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Activation of reward-relevant neurons in the caudate-putamen influences the development of medial prefrontal cortex self-stimulation: a moveable electrode mapping study

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Abstract. Two hundred fifty five medial prefrontal cortical (MPFC) and 187 caudate-putamen (CPu) sites were evaluated for intracranial self-stimulation in 67 animals using moveable electrodes and collecting trade-off functions between current and frequency. Eleven percent of the examined areas, located predominantly in the ventromedial aspects of MPFC and CPu, showed reliable self-stimulation and the average charge of 1.12 and 1.11 μC respectively, values that are in line with those reported for the Medial forebrain bundle. The distribution of charge, however, was greater than reported for the latter region, and ranged between 0.68 to 1.63 μC across sites. Some subjects were implanted with two electrodes, one aimed at the MPFC, and the other at the CPu, ventral tegmental area, or lateral hypothalamus. Only animals with CPu placements showed transference of self-stimulation to the MPFC, suggesting that these two regions might form part of the same reward substrate, a view that has anatomical, electrophysiological and recently behavioral support.

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Key words: brain-stimulation reward, striatum, forebrain, charge, threshold

INTRODUCTION

The psychophysical approach has been extensively used to document the characteristics of the reward sites located predominantly along the medial forebrain bundle (MFB; for review refer to Milner 1981, Stellar and Stellar 1985, Yeomans 1990). However, brain regions lying rostral to the MFB which also support intracranial self-stimulation, such as the medial prefrontal cortex (MPFC) and caudate-putamen (CPu), have not been systematically studied using this approach. The few experiments that have been conducted suggest that the more anterior brain regions are characteristically different from the MFB reward substrate (Justesen et al. 1963, Routtenberg 1971, Routtenberg and Sloan 1972, Corbett et al. 1982a, Schenk and Shizgal 1982, Prado-Alcala and Wise 1984, Schenk et al. 1985, Robertson 1989, Trzcińska and Bielajew 1992, Panagis et al. 1995). For example, it was reported that the currents necessary to elicit MPFC self-stimulation are higher than the ones usually employed for MFB stimulation, indicating a less excitable and sparser population of reward fibers than the one underlying MFB self-stimulation (Schenk and Shizgal 1982). Similarly, based on the range of current thresholds obtained in another study, it appears that the CPu reward substrate is heterogenous (Prado-Alcala and Wise 1984).

Another characteristic of MPFC and CPu self-stimulation that distinguishes it from that of the MFB is the gradual acquisition. It was shown that it typically takes several sessions for the behaviour to develop (Prado-Alcala and Wise 1984, Corbett et al. 1985) and in the case of the MPFC, it can be accelerated by applying non-contingent stimulation to the same site (Corbett et al. 1982b) or by initially shaping the animal to bar press on another self-stimulation region, such as the sulcal cortex, the lateral hypothalamus, or the ventral tegmental area (Corbett et al. 1982b, Robertson et al. 1982a, Robertson et al. 1986, Cobo et al. 1989). Once self-stimulation on the MPFC was established as a consequence of the experience of rewarding stimulation at another site, activation of the training site, either the sulcal cortex, the lateral hypothalamus, or the ventral tegmental area, was no longer necessary to maintain MPFC self-stimulation (Robertson et al. 1986).

Two hypotheses have been proposed to explain the mechanism underlying the slower acquisition. First, a kindling-like phenomenon, similar to synaptic potentiation, might facilitate acquisition, since treatment with anticonvulsant agents retards the development of brain stimulation reward in the MPFC (Robertson et al. 1982b, Balleine et al. 1989, Robertson, 1989, Corbett, 1990). Second, a reduction over time in an initial stimulation-induced behavioral inhibition might increase self-stimulation rates (Corbett et al. 1985). In other words, gradual emergence of MPFC self-stimulation might be explained by an habituation to the disruptive effects of stimulation (Corbett et al. 1982a).

