Research report

The substrate for brain-stimulation reward in the lateral preoptic area
I. Anatomical mapping of its boundaries

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Abstract

Given the putative role of the lateral preoptic area as a primary contributor of the cell bodies of origin of the descending pathway linking a subset of lateral hypothalamic and ventral tegmental area reward neurons, the distribution of self-stimulation sites in this structure was mapped in 22 animals using moveable electrodes and threshold procedures. Ninety-seven electrode sites were evaluated with placements ranging from just rostral to the midline convergence of the anterior commissure back to the transition zone between the lateral preoptic and lateral hypothalamic areas; of these, roughly 2/3 supported self-stimulation which was widely observed throughout the lateral preoptic area and medial forebrain bundle. In general, self-stimulation thresholds obtained from lateral sites were most stable, and progressively so approaching more caudal regions. Examination of the slopes of the period/current trade-off functions revealed a tendency for higher values in lateral and caudal sites; in contrast, dorsoventral excursions did not influence these estimates. Taken together, these data provide support for the notion that the substrate for brain-stimulation reward in the lateral preoptic area has a relatively homogeneous distribution that is more diffusely organized than that found in reward sites activated further caudally in the medial forebrain bundle.

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1. Introduction

The lateral preoptic area (LPO), situated rostral to the lateral hypothalamus (LH), has reciprocal connections with both the ventral tegmental area (VTA) and LH [33,40], and is linked to the latter by a short transition zone, frequently referred to as the bed nucleus of the medial forebrain bundle [29]; rostral and caudal parcellations correspond to the LPO and LH, respectively [15,16]. In view of its particular anatomical arrangement, the LPO is considered an important candidate for the cell bodies of origin of the descending pathway that links a subset of LH and VTA reward neurons [6,21,25,37,44]. However compelling this hypothesis, the LPO has not been systematically mapped for self-stimulation. For example, single cell recordings have shown that neurons in the LPO and the area between the preoptic and anterior hypothalamic nuclei respond antidromically to stimulation of the medial forebrain bundle (MFB) [32,36]. Cells responsive to activation of sites in the MFB that support self-stimulation are present in the lateral and medial preoptic areas, the ventral pallidum, and the parastraial nucleus, with a much larger region of antidromically driven neurons existing in and around the septal complex [38].

Metabolic markers have been used to further define regions selectively activated by self-stimulation electrodes in the MFB. Autoradiography with 2-deoxyglucose [13,34,35,39] has shown activation in the vertical limb of the diagonal band of Broca (included within the boundaries of the LPO in some studies [28]), the bed nucleus of the stria terminalis, the medial preoptic area with suppression of activity in the lateral habenula, as a consequence of self-stimulation in the anterior VTA [13]. Cytochrome oxidase [2] and glycogen phosphorylase
were aimed at the LPO; the flat-skull coordinates \( [31] \) During this phase, collection of full trade-off functions (TH22) which received a fixed electrode. The electrodes (Kinetrode Inc.) were implanted in all but one subject 2.4. Water were available ad libitum. Moveable electrodes a 12-h light / dark cycle with lights on at 07.00 h. Food and period functions at the baseline current were collected. Laboratories) were housed separately in plastic cages under achieved within two sessions, during which repeated rate / period functions at the baseline current were collected. 2.1. Subjects and surgery Twenty-two male Long–Evans rats (Charles River Laboratories) were housed separately in plastic cages under a 12-h light/dark cycle with lights on at 07.00 h. Food and water were available ad libitum. Moveable electrodes (Kinetrode Inc.) were implanted in all but one subject (TH22) which received a fixed electrode. The electrodes were aimed at the LPO; the flat-skull coordinates \( [31] \) ranged from 0.2 to 0.92 mm posterior to bregma, 0.6 to 2.0 mm lateral to the mid-sagittal suture, and 7.6 to 8.2 mm ventral to the skull surface reading at bregma. The anesthetic consisted of a combination of sodium pentobarbital (Somnotol, 65 mg/kg i.p.) and xylazine (Rompun, 0.05 ml i.m.); atropine sulfate (0.05 ml s.c.) was administered just prior to surgery in order to minimize respiratory distress.

