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The Effects of Electrolytic Lesions of the Lateral Preoptic Area on LH and VTA Self-Stimulation Behavior

Myriam Levy

A Thesis

in

The Department

of

Psychology

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ABSTRACT

The Effects of Electrolytic Lesions of the Lateral Preoptic Area on Lateral Hypothalamus and Ventral Tegmental Area Self-Stimulation

Myriam Levy

Much of the research in the field of self-stimulation has been aimed at identifying the directly-activated neurons subserving the rewarding effect of medial forebrain bundle (MFB) self-stimulation. Two experiments were carried out to assess the involvement of lateral preoptic area (LPOA) neurons. In experiment 1, recovery from refractoriness in the neural substrate for self-stimulation of the LPOA was estimated using psychophysical techniques. In 6 out of 7 rats, recovery from refractoriness began at around 0.5-0.7 ms and approached asymptote by approximately 2.2-29 ms. Stimulating electrodes were located in the LPOA and in the case of two rats, in the ventral pallidum (VP). Estimates of refractoriness were found to overlap with those previously established for sites in the anterior basal forebrain and for more posterior MFB self-stimulation sites. Nonetheless, the recovery curves obtained at the LPOA sites approached asymptote later than curves previously obtained at more caudal MFB sites. In experiment 2, the effects of electrolytic lesions of the LPOA on self-stimulation of the LH and VTA were assessed using curve-shift scaling. Changes

in reward effectiveness were inferred from lateral displacements of the rate-frequency functions collected at three currents. In two rats, increases in the required number, ranging from approximately 0.1 to 0.25 log10 units were observed. Effective lesions were located mostly in the LPOA and VP. However, in the case of the remaining 9 rats, the lesions failed to produce substantial, long-lasting changes in the required number. The results from these experiments suggest that neurons of the anterior basal forebrain contribute to the rewarding effect of MFB self-stimulation.

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Finally, I thank my husband Andreas for his words of wisdom and his words of love, which kept me on the right track. Without his help, patience, and understanding, the road to finishing this thesis would have been impracticable...

Dedication

I would like to dedicate this thesis to my family, whose endless love and support has made this endeavour possible.

TABLE OF CONTENTS

LIST OF FIGURES

In their seminal paper, Olds and Milner (1954), discussed the phenomenon of intracranial self-stimulation as providing a methodological basis for the study of the physiological mechanisms of reward. Their suggestion was based on the fact that electrical stimulation delivered to appropriate brain sites produced acquisition and extinction curves comparable with those produced by conventional rewards. Many attempts have been made since then to forge a link between the reward effect produced by brain stimulation reward (BSR) and that of natural reinforced behaviors such as feeding (Hoebel, 1969; Rolls, 1972). To that end, it has been found that the neurons involved in stimulation-induced feeding share common properties with those involved in BSR (Gratton & Wise, 1988). Wise (1980) has proposed that BSR results from activation of neural circuits underlying normal appetitive behaviors and maladaptive behaviors such as self-administration of drugs of abuse. Identifying the directly stimulated cells that carry the reward signal away from the electrode tip (first-order neurons), would allow us to put to a more rigorous test the relationship between BSR and motivation towards naturally occurring goal objects. The aim of this project is to find regions of the central nervous system important to stimulation-induced reward effects.

Mapping studies, testing for self-stimulation at different brain loci, provide a means of localizing structures important for BSR. Olds (1956), and Olds and

Olds (1963) established that stimulation in sites along the medial forebrain bundle (MFB), including the lateral hypothalamus (LH) and ventral tegmental area (VTA) was rewarding. To date, much research has been aimed at identifying neurons in the directly-activated substrate for the rewarding effect of MFB stimulation. The MFB is a fiber bundle consisting of more than fifty independent fiber pathways connecting the limbic forebrain with various structures of the midbrain through the hypothalamus (Nieuwenhuys, Geeraeddts, & Veening, 1982). Ascending monoamine pathways have been shown to coextend along the MFB (Lindvall, 1979; Ungerstedt, 1971).

Initially, first-order neurons were thought to be catecholaminergic. The coincidence of the trajectory of monoaminergic fibers and self-stimulation sites along with early reports that self-stimulation is influenced by catecholaminergic agonists and antagonists (German & Bowden, 1974; Olds, Killam, & Bachy-Rita, 1956; Olds & Travis, 1960; Stein, 1962) pointed to a possible involvement of catecholamine neurons in BSR. From among the catecholaminergic fibers, dopamine fibers were found to be of primary importance for BSR (German & Bowden, 1974; Phillips & Fibiger, 1973).

Numerous pharmacological studies investigating the neurochemical substrates of self-stimulation have been

carried out and have provided support for the involvement of ascending dopamine neurons, particularly the mesolimbic pathway, in MFB self-stimulation. This pathway terminates in limbic structures such as the the nucleus accumbens, septal area and caudate putamen (Fallon & Moore, 1978; Lindvall, 1979). It has been demonstrated that drugs that increase dopaminergic transmission facilitate self-stimulation while those that interfere with this transmission attenuate the reward effect (Fibiger, LePiane, Jakubovic, & Phillips, 1987; Gallistel, Boytim, Gomita, & Klebanoff, 1982; Gallistel & Freyd, 1987). Thus, on pharmacological grounds, dopamine neurons appeared to play a role in BSR. Nevertheless, psychophysical experiments demonstrated that first-order neurons have characteristics incompatible with those of dopamine neurons.

Psychophysical Approach

The various fiber pathways coursing through the MFB have very different physiological characteristics. If the characteristics of the reward-relevant axons are known, the search for their origin will be limited to a subset of the projections funnelling through the MFB. In that respect, psychophysical studies provide us with a portrait of neurons likely to carry the reward signal. This portrait becomes very useful during electrophysiological recordings from regions suspected to contain reward-relevant neurons. Cells

that are activated by stimulation of sites known to support self-stimulation, and with properties matching those derived psychophysically can be considered candidate cells, likely to be first-order neurons.

Psychophysical experiments often use the trade-off procedure whereby a change in one parameter of the stimulation is balanced by a change in another parameter so as to keep behavior at a constant level. For example, an increase in the pulse frequency produces a change in the behavior. To restore behavior to its previous level, the current must be reduced to a new value. By trading-off frequency and current at different values, a trade-off function is derived which describes the relationship between the two parameters. From this relationship, the spatiotemporal integration of the reward signal can be modelled.

Indeed, the trade-off between current and frequency has shown that, over an appreciable range of both parameters, there is a scalar relationship between the inverse of pulse frequency and current (Simmons & Gallistel, submitted). This is to say that doubling the current reduces pulse frequency by a factor of two. Assuming that a) all reward axons are uniformly distributed around a point source electrode, b) that the number of first-order neurons fired is proportional to the current, and c) that the product of the number of

axons fired and the pulse frequency equals the total number of firings, the reward magnitude is determined by the total number of action potentials regardless of their spatiotemporal distribution (Gallistel, Shizgal, & Yeomans, 1981). Thus, there is no difference between the rewarding impact produced by 1000 axons firing one action potential and 500 axons firing two action potentials.