In light of these proposals, the first aim of this experiment was to document the distribution of reward sites in the MPFC and CPu as reflected in their trade-off relationships between current and frequency; the second goal was to assess whether MPFC acquisition would be promoted by self-stimulation already established at a CPu placement. To that end, the animals were implanted with pairs of electrodes, the first one in the MPFC, and the second in either the CPu, the lateral hypothalamus, or the ventral tegmental area, with the latter two sites serving as controls. The three non-cortical electrode placements were defined as the "training sites". After stable frequency thresholds were obtained from one of the training sites, stimulation was applied to the MPFC alone. Note that before data collection commenced at the training site, the MPFC was screened for self-stimulation and shown not to support the behavior at the parameters tested. If following this manipulation, animals now bar-pressed for MPFC stimulation, the phenomenon in question was called the transference effect. Transference was defined as a persistent and consistent maintenance of self-stimulation, long after the effects of training electrode stimulation ceased, that is during the consecutive two to five testing sessions of the MPFC alone. It is important to point out that although many MPFC sites were subsequently tested in the same subject, by using moveable electrodes (Miliaressis, 1981) only the first one was examined for transference, since the behavior obtained from stimulating subsequent placements might have been confounded by the already established self-stimulation at the initial MPFC site.

METHODS

Subjects and surgery

Treatment of rats was in accordance with the guidelines of the Canadian Council on Animal Care, the National Institute of Health Guide for the Care and Use of

Laboratory Animals (NIH Publications No.8023), and the university policies pertaining to animal experimentation, approved by the University of Ottawa Animal Care Committee.

Sixty seven Long-Evans male rats (Charles River, Québec, Inc.) weighing between 297 and 365 g at the time of surgery were singly housed in shoe-box style cages and kept on a 12 h light/12 h dark cycle, with light onset at 7:00 h. Purina rat chow and water were freely available. Stereotaxic surgery was conducted under a combination of atropine sulfate (0.6 mg/kg, s.c.) sodium pentobarbital (60 mg/kg, i.p.) and Rompun (1.0 mg/ml Xylazine, i.m.) to reduce bronchial secretions and to produce deep and long-lasting anesthesia. Each subject was implanted with one of the following electrode assemblies - a single moveable electrode (Kinetrods Reg'd), ($n = 48$), a pair of electrodes, one fixed and one moveable ($n = 12$), or a pair of moveable ones ($n = 7$). The electrodes were made of 0.25 mm stainless-steel wire insulated with Epoxylite to the rounded tips. The stereotaxic coordinates were as follows: MPFC 1.7-5.2 mm anterior to bregma, 0.4-3.0 mm lateral to the midsagittal suture, and 1.0-5.1 mm below dura reading at Bregma; CPu 0.2-1.7 mm anterior to bregma, 1.4-2.6 lateral to the midsagittal suture, and 3.3-5.0 mm below dura; lateral hypothalamus 1.4-2.8 mm posterior to bregma, 1.7-2.0 mm lateral to the midsagittal suture, and 7.7-8.4 mm below dura; ventral tegmental area 4.8-5.3 mm posterior to bregma, 0.7-1.2 mm lateral to the midsagittal suture, and 8.0-8.2 mm below the dura (Paxinos and Watson 1986). The current return was provided by a stainless-steel wire, soldered to an Amphenol pin and wrapped around 3 or 4 stainless-steel skull screws. The whole assembly was secured to the skull and screws by dental cement.

Apparatus and procedure

About a week after surgery, the animals were screened for self-stimulation on a continuous reinforcement schedule. All testing was conducted in a 27 cm long X 36 cm wide X 51 cm tall wooden and Plexiglas cage, which was fitted with a lever on the right wall, situated 3 cm above the floor of the cage. Each lever press delivered a 500 ms train of 0.1 ms rectangular cathodal pulses; stimulation was provided by dual constant-current amplifiers (Mundl 1980), and in-house manufactured pulse generators. In order to prevent polarization at the tip between pulses, the outputs of each channel were shorted to ground via a low resistance path. During stimulation trials, the parameters, were monitored on an oscilloscope, by observing the voltage drop across a 1 KΩ resistor in series with the rat. The beginning of each trial and the available stimulation current were signalled by five trains of "priming" pulses, separated from each other by a 1 s interval. If after several screening sessions bar pressing was not evident, the rat was briefly anaesthetized with an inhalant fluothane (Halothane) and the moveable electrode lowered 0.32 mm using a microdrive (Kinetrods Reg'd); testing resumed 24 h later. This procedure continued until bar pressing was established, according to a minimum criterion of no less than five responses per minute. The currents used to screen for self-stimulation ranged from 200 to 1,200 μA and the frequency values from 20 to 200 Hz, across animals and sites. Once bar pressing was reliably observed, a descending order of frequencies was administered, separated from each other by 0.05 $\log_{10}$ steps, starting with a value that yielded maximum responding to one that produced no responses. This procedure was applied at three different currents. The frequency thresholds were then interpolated from each rate-frequency function by finding the frequency corresponding to 50% of the maximum response rate. The frequency thresholds were considered stable when their values did not vary by more than 0.1 $\log_{10}$ steps, across at least three consecutive sessions. The thresholds were then plotted against current to yield a frequency-current trade-off function. When stimulation-induced seizures occurred, the session was aborted and testing resumed after a 10-30 min interval. Occasionally, following a severe motor seizure (characterized by a complete loss of motor control), animals ceased responding altogether and testing had to be postponed until the next day.

