colony that has been maintained in our laboratory for the past 7 years. The colony consists of about 4000 animals. Some of these have been kept in a mixed population and have unknown parentage, but most are kept as separate individuals with known genealogies. This allows us to begin to examine the genetics of the immune response. The methods for rearing sea urchins in the laboratory have been described (*7*). The animals were maintained in a closed seawater system at 15° to 16°C.

Allografts were exchanged between pairs of animals by removing sections of test (shell), about 3 mm square, along with the overlying epidermis and surface structures such as spines and pedicellaria. The squares were taken from between the rows of tube feet. Grafts were placed on the host graft bed on the upper third of the animal. Control tests indicate that the position of the graft on the host does not affect the immune response. No special methods were used to anchor the grafts to the host, and about 10 percent did become dislodged and were scored as technical losses. The sea urchin usually deposits calcium carbonate along the border between the graft and host, and the graft becomes firmly attached to the host test within a few days. This host-graft interface sometimes fails to form in the case of rapidly rejected allografts, such as those between unrelated animals.

Five sets of animals were used. These were grouped on the basis of genetic relationship. The sets were: lab stock, which were laboratory animals of unknown genetic relationship to one another; second-generation inbred animals; third-generation inbred animals; wild-type animals grafted to third generation; and wild type grafted to wild type. Wild-type animals were sea urchins obtained from Pacific Bio-Marine Laboratories in Venice, California. Graft survival times are summarized in Table 1.

Control autografts were lifted from the animal to ensure that all connections between the graft and underlying tissue were completely severed, and then reinserted into the original graft bed. Some controls were done by excising two sections from the animal and transposing them. In other experiments, autografts were placed on the animal when the second sets and third-party grafts were done. All autografts healed in rapidly. Grafts were positioned relative to the madriporite to facilitate future identification.

The first response to the surgical procedure took place in 2 days, with the appearance of red pigmented cells on the graft. The brightly colored cells were frequently first observed in the border between the host and graft, and appeared to migrate onto the graft from the underlying host tissue. These cells were present in slight to moderate amounts on about 75 percent of the autografts. Their number decreased between the first and third weeks after surgery until they disappeared from the autografts. These pigmented cells were present in considerably greater density on the allografts and were observable throughout the rejection period. The red pigment within