2.2. Apparatus Electrical stimulation was delivered via an integrated circuit pulse generator, manufactured in house, and a constant-current amplifier [24]. The current was continuously monitored on an oscilloscope by reading the voltage drop across a 1 kΩ precision resistor in series with the rat. In order to prevent polarization at the electrode tip in the absence of a pulse, the output was shorted to ground via a 1 kΩ resistor.

2.3. Screening and training Three days after surgery, the animals were trained to lever-press on a continuous reinforcement schedule for 0.5 s trains of square wave monophasic pulses, 0.1 ms in duration, using conventional operant shaping techniques. If after two screening sessions no self-stimulation behavior was evident, then the electrode was moved a ventral distance of 0.08 to 0.24 mm, aided by a calibrated driver; the new site was evaluated the following day. This procedure continued until either bar-pressing was established or the full ventral travel of the electrode was used, based on the estimated location of the electrode tip from the Paxinos and Watson atlas [31]. During the training phase of the study, the animals were introduced to 60 s trials preceded by five priming stimulations, separated by 1 s, set at the same values as the trial parameters; the current was 200 μA. A descending sequence of periods was presented, starting with a value at which no response occurred. Each successive period was 0.1 log 10 unit below the previous one. The series continued until the bar pressing rate exceeded the criterion of 35 responses/min. A rate/period curve was plotted for each current and the period threshold, i.e. the period required to support the criterion response rate was determined. The testing phase, during which complete trade-off functions were generated, was initiated when stable thresholds were obtained. The criterion for stability was met when the standard error of the mean associated with the average period threshold across sessions did not exceed 10% of the mean value. Generally, stable thresholds were achieved within two sessions, during which repeated rate/period functions at the baseline current were collected.

2.4. Behavioral tests During this phase, collection of full trade-off functions
was conducted; the currents used ranged from 80 μA to 1000 μA, incrementing in 0.2 log_{10} units. The period thresholds, as previously described, were then plotted in order to generate the trade-off relationship between the period threshold and the current. If the period thresholds associated with a baseline current administered at the beginning and at the end of each session differed by more than 0.1 log_{10} units, the data from the session were discarded. When the trade-off functions were established for a range of currents, the electrode was moved and the site tested anew using the same procedure. An example of a rate/period function generated at different currents has been published elsewhere [19].

2.5. Histology

Following administration of a lethal dose of sodium pentobarbital, animals were perfused intracardially with saline, followed by 10% formalin. The brains were removed and stored in 10% formalin for at least 1 week. Coronal sections (40 μm) were placed on coated slides and stained with Cresyl Violet in order to better locate the electrode tips using the Paxinos and Watson atlas [31].

3. Results

3.1. Histology

The electrode placements were divided into five groups, based on anteroposterior position: the first was located anterior to the LPO, the second, third, and fourth groups were found within the LPO nucleus at roughly anterior, mid, and posterior levels, and the fifth group was situated posterior to the LPO, in the transition zone between the LPO and the LH.

The placements are shown in Fig. 1. Those of the first group (n=4 rats, 10 sites) were found to be just rostral to the midline convergence of the anterior commissure (Fig. 1, plate 1). This level is dominated by the caudate-putamen and the nuclei of the precommissural septum which includes the horizontal and vertical limbs of the diagonal band, the medial septum, and compartments 'a' and 'b' of the MFB [29]. The second group (n=7 rats, 24 sites) is made up of electrodes located around or near the anterior commissure and the fornix, as yet not bifurcated, are still evident. The LPO is bordered medially by the medial preoptic area, laterally by the ventral pallidum and substantia innominata, dorsally by the bed nuclei of the stria terminalis, and, ventrally by the nucleus of the horizontal limb of the diagonal band. Three subjects (20 sites) contribute to the third grouping of electrode sites (Fig. 1, plate 3). This level is characterized by a large, well-defined third ventricle, the movement of the medial preoptic area from the midline to a more lateral position, and a more dispersed optic chiasm. The LPO is bordered by the same structures as at the previous rostral level. The fourth group (n=4 rats, 19 sites) had placements at the intersection of the lateral ventricles with the midline (Fig. 1, plate 4). Here, the LPO is bordered medially by the medial preoptic area, dorsally by the bed nucleus of the stria terminalis,
laterally by the horizontal limb of the diagonal band, and
ventrally by the supraoptic nucleus at the base of the brain.
The fifth group of electrodes \( (n=4 \text{ rats, 25 sites}) \) was
situated posterior to the LPO at the level of the anterior,
lateroanterior, and lateral hypothalamic areas (Fig. 1,
plates 5 and 6).