Behavioral tests

The animals were implanted with a two-electrode assembly comprising one MPFC moveable electrode and either a lateral hypothalamic (3 rats), ventral tegmental area (3 rats), or CPu (5 rats) moveable electrode. Initially the animals were shaped to bar press for MPFC stimulation at currents ranging from 50 to 1,000 μA across subjects over five consecutive sessions, adopting the following testing protocol used in other studies of this nature (Corbett and Stellar 1983). After a minimum of shaping, the stimulation was made available at fixed values for 30 min and the number of bar presses recorded. All of the animals showed only minimal and

transient responding at the selected currents; rates of bar pressing ranged from 0 to 6 per 30 min session. During this period no stimulation was delivered to the training electrode. Once the five-day protocol was completed, stimulation tests began at the training site. Trade-off functions between current and frequency were collected at currents that ranged from 300 to 1,000 μA for the CPu, 400 to 800 μA for the lateral hypothalamus and 630 to 1,000 μA for the ventral tegmental area. Four to six frequency thresholds were collected at three current values - low, medium, and high. For the most part, adjacent current values were in 0.1 $\log_{10}$ unit steps apart. If self-stimulation on the training electrodes was hampered by seizure activity or motoric side-effects, the electrode, if possible, was lowered to the next site. Immediately following the last stable frequency-threshold determination on either the CPu, lateral hypothalamus, or ventral tegmental area electrode, stimulation was applied to the MPFC site, at parameters which produced the lowest frequency threshold at the training site. In some instances, the currents had to be adjusted in order to produce optimal responding and also to eliminate any factor that interfered with bar pressing, such as motor effects and seizures. Once reliable self-stimulation was established, trade-off functions between current and frequency thresholds were collected for a particular training site and for the MPFC. Then the total charge was calculated for each trade-off function between frequency and current, using the following formula (Gallistel 1978):

$$Q = INd$$

where
Q = the charge in μC
I = the current in μA
N = the number of pulses in the 500 ms stimulation train
d = the pulse duration in sec (0.0001)

This formula is somewhat simplified as it does not take into account the minimum current needed to evoke self-stimulation; however, it is useful as a comparison of the charge values obtained at other sites which have been evaluated using the same approach. When the reciprocity between frequency threshold and current is equal, the charge values across currents should remain constant, provided that the minimum current is close to zero and the trade-off function between current and frequency is expressed in log values. The advantage of using charge lies in the fact that it combines several currents and frequencies from the trade-off function obtained by stimulating one particular site, into a single representative value for the whole curve, and thus constitutes a valid reflection of the rewarding value of stimulation (Wise 1996). Such manipulation is especially useful when only a few data points are obtained, as was often the case here, which prevents the traditional representation of the changes in frequency thresholds as a function of current (e.g. Panagis et al. 1995).

Histology

Upon completion of all behavioral tests, the subjects were injected with a lethal dose of sodium pentobarbital (usually 1 ml of a 60 mg/kg dose, i.p.) and then perfused intracardially with 0.9% saline, followed by 10% formalin. The brains were immediately removed and placed in the formalin solution. Approximately two days later the tissue was cut into 40 μm sections and treated with a Nissl stain to indicate cell bodies. The location of the electrode tips was determined based on the Paxinos and Watson (1986) atlas. If the subject was implanted with a moveable electrode, the individual anatomical sites were estimated from the difference between the consecutive microdrive readings and the last position of the electrode tip; that is in order to estimate the depth of an electrode at site 0 (the site of initial implantation), the difference between the last position of that electrode and the number of equidistant 0.32 mm consecutive moves was derived.

RESULTS

Histology

Sixty seven animals were screened for self-stimulation on both the MPFC and the CPu. Ten rats, altogether tested at 20 MPFC and 29 CPu sites, demonstrated self-stimulation, ranging from transient behaviour interrupted by epileptogenic activity to consistent bar pressing. Figure 1 shows the 187 and 255 individual sites evaluated in the MPFC and CPu respectively.