The trade-off procedure can be used only if the stimulus-response relationship is monotonic. Monotonicity implies that each input to the system yields one output and one output only; linear and sigmoid relationships are monotonic. A non-monotonic relationship, for example a "U-shaped" input-output curve, whereby the same output can be achieved by more than one stimulus input, violates the previously mentioned assumption which is at the heart of trade-off experiments. The BSR system has been shown to possess this property of monotonicity over a unique domain of various stimulation parameters (Gallistel, 1978). For instance, the rate-frequency curve relates the rate of responding to the pulse frequency, while keeping all other stimulation parameters constant. This curve is monotonic over a range of frequencies corresponding to the initiation and levelling off of maximum responding, termed the dynamic interval.

Refractory period experiment

One of the neuronal characteristics that can be inferred by means of the psychophysical approach is the refractory period: the time required for an axon to recover after firing one action potential before another action potential can be fired. If an axon is stimulated twice, first with a conditioning pulse (C-pulse) and then with a test-pulse (T-pulse), two action potentials will be generated, given that enough time, equal to or greater than the refractory period, has elapsed before the administration of the T-pulse. In conventional single-unit recordings, the refractory period of an axon can be measured by estimating the minimum amount of time required before the administration of the T-pulse produces a second action potential. As the time interval between the C and T pulse is lengthened, the T-pulse will eventually be administered after the state of refractoriness of the axon has dissipated, and will fire the axon.

The electrophysiological technique of determining refractory periods has been modified to allow for the behavioral determination of the estimates of the refractory periods of the directly-stimulated neurons (Deutsch, 1964; Yeomans, 1975). In these experiments, trains of C-T pulses are administered through a stimulating electrode. The number of pulse pairs per train is systematically varied so as to construct a rate-frequency curve; by interpolation, the

number of pulse pairs required to support a half-maximal level of responding (required number) is derived from this curve. The required number of pulse pairs is subsequently compared to the number of single pulses required to support a similar level of responding. When the C-T interval is very short, such that T-pulses are administered while all the directly-activated neurons are refractory from the preceding C pulses, none of the T-pulses will be effective in eliciting action potentials. In this case, the required number of pulse pairs will equal the required number of pulses. Responding for these higher frequency trains of paired-pulses at the shorter C-T intervals will be similar to responding under the single C-pulse condition. Thus, the threshold under this paired-pulse condition will not differ from the threshold observed under the single C-pulse condition.

However, if the time interval between the C and T pulses is sufficiently long for the T-pulses to be delivered after all of the directly-activated reward neurons have recovered from refractoriness from the preceding C-pulses, the T-pulses will be as effective in eliciting action potentials as the C-pulses. Responding for trains of these paired-pulses will therefore differ from the responding for trains of single pulses. The required number of pulse pairs will be half of the required number of single pulses. The scaling formula devised by Yeomans (1975) expresses the effectiveness of the T-pulses as a proportion of the effectiveness of the C-pulses

($E = N_{sp}/N_{Ct} - 1$), where E = effectiveness of the T-pulse, N_{sp} = threshold for single pulses, and N_{Ct} = threshold of pulse-pairs. In the case where none of the reward-relevant fibers are fired by the T-pulse, the effectiveness value would be equal to 0. If all the neurons are fired by the T-pulse, then the effectiveness value would be equal to 1. Since the refractory periods within a population of neurons vary, the function that relates C-T interval to the effectiveness of the T-pulse in producing action potentials rises gradually and assumes a sigmoidal shape.

Using this technique, investigators have examined the refractory periods of various brain sites that support self-stimulation. Refractory period curves for diencephalic MFB sites have generally shown a sharp rise between C-T intervals of 0.5 to 1.5 ms (Bielajew, Jordan, Ferme-Enright & Shizgal, 1981; Bielajew & Shizgal, 1982; 1986; Macmillan, Simantirakis & Shizgal, 1985; Rompré & Miliaressis, 1980; Yeomans, 1975). The characterization of reward-relevant neurons has been extended to areas in the anterior basal forebrain, such as the lateral preoptic area (LPOA), the horizontal limb of the diagonal band (HDB), and the nucleus accumbens (Acb) (Fouriezos, Walker, Rick, & Bielajew, 1987; Bielajew, Thrasher, & Fouriezos, 1987). The results show a rise in effectiveness beginning at C-T intervals of 0.2 to 0.4 ms and reaching asymptotic effectiveness at test intervals between 4-5 ms. For two rats, with electrodes located in the LPOA,

this value ranged between 1.6-2.0 ms. As can be observed, the refractory periods in the basal forebrain are relatively longer than those reported at more caudal sites along the MFB. Estimates of refractory periods in the prefrontal cortex (Schenk & Shizgal, 1980), periaqueductal grey (Bielajew et al., 1981), midline hindbrain and thalamic (Rompré & Miliaressis, 1985) brain-stimulation sites also appear to be longer than those estimated at more caudal MFB sites. The observed difference between the refractory period estimates could be attributed to additional populations of neurons with slower refractory periods contributing to the reward signal at different sites. Nevertheless, it should be emphasized that the refractory period curves, obtained from sites that support self-stimulation, overlap in a considerable portion of their range.

Portrait of the reward substrate

Refractory periods are but one of the characteristics of the first-stage neuron that can be determined using psychophysical techniques. By delivering the C and T pulses through two distinct electrodes, one can perform the behavioral version of the collision technique which is used to infer the connection, via reward-relevant fibers, between two sites that support self-stimulation. Shizgal, et al. (1980) and Bielajew and Shizgal (1982) have shown that at least some of the reward-relevant fibers connect the LH and

VTA. Combining the collision technique with the refractory period test has allowed experimenters to estimate the conduction velocity of the axons carrying the reward signal. Those range between 1 and 8 m/s (Shizgal & Murray, 1989). Taking into account the established relation between fiber diameter and conduction velocity (Waxman & Bennett, 1972), reward-relevant fibers appear to be small and myelinated. A variation of the collision technique has been used to demonstrate the directionality of the rewarding signal. At least some of the neurons responsible for the reward effect of MFB stimulation give rise to axons that descend from the LH and project at least as far as the VTA (Bielajew & Shizgal, 1986). Electrophysiological single-unit recordings anterior to the LH have shown that cells in areas such as the septum and the substantia innominata (SI) are fired by stimulation of the LH or the VTA and possess characteristics similar to those obtained psychophysically (Murray, 1993; Rompré and Shizgal, 1986; Shizgal, Schindler, & Rompré, 1989).