3.2. Mapping of self-stimulation sites

The composite drawing in Fig. 1 shows both the positive
and negative self-stimulation sites; where appropriate,
intersecting electrode tracks are combined. Self-stimulation
was observed at 60 (61%) of the 98 placements that were
evaluated. These included five sites from which threshold
determinations could not be obtained because the threshold
response rate or the stability criteria were not met (see
Materials and Methods). Positive sites are denoted by
closed circles; open circles mark those locations at which
self-stimulation could not be elicited. Maximum response
rates tended to be twice the criterion of 35 responses per
minute across rats. The presence or absence of self-stimu-
lation did not predict seizure and associated motor activity
which were observed in both groups. The typical pattern of
behavioral responses (sniffing, chewing, grooming, etc.)
was more likely to accompany positive rather than nega-
tive sites, but a thorough evaluation of the behavioral
profile was not undertaken. It may be that additional
screening sessions would have increased the yield of
positive sites; however, our experience with the LPO to
date does not suggest that a gradual acquisition of self-
stimulation characterizes this structure.

Subjects with similar placements produced consistent
results. The one exception was in the most anterior
stimulation site (see Fig. 1, plate 1) where the two subjects
that did bar-press had electrode tracks that were indis-
tinguishable from the two subjects that failed to respond to
the stimulation; however, the positive sites in this case did
not support stable self-stimulation.

In general, medial sites, including the medial preoptic
area and nucleus, did not support self-stimulation (Fig. 1,
plates 2, 4, 5, and 6); if bar-pressing was observed, it was
not robust enough to generate trade-off functions. In
contrast to the medial preoptic area, the majority of LPO
sites was associated with vigorous self-stimulation (plates
2, 3, and 4). The bed nucleus of the stria terminalis, a more
dorsal structure, was also positive for self-stimulation
(plate 2) at least at its more rostral level. Robust self-
stimulation was generally elicited from the compartments
of the MFB and the LH (plates 2, 3, and 5); high response
rates were associated with lateral sites, particularly in more
caudal regions.

3.3. Characteristics of the substrate for self-stimulation
in the LPO

Fig. 2 demonstrates the consistency of the trade-off
between period threshold and current for a representative
animal in which seven sites were evaluated from a total of
51 across animals. Except for the most ventral placement
in this example, the relationship between current and
period was roughly constant with electrode move; this
pattern was observed in all animals.

While there was no obvious slope variation in the
trade-off functions generated at different dorsoventral sites,
this does not provide any interpretation about the ordinate
position of the curves. To examine this, we plotted for
each subject the period thresholds as a function of dor-
soventral electrode position for a low and high current.
This compresses the full trade-off functions such as shown
in Fig. 2 to two points representing roughly the extremes
of the curves. Fig. 3a, b, and c illustrate these results in
individual animals, with the subjects organized according
to anteroposterior coordinates. As noted above in subject
TH21 (see Fig. 2), the typical displacement of the curves
associated with each current was obtained given the
Fig. 3. The period thresholds obtained at different electrode depths corresponding to a low current, 200/250 μA, and a high current, generally 1000 μA, for all subjects with thresholds that met the stability criterion are shown on the right side. Each subject is identified by the alphanumeric designation on the left side of the figure, next to the corresponding plate traced from the Paxinos and Watson [31] atlas. The anteroposterior coordinate of each group appears at the top of each figure (−0.40 to −1.30).
characteristic trade-off between current and period — as current increases, there is a compensatory increase in period (or decrease in frequency); this effect was generally maintained as the electrode was lowered, suggesting no change in the trade-off property of the substrate at different depths. Finally, electrode position produced little alteration in reward efficacy except in the mid-LPO region (note dorsoventral coordinate of \(-8.2\) mm) where the high current in particular gave rise to higher period thresholds, interpreted as an increase in the rewarding effect of the stimulation.