There was about an 11% success rate of obtaining self-stimulation in both regions. The cluster of positive sites for the MPFC was found in the anterior cingulate (Cg1, Cg3) frontal cortex areas 1 and 2 (Fr1, Fr2) and the orbitofrontal (MO/VLO) portions, and for the CPu, predominantly in the ventromedial region; some positive areas were also found in the ventrolateral CPu, results in accordance with previous findings (Routtenberg 1971,

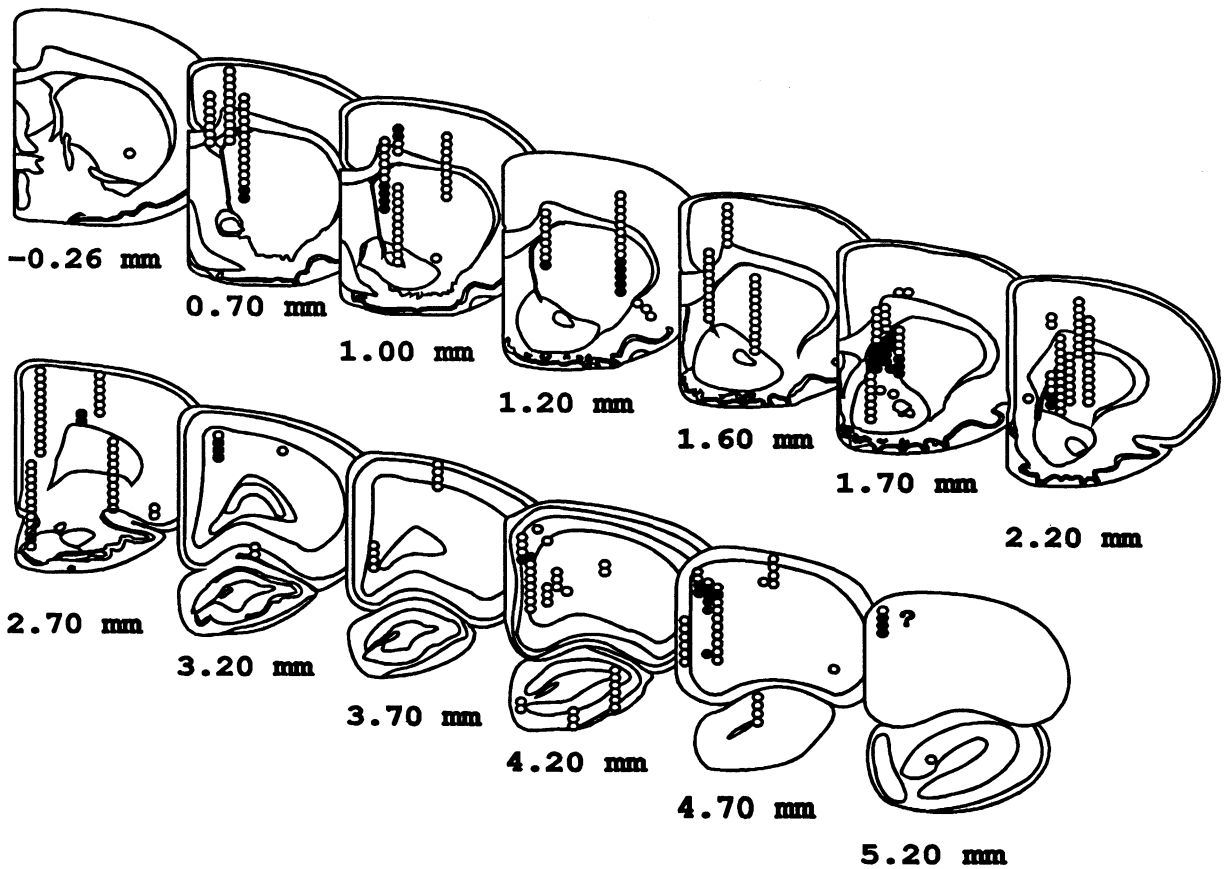

Fig. 1. The locations of all the CPu and MPFC sites evaluated for self-stimulation; the sites are arranged from the most posterior (upper left) to most anterior (lower right) placements. The circles correspond to the histological verification of the electrode tips; unfilled circles indicate placements from which self-stimulation was not obtained; the filled ones mark positive self-stimulation sites. The clipart sections are from the program NeuroGraphics - The Rat Brain, which is based on the Paxinos and Watson (1986) atlas, with additional plates. The question mark on the coronal section labelled +5.2 reflects uncertainty as to the exact position of that electrode tip.