The Lesioning Approach

Psychophysical studies have advanced our knowledge of the substrate of the circuitry underlying BSR. For instance, the findings from these studies rule out ascending, unmyelinated axons (such as those of catecholaminergic neurons) as the principal components of the directly-

activated fibers. However, the psychophysical approach alone cannot establish the origin or origins of the fibers directly activated by BSR especially when considering the fact that a third of the myelinated axons in the MFB could be considered as likely candidates. That is, it is possible that a particular area in the brain contains cells that have anatomical and physiological characteristics similar to those of directly-activated neurons, yet are not involved in the rewarding effect. Lesions can be used as a direct means of assessing if candidate neurons are responsible for the reward effect. If damaging or destroying a particular structure thought to contain neurons with appropriate characteristics, decreases the reward effect, then it is likely that this structure contains first-order neurons.

Interestingly, many lesion studies have failed to demonstrate a significant reduction in the reward effect of self-stimulation following lesions in various brain nuclei. This failure could, to a certain degree, be attributed to poor measurement techniques that did not appropriately quantify changes in the rewarding impact of the stimulation. Early efforts to identify the substrate for BSR often involved the use of rate of responding to quantify the effects of lesions. However, this method has been shown to confound changes in reward with changes in the animal's ability to perform the required response (Edmonds & Gallistel, 1974; Hodos & Valenstein, 1962; Miliaressis, et

al., 1986; Stellar, Waraczynski & Wong, 1988). The alternative to response-rate measures accepted by many currently-active researchers is the curve-shift paradigm, which permits changes in the reward efficacy to be at least dissociated from performance-affecting variables (Edmonds & Gallistel, 1974; Miliaressis, Rompré, Laviolette & Coulombe, 1986).

The Curve-Shift Paradigm

In the curve-shift paradigm, the rate of lever-pressing is measured over a range of frequencies such that the rat's response varies from zero to a maximal level. Lateral shifts in the rising portion of the rate-frequency function along the abscissa reflect changes in the rewarding impact of the stimulation while changes in asymptotic rates seem to indicate changes in the animal's capacity to respond. Decreasing the current, at least over a substantial range, and therefore decreasing the number of reward-relevant fibers activated, produces a log parallel shift of the rate-frequency function towards higher pulse frequencies while leaving asymptotic rates intact (Edmonds, Stellar, & Gallistel, 1974; Simmons & Gallistel, submitted). Furthermore, validation studies have shown that manipulations that interfere with the animal's performance abilities, such as making the animal run up a gradient, injections of drugs, or weighting the lever, significantly decrease the asymptotic

rate of responding, while producing relatively small (Edmonds and Gallistel, 1974; Fouriezos, Bielajew, & Pagotto, 1990; Miliaressis, et al., 1986) or no change in the lateral position of the curve (Stellar, et al., 1988). Thus, the curve-shift method is particularly sensitive to the effects of a lesion on reward efficacy, while changes in the response rate reflect a wide range of lesion-induced deficits.

Lesion Experiments Using the Curve-Shift Method

The lesion experiments using curve-shift scaling have only been partially successful in demonstrating substantial increases in threshold for self-stimulation. Colle and Wise (1987) have found that large forebrain ablations, which included all or part of the frontal cortex, Acb, septal area and olfactory tubercule, ipsilateral to the LH stimulating electrode resulted in only modest increases in the required number thresholds. Lesions in half of the subjects did produce substantial increases in the required number (0.15 log10 units) but the values subsequently returned to baseline over several weeks of testing.

A concern pertaining to large lesions is that they might remove both agonistic and antagonistic reward systems. If such is the case, no major changes in the rewarding impact of the stimulation should be expected. Indeed, the postulate of antagonistic systems has some relevance in explaining the

decreases in threshold that Waraczynski (1988) obtained in some of her subjects. The decrease in threshold might be due to compensatory behavioral effect produced by lesioning neurons antagonistic to the first-order neurons. She also demonstrated that knife-cuts in the diagonal band/medial septal region and in the MFB just anterior to the stimulating electrode, including the medial preoptic area (MPOA) failed to produce substantial, consistent changes in threshold. Moreover, some of her cuts actually increased the reward effectiveness. Her conclusion was that the directly activated substrate for MFB self-stimulation does not stem from neurons located rostrally to the LH.

To address the concern associated with making large lesions, smaller and better defined electrolytic lesions were tried. Electrolytic lesions of the amygdaloid complex (Waraczynski, Conover, and Shizgal, 1992) and the dorsomedial hypothalamus (Waraczynski, Ng Cheong Ton, and Shizgal, 1990) failed to produce any marked changes in reward efficacy of LH and VTA self-stimulation. To investigate the possibility that the intrinsic hypothalamic neurons have an important role in BSR, Stellar, Hall, and Waraczynski (1991) performed excitotoxin lesions which covered the LH. Nevertheless, their results were rather disappointing insofar as large effects were only seen when demyelination encroached on the tip of the stimulating electrode.

Although the results from the abovementioned studies were reported as negative, a certain number of lesions were in fact effective. Careful examination of the sites whose removal was effective in degrading reward might reveal an area worth of further investigation. For instance, in Waraczynski's (1988) study a group of rats with knifecuts in the posterior LPOA had appreciable rightward curve-shifts. A closer examination of Stellar's et al. (1991) data reveals that shifts of approximately 0.1 $\log_{10}$ units were obtained in the absence of demyelination around the electrode tip. These effective lesions produced damage to several areas including the LH and the LPOA. Janas and Stellar (1987) have found relatively substantial and consistent changes in BSR effectiveness with unilateral knife cuts transecting the anterior MFB, at the level of the caudal LPOA. These effects ranged from 0.2 to 0.5 $\log_{10}$ units. In all, it seems that the region anterior to the LH electrode, including the LPOA, might play a role in MFB self-stimulation.

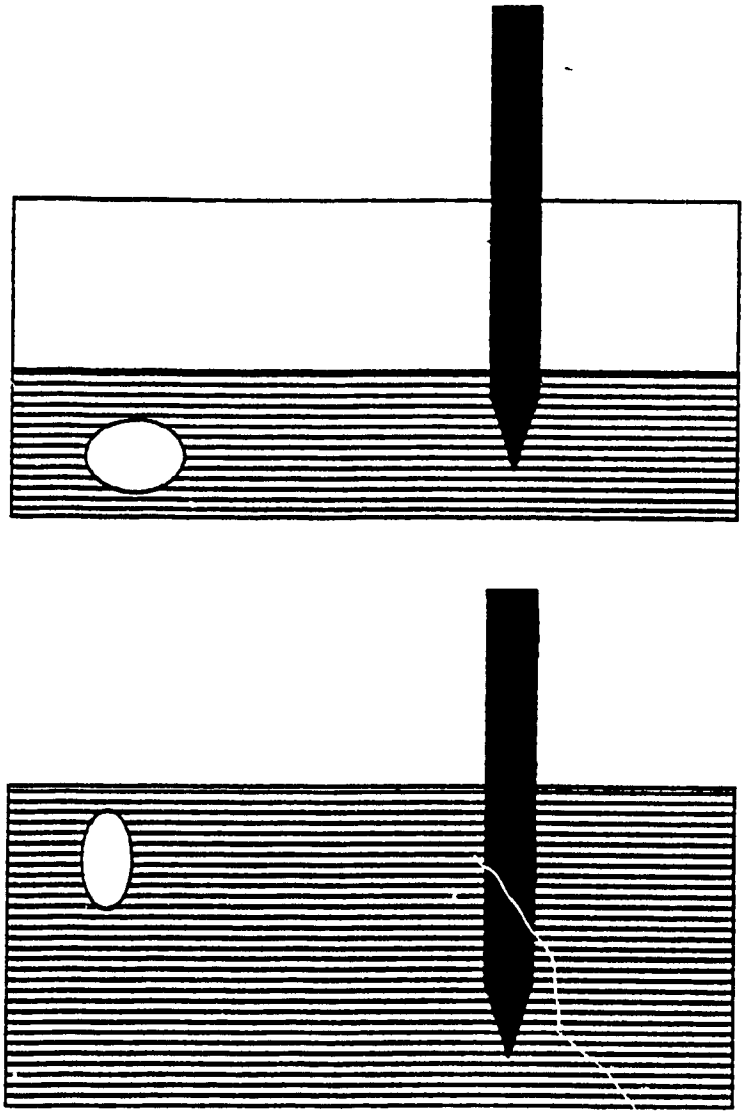
Murray and Shizgal (1991) made electrolytic lesions at the border of the anterior LH (ALH) and the LPOA and found increases in the threshold for LH or VTA self-stimulation of approximately 0.1-0.2 $\log_{10}$ units in five out of seven subjects. In the case of one rat, thresholds were measured at two currents. Following the lesion, a long-lasting increase in the threshold was observed at the lower current only. This finding could explain some of the inconsistencies