A regression analysis was conducted on the individual trade-off functions, which were generated at each dorsoventral site. Only in those cases where the \(r^2\) value exceeded 0.80, an indicator that the data were reasonably linear, were slope values included in the comparison. The \(r^2\) value did not exceed 0.80 in five (or roughly 10%) of the trade-off functions. In three of these, the curves that failed to meet this criterion were observed at dorsal stimulation sites.

Taking into consideration the three-dimensional coordinates of the stimulation sites, the regression slopes were examined for patterns of change in the anteroposterior, mediolateral, and dorsoventral axes. Because no particular order in the slope values obtained from excursions in the dorsoventral direction for any subject were noted, we collapsed these slope values to generate an error term and arranged the average value for each subject in Fig. 4, according to group (which is based on the anteroposterior dimension — see description above), and mediolateral coordinate. Displayed in this manner, the data suggest a tendency for slope values to increase with distance from the midline, particularly in more caudal placements. Groups 2 and 3, which had more sites associated with each mediolateral coordinate than the other two groups, were examined for statistical significance using a simple 1-way ANOVA design. The overall test was significant only in Group 3 \((F_{2,16} = 24.56, P = 1.3 \times 10^{-7})\). The post-hoc comparison revealed a difference between the most lateral slope (2.00) and those of the coordinates 1.10 and 1.40 \((F_{1,16} = 48.16, P = 3.0 \times 10^{-6})\), which did not differ from each other. Nonetheless, a linear trend was observed in this group \((F_{1,16} = 30.26, P = 4.8 \times 10^{-5})\). The single subject in the LH group (Group 5) was found to have a comparable slope value to those reported in other LH placements [18].

4. Discussion

The LPO is a structure that has been examined in a variety of experimental paradigms intended to uncover the origin of the cell bodies of the descending pathway linking LH and VTA reward neurons [5,6,26]. While the results of lesioning of cells in the LPO [21,25,44], recording from cells in the LPO [27,32,36], and measuring the metabolic activation of LPO neurons [1,10,13,20,22,28,34,35], all of these manipulations following MFB self-stimulation, have suggested that the LPO may play a key role in the function of this reward pathway, a systematic investigation of self-stimulation in this area has not been reported. The results of this study suggest that self-stimulation can be elicited throughout the LPO; however, the slopes of the trade-off
functions appear to vary more as a function of anteroposterior and mediolateral position than electrode depth. Specifically, the trade-off profiles tended to be marked by higher slopes as more lateral and caudal sites were sampled, values that match the ones that we have obtained in our laboratory for the substrate underlying LH and VTA self-stimulation. Several factors may contribute to this finding, including density and differences in the excitability and/or value of the postsynaptic signals generated by the relevant neurons [11,45,46]. Recovery from refractoriness as an index of excitability suggests that there are no differences across LPO sites in this measure [3,12]. Specific tests for assessing changes in postsynaptic influence were not carried out in this study. However, several aspects of this study favour the first hypothesis, that our data reflect changes in the density of the relevant substrate. Based on anatomical descriptions of the MFB, this view carries face validity, in that the lateral progression of positive sites paralleled the rostrocaudal trajectory of the MFB [29,43]. On other grounds, it seems the most reasonable explanation. For example, we have consistently observed that LPO currents are higher than the values required to elicit self-stimulation from either the LH or the VTA. This is best examined in situations where currents are selected in order to match period thresholds between sites, such as is required in behavioral collision experiments (two electrode designs); this manipulation is needed so that the degree of summation between two sites that are concurrently stimulated can be evaluated [37]. In the subsequent article [3], the nature of the axonal connectivity was assessed between the LPO and VTA; with minor exception, the LPO current was 2 to 5 times the VTA value. Similarly in previous collision studies [4,5,37], aimed at examining LH and VTA linkage, the LH current was typically twice that of the VTA. The few LPO sites at which direct axonal connectivity with the VTA was demonstrated were located at relatively anterior LPO levels and were characterized by shallower trade-off functions than that obtained in the VTA [3]; note that at least some of these neurons are common to both sites. While there may be other explanations for this phenomenon, the most parsimonious one in our view is that the caudal and lateral projections of the substrate underlying reward in the MFB are more dense.