Routtenberg and Sloan 1972, Prado-Alcala and Wise 1984, Panagis et al. 1995). The highest concentration of positive self-stimulation sites in the MPFC was found in the Cg3 region at 4.7 mm in front of bregma, and for the CPu, in the vicinity of the lateral septum at 1.7 mm anterior to bregma. Thus, the greatest concentration of positive self-stimulation sites is clustered in the anterior MPFC and CPu regions. Among striatal placements, the nucleus accumbens (core), an inner region of this structure, was found not to support self-stimulation, which is in agreement with previous work that suggests that this area is non-limbic, as opposed to the nucleus accumbens (shell), an outer portion, which is thought to be limbic in function (Deutch et al. 1992).

Figure 2 shows the histological placements of the training electrodes. The frequency thresholds varied from 26 to 50 Hz for the lateral hypothalamus, 15 to 66 Hz for the ventral tegmental area, and 16 to 84 Hz across the MPFC and CPu. In all but one case (subject CH58), the acquisition of MPFC self-stimulation was not facilitated by prior rewarding MFB stimulation (subjects CV37, CH39, CV44, CV45, CH57, and CH59). However, a transference effect was observed following rewarding stimulation of the perifornical nucleus (subject CH58). Further, rewarding stimulation applied to three out of four training sites in the CPu appeared to facilitate the acquisition of ipsilateral MPFC self-stimulation (subjects CP46, CP64 and CP66, but not CP62), as confirmed by the appearance of reasonable rates of bar pressing. Lastly, no transference of the behaviour to the MPFC was observed following stimulation of a contralateral CPu site (subject CP65).