observed in other lesion experiments. It suggests that the alignment between the lesion and the stimulating electrode might be of importance.

When the axons of the lesioned neurons pass close to the tip of the stimulating electrode, the effect of the lesion can be observed at a low current. In the case where the axons of the lesioned neurons are far removed from the electrode tip, a low current would not reveal any effect. Increasing the current will increase the field of effective stimulation, and thus recruit more reward-relevant axons, including those actually damaged by the lesion. Thus, high currents increase the chance of overlap between the stimulation field and the lesioned axons (See Figure 1).

Murray (1993) further investigated the role of anterior basal forebrain neurons in MFB self-stimulation. Using the three-current design, she assessed the impact of ALH electrolytic lesions on more posterior MFB stimulation sites. She found that in 5 out of 14 subjects, lesions of the anterior LH and surrounding regions resulted in long-lasting increases in the required number of pulses, ranging from 0.1 to 0.25 log10 units, for LH and VTA self-stimulation. These results lend further support to the idea that neurons in anterior regions of the basal forebrain contribute to the rewarding effect of stimulating more posterior MFB sites.

Fig. 1 Effect of current on orientation of lesion and stimulating electrode. In the first panel, the lesioned axons and the tip of the electrode are aligned, allowing for the detection of an effect at the low current. In the second panel, the lesioned axons are removed from the fibers close to the electrode tip. In this case, increasing the current results in an activation of more reward-relevant axons, including those damaged by the lesion. An effect is more likely to be observed at a high current. The lesion is indicated by the open ovals and the stimulating electrode is drawn in black. Stimulated fibers are represented by horizontal lines.



Rationale for the present experiments

A reevaluation of previous lesion studies has revealed the possible involvement of the ALH and the LPOA in MFB self-stimulation. Murray and Shizgal (1991) and Murray (1993) have provided direct evidence for the role that the anterior LH plays in BSR. However, the importance of the LPOA remains to be determined. In that light, two experiments were conducted to assess the hypothesis that neurons of the anterior LPOA are part of the directly-activated substrate for self-stimulation of more caudal MFB sites. In the first experiment, estimates of the refractory periods of reward-neurons activated by LPOA stimulation were obtained using psychophysical methods. These estimates were compared to previously determined estimates of other reward-relevant neurons of the MFB. The second experiment assessed the hypothesis that the LPOA contributes to the substrate for self-stimulation of more caudal MFB sites. Changes in self-stimulation of the LH and VTA were measured, at three different currents, following an electrolytic lesion of the LPOA. If the neurons in the LPOA are involved in the directly-activated substrate, then it is expected that the lesions will shift the rate-number curves towards higher pulse numbers, at least at some currents.

Experiment 1

Previous work has suggested that neurons in the LPOA contribute to the rewarding effect of stimulating the LH and the VTA (Janas & Stellar, 1987; Murray & Shizgal, 1991; Waraczynski, 1988). Furthermore, evidence from a collision experiment has shown that the LPOA and LH are directly-linked (Bielajew, et al., 1987). In addition, refractory periods obtained from LPOA sites overlap with those obtained from more posterior sites (Bielajew, et al., 1987; Fouriezos, et al., 1987). In the present experiment, refractory period estimates were collected from stimulation of more anterior parts of the LPOA. These were compared to refractory periods of the anterior basal forebrain and more posterior MFB sites, obtained in previous experiments. If these sites share a common reward substrate, it is expected that their refractory periods will be similar.

Method

Subjects

Seven male rats of the Long Evans strain (Charles River Breeding Farms), weighing 350-500 g at time of surgery were used as subjects. They were individually housed in plastic cages with wire lids, given unlimited access to standard lab chow and water, and kept on a reverse 12 hour dark/light cycle. All testing took place during the dark period of the

cycle.

Surgery.

Atropine sulphate (0.5 mg/kg, i.p) was administered to the animals 20 minutes before anaesthesia to reduce mucous secretions. Animals were deeply anaesthetised using sodium pentobarbital (Somnotol, 65 mg/kg, i.p.) with supplements administered as required.

The anaesthetised rat was secured in a stereotaxic instrument and the incisor bar adjusted so as to level the skull. Electrodes were made from a 0.25 mm diameter stainless steel rod, insulated with Formvar except for the tip, with an insulated wire soldered to the shaft, 11 mm from the tip. An electrode was aimed at the LPOA, using the following coordinates: 0.3-0.4 mm posterior to bregma, 1.8-2.5 mm lateral to the mid-sagittal suture and 7.2-7.6 mm ventral to the dura. A stimulating electrode was also implanted in the lateral hypothalamus with the following coordinates: 2.8 mm posterior to bregma, 1.7 mm lateral to the mid-sagittal suture, and 7.5-7.8 mm ventral to the dura as well as in the ventral tegmental area (VTA): 4.8 mm, 0.9 mm, and 7.5-7.8 mm respectively. The LH and VTA electrodes were used in Experiment 2 only. The anode was an uninsulated wire with a male amphenol pin crimped to one end, wrapped around four to five jeweller's screws embedded in the

skull to support the electrode assembly.

Once the electrodes were implanted, dental acrylic was used to cement them to the skull and skull screws. The amphenol pins for each electrode and the ground wire were inserted into a nine-pin, externally threaded connector and cemented to the rat's head with dental acrylic. Using an internally-threaded ring, this connector was attached, during testing, to a matching connector mounted at the end of the stimulation cable.