Self-stimulation could be elicited from activation of sites located throughout the LPO and compartments ‘a’ and ‘b’ of the MFB [29]. For all groups, except the most anterior one, the dorsal sites were less likely to support the behavior and even more so if they were medially situated. The presence or absence of self-stimulation at a particular location was generally consistent between animals with the exception of the most anterior group; the behavior obtained from these particular placements never exceeded either the criterion response rate or the stability requirement. These erratic results may reflect a paucity of reward-relevant neurons in this area or the recruitment of inhibitory or competing systems which interfere with the elicitation of self-stimulation.

The location of positive and negative self-stimulation sites, extending from the area anterior to the midline convergence of the anterior commissure caudally to the transition area between the LPO and LH, suggests a projection that moves more laterally in its caudal direction. For example, it was possible to elicit self-stimulation from a medial site, 0.9 mm lateral to the midline, in the anterior LPO group, while in the LH group, negative self-stimulation sites were observed up to 1.6 mm lateral to the midline. Indeed, except for the most anterior group, lateral placements always supported self-stimulation while medial sites were far less likely to do so. Note that the latter conclusion is based on only 36 sites, of which 8 were positive, and therefore warrants additional investigation.

The period/current trade-off function produces estimates of both the neural density and the spatial boundaries of the relevant substrate [46]. Through the use of moveable electrodes the trade-off functions of a single subject can be examined for both a departure from linearity and the current at which the curve begins to asymptote; these observations can be used to infer the spatial limits of the reward substrate for that subject [7,11,42]. No consistent pattern was found in either the correlation between electrode placement and the observation of a curvilinear function or the current intensity at which the curve began to asymptote for the subjects in this study.

The relative density of the behaviorally relevant neurons can be inferred from the slopes of the regression lines relating period threshold to current intensity. These values did not differ greatly across LPO stimulation sites, especially when the average of the slopes within each group (anterior, middle, and posterior LPO) were compared. Within individual groups, differences in slope were detected only in Group 3, which showed a predominant linear trend. In contrast, the average slope value across sites for the single subject in the LH group, which was comparable to slope estimates obtained at other LH placements [18], was substantially greater, suggesting that the reward neurons in the LPO are less densely packed than in the LH.

Within the LPO, certain trends were observed that suggested an increase in neural density as more caudal sites were stimulated. Thresholds increased as the stimulation site moved caudally (see Fig. 4, groups 2–4); particularly at high currents, the threshold rose by an average of 39%. This pattern is consistent with the anatomical literature [15,29] which depicts a profile of greater neural density at more caudal LPO sites.

Of particular relevance to the interpretation of this study is the assumption that neurons with varying thresholds for activation are homogeneously distributed within the effective stimulation field. It has been shown that the LPO comprises mainly diffuse, thin, and poorly myelinated fibers, in contrast to the LH which contains a wide variety of fibers ranging from small unmyelinated to small or
coarsely myelinated neurons extending to the VTA [43]. A potential difference in composition between LPO and LH reward neurons is supported by the behaviorally derived measures of refractoriness and estimates of conduction time between these two structures [6,12]. If these LPO neurons, thin and poorly myelinated, are the ones that underlie self-stimulation, they should be more difficult to activate than corresponding LH ones; slope differences, similar to those obtained in this study, could be observed and not be due to relative differences in neural density [45]. Given this concern, it would be useful to determine if the relevant LPO neurons are the same ones that course downstream through the MFB. One direct manner to determine whether the same neurons underlie self-stimulation in the LPO and more caudal MFB sites is the use of the behavioral collision test [3]. The following paper presents the results from such an experiment.

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References