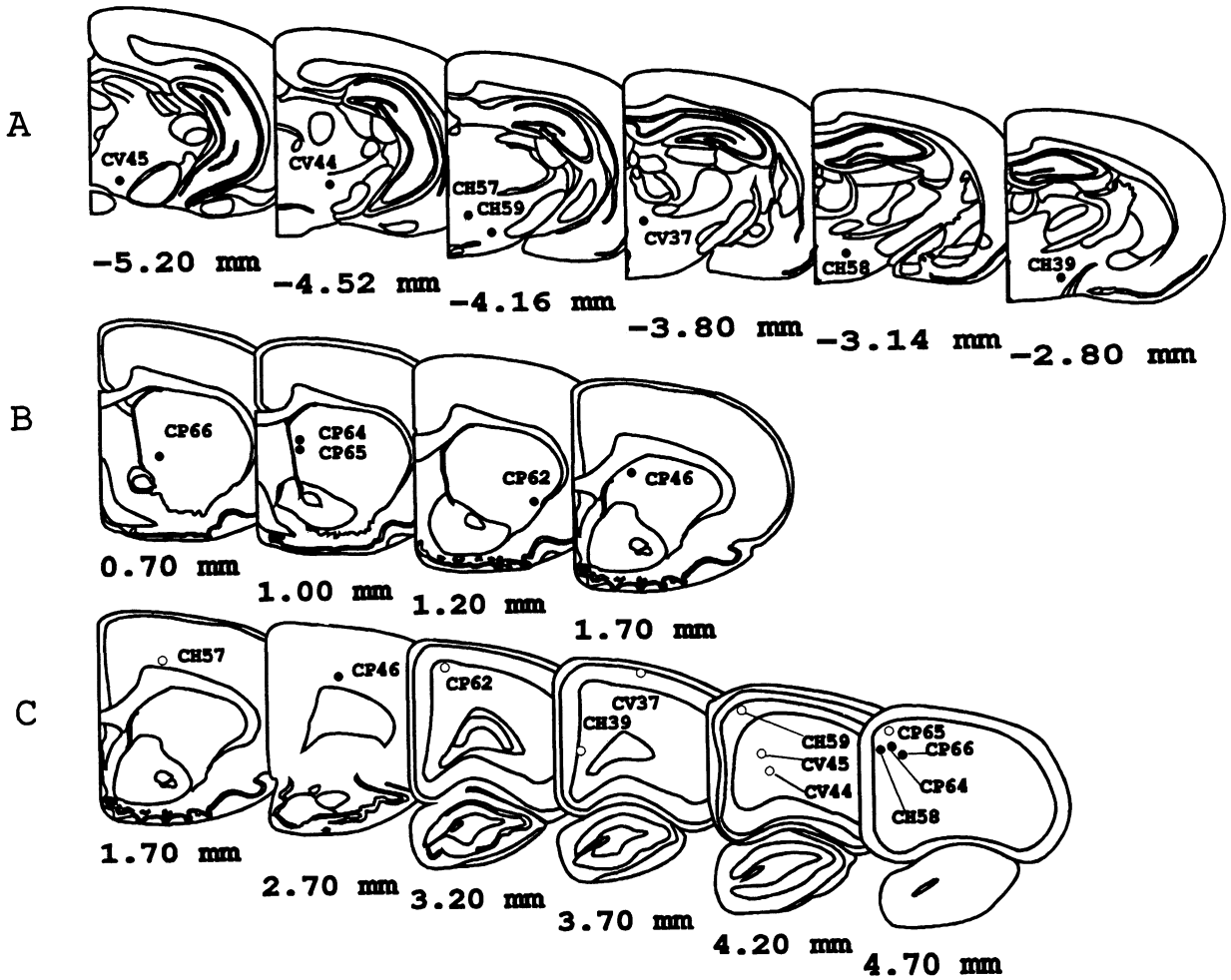

Fig. 2. The location of the electrode placements in the transference experiment; the sites are assembled from the most posterior (top left) to the most anterior coronal plate (bottom right). Panel A illustrates the sections containing the electrode tips aimed at the lateral hypothalamic and ventral tegmental areas, panel B, the CPu placements, and panel C, the MPFC placements. The alphanumeric located beside each site refers to the identity of the subject. The filled circles indicate the sites that gave rise to self-stimulation and the unfilled circles indicate sites where self-stimulation was not obtained.

Charge values

The average charge values of 1.12 and 1.11 μC obtained across all MPFC and CPu sites respectively, were not statistically different, and ranged from 0.81 to 1.63 μC for the MPFC and 0.68 to 1.60 μC for the CPu. The pattern in Figure 3 demonstrates that both MPFC and the CPu show a similar distribution of total charge values across the posterior-anterior plane; charge increased progressively from the most posterior MPFC and CPu sites until it reached a peak at roughly 1.2 mm in front of bregma for the CPu (the last plate before the appearance of tenia tecta and the first section where nucleus accumbens core is shown in two separate parts), and at 4.2 mm for the MPFC (the first coronal plate where regions Fr1, Fr3, dorsal transition zone, forceps minor, and the corpus callosum are no longer visible); it then decreased gradually to reach the lowest value in the most anterior MPFC and CPu regions. The lowest charge values were found at 2.2 mm (the first coronal section where regions Cg3, IL, VLO, LO and dorsal peduncle appears and where tenia tecta and lateral septal nucleus are no longer visible), and 5.2 mm in front of bregma (the plate where the internal granular layer, internal and external plexiform layers of the olfactory bulb can be seen, and where the regions Cg1 and Cg3 are no longer visible), for the CPu and MPFC respectively (Paxinos and Watson 1986). A decrease in required charge is interpreted as an increase in the rewarding value of the stimulation and vice versa (Gallistel 1978, Harris and

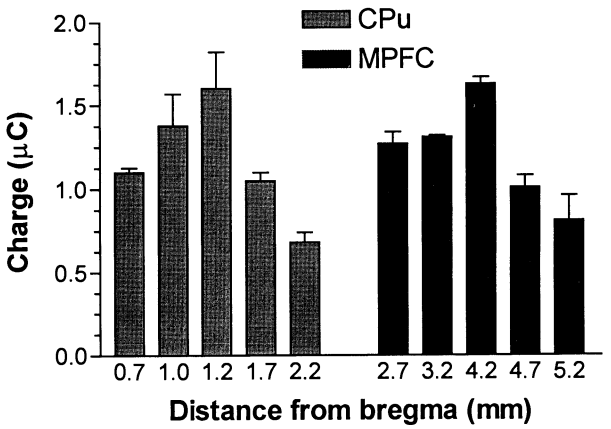

Fig. 3. The average charge ± SEM for all CPu and MPFC self-stimulation sites as a function of distance from bregma. The charge values associated with each current were averaged to represent the total charge for a particular site. In order to illustrate an overall pattern, the average of all charge values was calculated to yield a single representative value for each coronal section.

Bielajew 1991). The charge values are derived from the frequency-current function. The slope of this function is believed to reflect the density of the relevant fibers at the site of stimulation, the higher the density the greater the number of relevant fibers recruited, which ultimately is translated into a smaller required current for self-stimulation (Sax and Gallistel 1991). As an aside, when the frequency thresholds at a given current are plotted as a function of distance from bregma the same pattern as described above is obtained when charge is graphed as a function of distance from bregma (personal observation).