Once the animal had recovered sufficiently from anaesthesia, a dose of 5.0 mg/kg of morphine sulphate was administered (i.p.) to reduce post-surgical pain. Subjects were given at least five days post-operative recovery before initial testing was undertaken.

Apparatus

Screening/Training Apparatus.

Subjects were screened for self-stimulation in wooden boxes measuring 25 cm(w) x 25 cm(d) x 70 cm(h), with Plexiglas front panels and wire mesh floors. At the centre of the left wall was a Lehigh Valley rodent lever placed at 5 cm above the floor. Approximately 5 cm above the lever was a yellow light, 1.5 cm in diameter. The light was turned on

whenever stimulation was available, but turned off for the duration of each stimulus train. The stimulation cable attached to the 9-pin connector on the animal's head was connected to the stimulator by a 7-channel slip-ring commutator mounted in the centre of the ceiling of the box. This allowed the animal to move freely about the cage.

Pressing the lever triggered a 0.5 s train of cathodal, rectangular pulses, 0.1 ms in duration. Train duration remained constant throughout all phases of the experiment. The temporal parameters of the stimulation were controlled by integrated circuit pulse generators with the specific values set manually. The amplitude of the pulses was set by dual constant-current amplifiers (Mundl, 1980), and monitored on an oscilloscope by measuring the voltage drop across a 1 kΩ resistor in series with the rat. Charge accumulation at the brain-electrode interface was minimized by a circuit that shorted the stimulator outputs through a 1 kΩ resistor when no pulse was present.

Testing Apparatus.

Testing was conducted in computer-controlled operant chambers that were similar to the manually-operated ones. Only those aspects that differ will be described.

The test chambers were Plexiglas boxes measuring 25

cm(w) x 25 cm(d) x 75 cm(h) with hinged doors on the upper half of front panels and wire mesh floors. Two levers were mounted on opposite walls of the boxes, 5 cm from the floor and 5 cm from the nearest corner. A yellow light, 1.5 cm in diameter was mounted 3 cm above one lever and a red light positioned similarly above the other lever. Only the lever under the yellow light was used. The test chambers were enclosed in 50 cm x 50 cm x 90 cm Plywood boxes insulated with 2.5 cm of Styrofoam. A Plexiglas window in the removable front panels allowed viewing of the animals from a separate room using a remote-controlled video camera. A 40 W bulb illuminated the test chamber, and an 11.5 cm fan provided ventilation.

The parameters of stimulation for each of the test cages were controlled by a microcomputer with a custom-built interface. A bank of relays controlled by the parallel port of the microcomputer determined which electrode delivered the stimulation. Stimulation current was determined by a digital to analog converter attached to a voltage-controlled constant current amplifier (a modified version of Mundl's (1980) design).

Procedure

Screening.

Initially, subjects were screened for self-stimulation of the LPOA site using a current of 200 uA and a train of 50 pulses. When the stimulation appeared to rouse the animal, that is if increased sniffing and exploring were observed, or if the stimulation elicited no observable response, the current and number of pulses were increased and attempts were then made to shape reliable self-stimulation at a rate of at least 50 presses a minute. If stimulation via any electrode the stimulation was aversive, that is if the animal attempted to escape from the stimulation, or if the animal could not be shaped to press the lever within three daily sessions, the electrode was not used.

Training.

Once the animal acquired the lever-pressing response, the number of pulses and the current were set so as to produce consistent and vigorous behaviour. The animal was then allowed free access to the lever for approximately one hour. Once this behaviour was established, the animals were trained on subsequent sessions using the following procedure. Prior to each trial, the rat received 5 trains of non-contingent priming stimulation with the same parameters as

the ones used for the reward stimulation. The light above the lever was illuminated indicating that the lever was armed. Following the priming, there was a 30 s period during which the subject could obtain the stimulation by pressing the lever. At the end of this period, the lever light was turned off and the stimulation withdrawn until the animal no longer pressed the lever. Another trial was then started, beginning with the priming stimulation. This was continued until the animal reliably returned to the lever following the priming, and quit when the lever-light was extinguished. When this was achieved, rate of responding was recorded over a range of stimulation parameters to be used in later testing.

Self-stimulation performance was stabilized by repeatedly determining the number of stimulation pulses required to maintain bar-pressing at a half-maximal rate. After a brief warm-up period, the same paradigm was used but the the number of pulses per train was decreased every trial in 0.05 log10 unit steps until the animal failed to respond more than 10 times on two consecutive trials. The required number, defined as the number of pulses that would support a half-maximal rate of responding, was calculated by interpolation. After four to five determinations, the testing phase was initiated.

Testing.

Estimates of the refractory periods of the neurons stimulated by the LPOA electrode were collected using the computer-controlled apparatus. One current (400 uA-800 uA depending on the subject) that produced reliable self-stimulation was tested in each subject. Stimulation was delivered through the LPOA electrode and the time between the delivery of the first pulse (C or conditioning pulse) and the second pulse (T or test pulse) was varied (C-T interval). These C- and T-pulses were of equal amplitude. A rate-number curve was collected for each of the C-T intervals tested (paired-pulse condition). Nine to eleven C-T intervals were tested in each session, ranging from 0.2 to 6.4 ms. Two single-pulse rate-number curves were collected at the start of each testing session, one single-pulse curve in the middle of the session, and one at the end of the session. The first single pulse rate-curve was considered a warm-up and discarded from the data analysis. The procedure for determining rate-number curves was similar to the one described in the training phase of the experiment except that in the paired-pulse condition, the number of pulse-pairs was decreased across trials instead of the number of single pulses. A different C-T interval was tested during each double-pulse determination and the order of presentation was chosen so that long and short C-T intervals were interdigitated. The C-T intervals were randomly presented in

one of two orders, with the second the reverse of the first. Between 7 and 17 sessions were run for each subject.

Data Analysis.

In a given session, the required number of single pulses was used to estimate the relative effectiveness of the paired-pulses. Effectiveness values were obtained for each C-T interval using Yeoman's formula:

$$E = [Nsp]/Nct-1]$$

where

E = effectiveness of the T-pulse

Nsp = average of the required number of single pulses for that session.

Nct = required number of pulse pairs for a given C-T interval

The average effectiveness value for each C-T interval was then plotted as a function of C-T interval. The C-T intervals corresponding to 5% and 95% of the range of recovery were calculated by interpolation and plotted on these graphs.

Histology

Histology was obtained following the completion of

Experiment 2 (See experiment 2 for Histology). The estimated centroid of the largest coronal cross-section of the lesion made in Experiment 2, was used as the location of the LPOA electrode tip for rats tested in Experiment 1.