It appears that the sites supporting the most reliable self-stimulation are clustered at 4.7 and 1.7 mm in front of bregma respectively, in the ventromedial MPFC and CPu. Interestingly, these sites are also the ones represented by charge values of nearly 1 μC. It was previously reported that charge values obtained for sites along the MFB, at roughly the same parameters as used here, are also around 1 μC (Gallistel 1978, Bielajew et al. 1987). In the present study, an average charge of 1.35 μC was obtained for the ventral tegmental area and 1.04 μC for the lateral hypothalamic placements.

Behavioral observations

All animals gradually developed severe motor seizures; however, there were differences in the seizure profile observed at the two sites. The MPFC seizures were generally less frequent, developed more slowly, but tended to be more disruptive to self-stimulation, as reflected in elevated thresholds, or at times complete cessation of self-stimulation. The CPu seizures, on the other hand, appeared abruptly, were more frequent and severe, but less disruptive to thresholds overall. The animals usually recovered quickly and returned to the lever. Reducing the train duration to either 300 or 250 ms, thus shortening the time of stimulation delivery, has been used in some studies as a strategy to reduce the possibility of seizure occurrence (Balleine et al. 1989, Rick and Fouriezos 1992). Similarly, applying an anticonvulsant agent, brotizolam (5 mg/ml or 7.5 mg/ml), has been shown to reduce the severity of seizures and the threshold variability at self-stimulation sites in the MFB associated with seizures (Harris and Bielajew 1991). However, neither of these measures was effective in this study. Interestingly, it has been reported that drugs known to suppress seizure activity also reduce self-stimulation rates (Reid et al. 1964, Weinreich and Clark 1970). Further, some subjects appear to "titrate" the level of stimulation by decreasing their rate of bar pressing, so as to prevent the seizures from re-occurring. Occasionally, in this study, a decrease in the incidence of seizures throughout the session was also accompanied by progressive elevation in self-stimulation thresholds. It has been pointed out that MPFC stimulation may lower seizure thresholds, which would in turn cause the animal to perform more slowly in order to avoid a seizure (Lenzer 1971).

The other secondary effects of stimulation included occasional biting of the lever, coprophagy, grooming, yawning, and motoric effects unrelated to seizures, such as circling.

DISCUSSION

It appears that the high required currents, generally low success rate in establishing reliable self-stimulation, and uneven distribution of charge values obtained at these forebrain self-stimulation sites may indicate a recruitment of smaller, less excitable, or sparsely distributed reward-relevant fibers, which corroborates other studies of this nature (Prado-Alcala and Wise 1984, Schenk et al. 1985). It appears that the average minimum current for MPFC self-stimulation is about 300 μA (Schenk et al. 1985), which is significantly higher than the value for most MFB sites - around 100 μA (Gallistel 1978). It is thus possible that these dissimi-

larities in current between the MPFC and MFB reflect differences in the density of reward-relevant neurons and possibly in the post-synaptic integration of reward signals in the two substrates (Schenk et al.1985).

The fact that transference of lever pressing to the MPFC was not observed following rewarding stimulation of the lateral hypothalamic or ventral tegmental area placements, but did occur following rewarding CPu stimulation under the experimental protocol used in this study, lends further support to the idea that the MFB and the anterior forebrain regions may constitute separate reward circuits. These conclusions are strengthened by previous research which has shown that behaviorally derived refractory periods associated with the MPFC and CPu sites are longer than the ones usually obtained for the MFB, both beginning and ending later (Schenk and Shizgal 1982, Trzcińska and Bielajew 1992), indicating that different populations of fibers underlie self-stimulation at the two sites. Near the end of this study, a pilot test was conducted in which double pulses were delivered concurrently to an MFB electrode that supported self-stimulation and an ipsilateral MPFC site that did not (subject CH39); this strategy did not hasten the acquisition of MPFC self-stimulation. It thus appears that co-stimulation of the MPFC in conjunction with any self-stimulation site is not sufficient to evoke this behavior during a single shaping session. One possibility is that in order for the transference effect to occur, the two sites must be axonally connected, a hypothesis explored recently (Trzcińska and Bielajew 1998, in press), and supported by the observation that rewarding stimulation of a contralateral CPu placement did not give rise to transference of acquisition to the MPFC (subject CP65). It thus appears that in order to observe the acquisition of MPFC self-stimulation, it is not sufficient to simply incorporate bar pressing into the animal's behavioral repertoire. The site at which the rewarding stimulation is first encountered and its possible anatomical relation to the MPFC could be the determining factor. In relation to the MFB placements, one experiment showed that non-contingent lateral hypothalamic stimulation does not facilitate MPFC self-stimulation (Robertson et al. 1982a). In another study, bar pressing for non-contingent lateral hypothalamic stimulation hastened MPFC acquisition (Corbett et al. 1985). However, the latter group did not assess the MPFC sites prior to the delivery of hypothalamic stimulation, as was done here. It is conceivable that in that study the observed transference was a function of the specific MPFC placements, stimulation of which might have supported self-stimulation anyway, and not related to experience on the lateral hypothalamic site *per se*. In the present experiment, non-contingent stimulation was not employed to facilitate the acquisition of MPFC self-stimulation, as was done in other studies (Balleine et al. 1989, Corbett 1990), because rats either find this type of stimulation slightly aversive (Steiner et al. 1969, Ettenberg et al. 1981), or simply prefer to control the rate at which rewarding stimulation is administered (Tsang and Stutz 1984).