Results

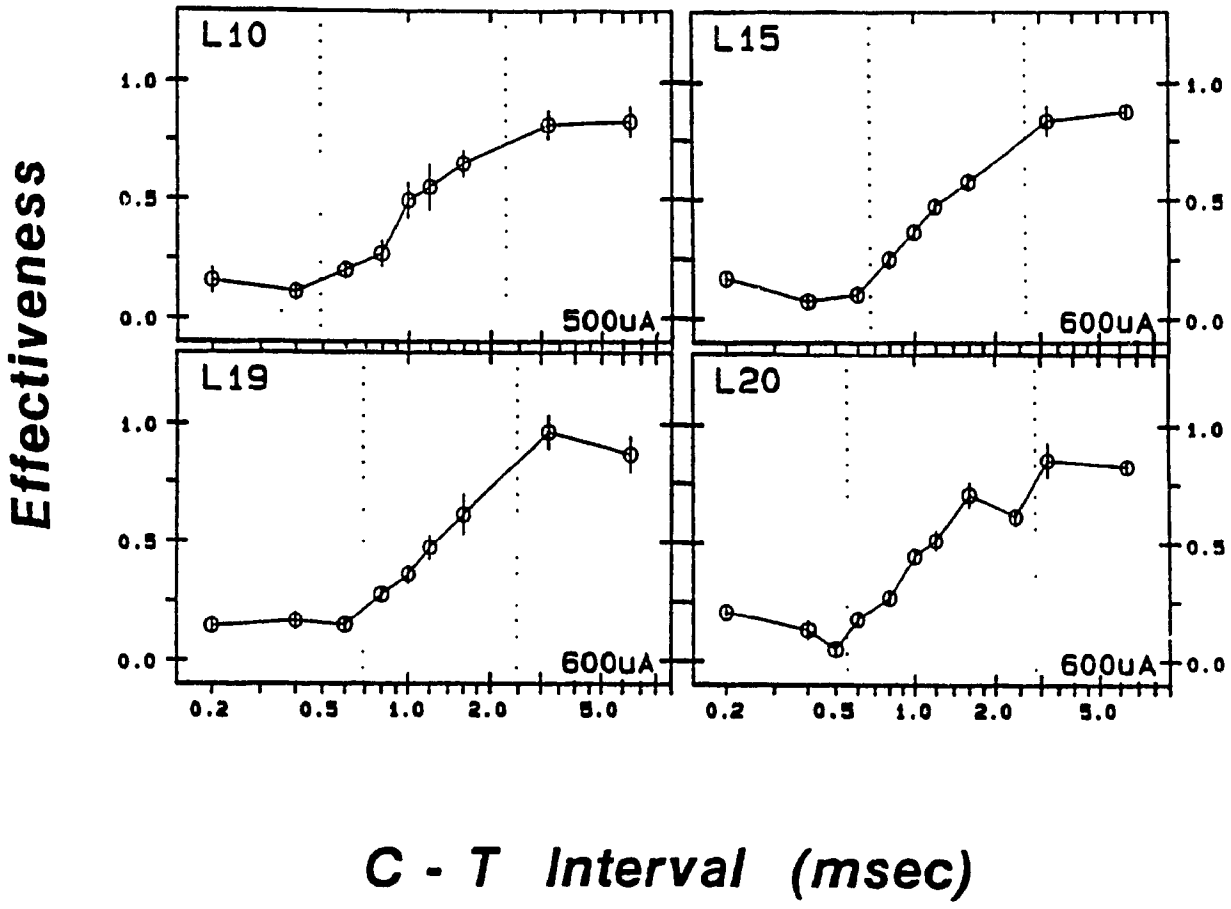
Refractory Period Data

Refractory period data for the 7 subjects tested are shown in Figures 2-3. T-pulse effectiveness values are shown on the y axis, and C-T intervals are shown on the x axis. The vertical dotted lines on the graphs denote the C-T intervals, in ms, corresponding to 5% and 95% of the range of recovery. The current is indicated in the bottom-right corner of each graph.

The beginning of recovery was estimated to occur between C-T intervals of 0.5 and 0.7 ms based on the 5% recovery point. The end of the recovery occurred between C-T intervals of 2.2 and 2.9 ms as indicated by the 95% recovery point for all subjects except for L23 where recovery was not 95% complete until a C-T interval of 4.5 ms. Table 1 shows the 5% and 95% range of recovery estimates. Note that the estimated beginning of recovery varies little across subjects, whereas the end of recovery is more variable.

In the case of several of the refractory period curves, the rise in effectiveness values stopped or slowed temporarily (L20, L23, L30). In all cases, these plateaus occurred at C-T intervals of 1.6 to 2.5 ms.

Figs. 2-3 Refractory period data for subjects L10, L15, L19, L20, L23, L30 and L31. The alphanumeric label in the top-left of each figure identifies the subject, and the current is given in the lower right-hand corner. The vertical dotted lines represent 5% and 95% of the range of recovery. Data points without error bars correspond to cases where the standard error of the mean (s.e.m.) was less than the radius of the symbol.


L10
500uA
L15
600uA
L19
600uA
L20
600uA
1.0
0.5
0.0
0.2
0.5
1.0
2.0
5.0
Effectiveness
C - T Interval (msec)

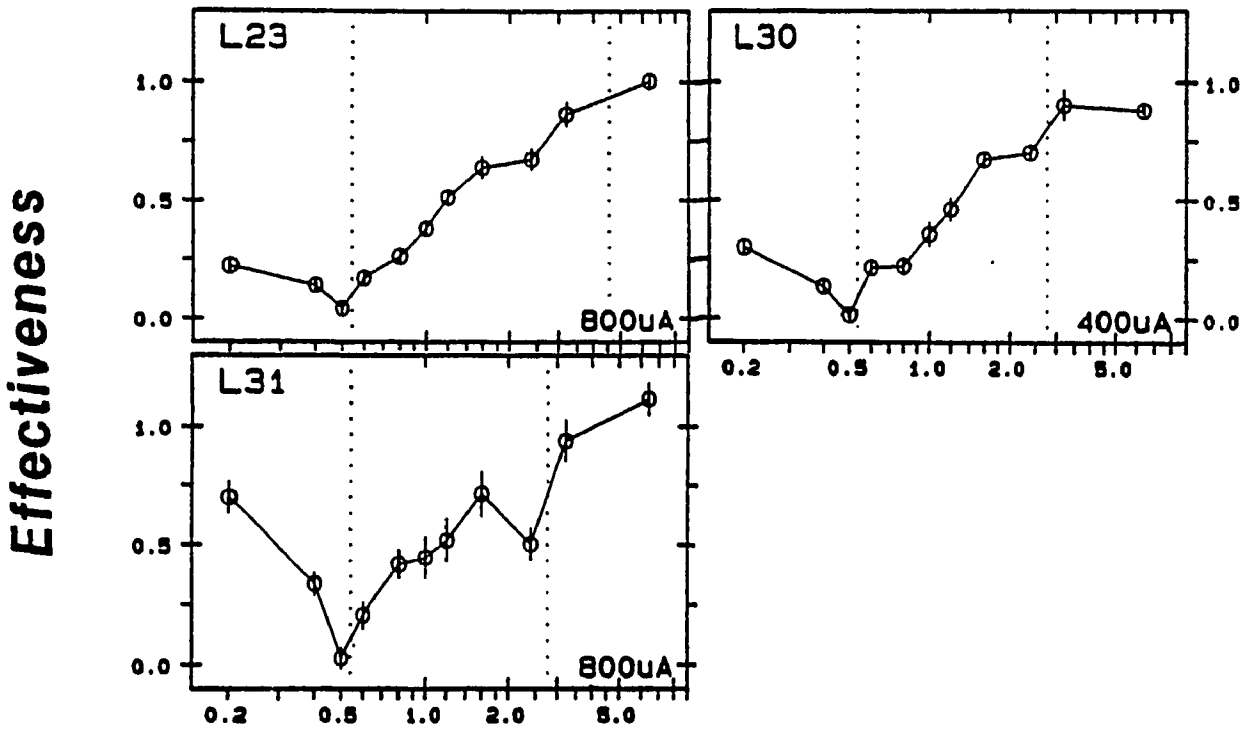

L23
800uA
L30
400uA
L31
800uA
1.0
0.5
0.0
0.2
0.5
1.0
2.0
5.0
Effectiveness
C - T Interval (msec)

Table 1. Values for 5% and 95% of the range of recovery for subjects in refractory period experiment.

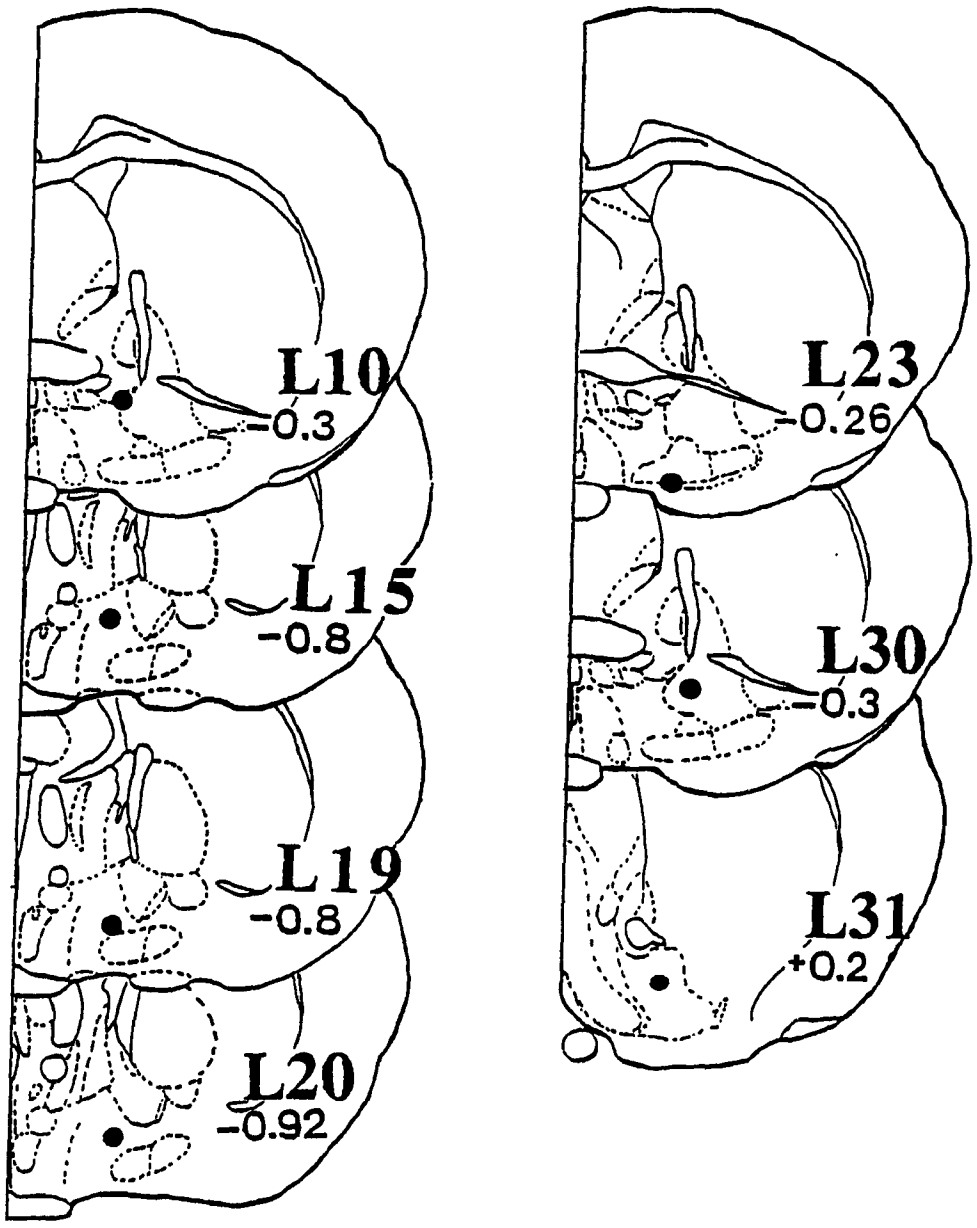
		PERCENT RECOVERY	
RAT	**CURRENT**	**5% RECOVERY**	**95% RECOVERY**
L10	500uA	0.49	2.26
L15	600uA	0.67	2.62
L19	600uA	0.68	2.47
L20	600uA	0.54	2.86
L23	800uA	0.54	4.57
L30	400uA	0.53	2.78
L31	800uA	0.54	2.75

Stimulating electrodes

Estimates of the placement of the stimulating electrode in Experiment 1 were taken as the middle point of the largest cross-section through the lesion made in experiment 2 (Figure 4). However, at best, these estimates provide only a rough approximation of the location of the tip.

In most cases, the estimates for the electrode tips were scattered around the LPOA. In the case of L30 and L31, the tip was located in the VP. In the case of L23, where slower refractory periods were observed, the electrode was located in the HDB.

Fig. 4 Estimated location of electrode tips in the refractory period experiment based on the location of the lesion. Stimulating sites are indicated by filled circles on tracings of coronal plates from the Paxinos and Watson atlas (1986). The distance of each plate from bregma is given in the bottom corner of each plate, along with the alphanumeric label identifying the subject.


L10
-0.3
L15
-0.8
L19
-0.8
L20
-0.92
L23
-0.26
L30
-0.3
L31
+0.2

Discussion

Refractory Periods

The majority of the refractory period curves obtained from stimulation of the LPOA sites began to rise over C-T intervals of 0.5 ms and reached asymptote at C-T intervals of 2.2 to 2.9 ms. In one case, recovery from refractoriness was complete at 4.5 ms (L23). The time course of the recovery at the LPOA, in this experiment, overlaps but is not identical to the recovery profile previously obtained from stimulation of this region. Bielajew, et al. (1987) have found that refractory periods for LPOA sites ranged from 0.4 to 6.3 ms. Fouriezos, et al (1987) obtained refractory period determinations for two rats with electrodes located in the LPOA. In the case of one rat, asymptotic effectiveness was obtained at C-T intervals of 4-5 ms, while in another, recovery occurred earlier, at around 1.6-2 ms. The disagreement with the present results concerns the asymptotic effectiveness which, between rats in other studies, was found to vary by approximately 3 ms. This variability is surprising in view of the stability of refractory period determinations observed in this study. It is possible that the reward-relevant neurons located at the LPOA are not homogeneous. If this is the case, small differences in electrode location might result in the differential recruitment of reward-relevant axons having longer refractory

periods. Unfortunately, the validity of this hypothesis cannot be assessed because, in the case of the data presented here, a lesion was made after collecting refractory period curves. Thus, the exact location of the electrode tips is not available. In the case of L23, where longer refractory periods were obtained, the stimulating tip was located in the HDB. These results are consistent with those of Fouriezos, et al. (1987), who found similar refractory periods in this area. Movable electrodes could provide a means of testing the hypothesis that small changes in electrode location can account for the variability in refractory period estimates.