As far as the ventral tegmental area is concerned, studies have found that electrical stimulation of this region and a microiontophoretic application of dopamine into the prefrontal cortex result in an inhibition of spontaneously firing cortical cells (Ferron et al. 1984, Sesack and Bunney 1989). This inhibitory effect of ventral tegmental area upon the cells in the MPFC may be an explanation for why transference from this region to the MPFC does not occur.

Alternatively, the reason why transference was not observed from stimulation of all but one MFB placement, may be due to the fact that most target cortical areas, except for subjects CH39 and CP65, were located outside the Cg3 region. Anatomical studies suggest that the Cg3 area appears to be limbic in function, while other cortical regions support primarily motor and somatic functions (Kolb and Tees 1990). This may explain why a transference effect was observed following stimulation of the perifornical hypothalamus in subject CH58; its target cortical site was located in the Cg3 region. McGregor and his associates (1989) found that in some animals the acquisition of MPFC self-stimulation was rapid and seemingly no different from that of the MFB. In others the acquisition may have depended on the dissipation of an initially aversive or motorically suppressive effect induced by MPFC stimulation (McGregor et al. 1992). However, most studies indicate that stimulation of the MPFC does not seem to produce aversive effects, even at high currents. It may appear that the emergence of rewarding MPFC stimulation depends on the precise locus of stimulation (Robertson 1989).

The results of the present study also suggest that a kindling-like mechanism through activation of a related structure, such as the CPu, might underlie MPFC self-stimulation (Corbett et al. 1982a, Robertson et al. 1982b). This hypothesis is particularly interesting in light of the fact that self-stimulation of the MPFC and CPu is almost always accompanied by seizures, which become progressively more severe and as a consequence

more disruptive to the testing regimen (Herberg and Blundell 1969). There is some evidence that the epileptogenic activity might be crucial to the learning process involved in MPFC self-stimulation, because seizures are often more prominent following and not preceding acquisition of self-stimulation (McGregor 1992); prior to acquisition, arrest-like behavior is more prominent than the epileptogenic activity. In addition, experimental evidence suggests that the MPFC has subcortical and limbic cortical connections that may be instrumental in the propagation of seizure activity, which may contribute to the termination of self-stimulation in certain reward areas, as shown by generally low response rates for MPFC self--stimulation (Herberg and Blundell 1969). Further, it seems that the CPu receives convergent seizure activity from the MPFC, orbital and insular cortices, amygdala, and the hippocampus, the latter two constituting highly epileptogenic structures in the brain (Beckstead 1979, Parent and Hazrati 1995). In addition, midline CPu and MPFC stimulation appears to promote epileptogenic activity (Handford and Ackermann 1993).

Other evidence points to the fact that kindling of one limbic site alone can greatly increase seizure susceptibility of other secondary sites (Duchowny and Burchfiel 1981, Burchfiel et al. 1982), which suggest that kindling can modify neuronal excitability beyond the actual region of stimulation. Also, if the transfer site (MPFC) is monosynaptically linked to the primary site (CPu) then it might be rapidly or immediately kindled by the input from the primary site and subsequently demonstrate a similar pattern of discharge, or in this case a similar pattern of response acquisition (Spiller and Racine 1994). In addition, it is not simply striatal stimulation *per se* which facilitates the acquisition of MPFC self-stimulation; the CPu stimulation must be rewarding. This conclusion is supported by the observation that stimulation of CPu sites which showed high epileptogenic activity but no evidence of self-stimulation failed to facilitate MPFC self-stimulation; this occurred in two animals.

Based on the evidence presented here, including the overlap in charge values and the phenomenon of transference, it appears that MPFC and CPu form part of the same, yet distinct reward substrate from that of the MFB.

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