As mentioned in the introduction, collision-like effects were observed between self-stimulation sites in the LPOA and the LH (Bielajew, et al., 1987). Furthermore, lesions that impinged on the LPOA have been shown to increase the required number for self-stimulation of the LH and VTA (Janas & Stellar, 1987; Murray & Shizgal, 1991; Waraczynski, 1988). The rationale behind collecting refractory period estimates from the LPOA is that this region appears to contain reward-relevant cells whose fibers project through the LH. If such is the case, one would expect these estimates to compare to the excitability properties of neurons in the LH. Previous refractory period estimates obtained from stimulation of the middle or posterior LH range from 0.4 to 1.5 ms (Bielajew & Shizgal, 1982; Bielajew, Lapointe, Kiss, and Shizgal, 1982; Macmillan, et al., 1985; Rompré & Miliaressis, 1980; Shizgal

& Murray, 1989; Yeomans, 1979). The curves in this case, approach asymptote faster than the ones obtained for the LPOA. The overlap is consistent with the notion that a common population of neurons is activated by rewarding stimulation of the LH and LPOA. However, there appears to be a population of neurons with slower refractory periods that contribute to the rewarding effect of stimulating the LPOA, yet is absent from MFB sites caudal to the anterior-posterior level of the ventromedial hypothalamus. This slower component seems to play a role in the refractory period estimates from sites anterior to the LH, as close as the ALH and as anterior as the Acb, including the diagonal band (Bielajew, et al. 1987; Fouriezos, et al., 1987).

Additional evidence for the recruitment of slower fibers in anterior sites stems from collision experiments. Collision-like effects have been demonstrated when stimulating the LPOA and the LH suggesting a continuity of fibers between these two sites (Bielajew, et al., 1987). However, the collision intervals in this case were relatively longer than those seen in collision between posterior sites such as the LH and the VTA (Bielajew & Shizgal, 1982; Bielajew & Shizgal, 1986; Shizgal, et al., 1980). Furthermore, in the case of some subjects, step-like increases in the effectiveness values of the collision curves were observed. In some cases, these abrupt increases were observed at C-T intervals of approximately 1.0 ms and again

at C-T intervals of approximately 7 ms. These data further suggest that a more heterogeneous population of reward-relevant fibers are recruited at the anterior MFB sites with neurons exhibiting slower conduction properties.

In some subjects, at C-T intervals of 1.6 to 2.5 ms, a plateau in effectiveness values was observed. This plateau may suggest a discontinuity in the recruitment of reward-relevant population fibers stimulated by the LPOA electrode. Gratton and Wise (1985), Rompré and Miliaressis (1987), and Murray (1993) have also observed these plateaus for stimulation sites in the metencephalon, posterior and anterior LH sites, respectively. In these cases, the plateau occurred a different C-T intervals suggesting the contribution of different subsets of reward-relevant neurons.

In support of the notion that different populations of fibers are recruited, it has been observed that maximum effectiveness values were observed at C-T intervals as short as 1.2 ms some LH sites (Gratton & Wise, 1985; Rompré & Miliaressis, 1980). Furthermore, at self-stimulation in the MFB and basal forebrain recovery from refractoriness has been shown to begin at C-T intervals as long as 1.0 ms (Fouriezos et al , 1987).

Experiment 2

Psychophysical data have suggested that directly-activated neurons within the LPOA have characteristics that overlap those of more posterior MFB self-stimulation sites (Bielajew, et al., 1987; Fouriezos, et al, 1987). The fact that collision can be obtained between the LPOA and the LH suggests a continuity of fibers between these two sites (Bielajew, et al., 1987). Lesions of this area have been previously shown to attenuate the rewarding effect of LH and VTA self-stimulation (Janas & Stellar, 1987; Murray & Shizgal, 199; Waraczynski, 1988). However, no systematic investigation of the LPOA was carried out. The present experiment assessed the hypothesis that neurons within the LPOA contribute to the rewarding effect of self-stimulation of the LH and the VTA. Some of the subjects previously tested in Experiment 1 were also tested in Experiment 2. Electrolytic lesions were made, and changes in the rewarding effect of the stimulation were inferred from lateral displacement of rate-frequency curves. To maximize the chances of detecting effects, these rate-frequency curves were obtained at three different currents.

Method

The apparatus and procedures used in Experiment 2 were similar to those described in Experiment 1. Only those

aspects of Experiment 2 that differ from experiment 1 will be described below.

Subjects and Surgery

Thirteen male rats were included in this phase of the experiment. Five of these subjects were previously used in Experiment 1 (L19 and L23 were excluded). A stimulating electrode was implanted in the LH with the following coordinates: 2.8 mm posterior to bregma, 1.7 mm lateral to the mid-sagittal suture, and 7.5-7.8 mm ventral to the dura, as well as in the VTA: 4.8 mm, 0.9 mm and 7.5-7.8 mm respectively, at the same time as the LPOA electrode, used as the lesioning electrode in this experiment, was implanted.

Procedure

During a daily session, four rate-number curves were collected at each of the currents determined during training, using the LH and VTA electrodes that supported self-stimulation. Three currents, equally spaced in logarithmic units, were used. If possible, the currents were chosen so that they spanned a 0.60 log10 unit range (0.30 log10 unit increments). Currents ranged between 200 and 1260 uA. If animals could not be trained with these currents, the increments between the currents were varied.

One session per day was run per stimulating electrode. The procedure for collecting data for each rate-number curve was the same as in the training phase described in Experiment 1. Only those aspects that differ will be described.

Each data point was obtained by determining the rat's response rate during 30 s trials. In some cases, the rat's response rate was very low, so the length of the trial was increased to 40 or 60 s trials. Prior to each trial, the overhead light was extinguished for 0.5 s followed by 5 trains of non-contingent priming stimulation. In rats where strong motor effects were present, only 2 trains of stimulation were delivered. This priming stimulation had the same parameters as the reward stimulation. The starting number of pulses was chosen so that performance during the first three trials would be at an asymptotic level. The number of pulses was then decreased systematically in 0.05 log unit steps until the rat failed to respond more than ten times on two successive trials. The LH and VTA electrodes (if applicable) were always tested in the same order.

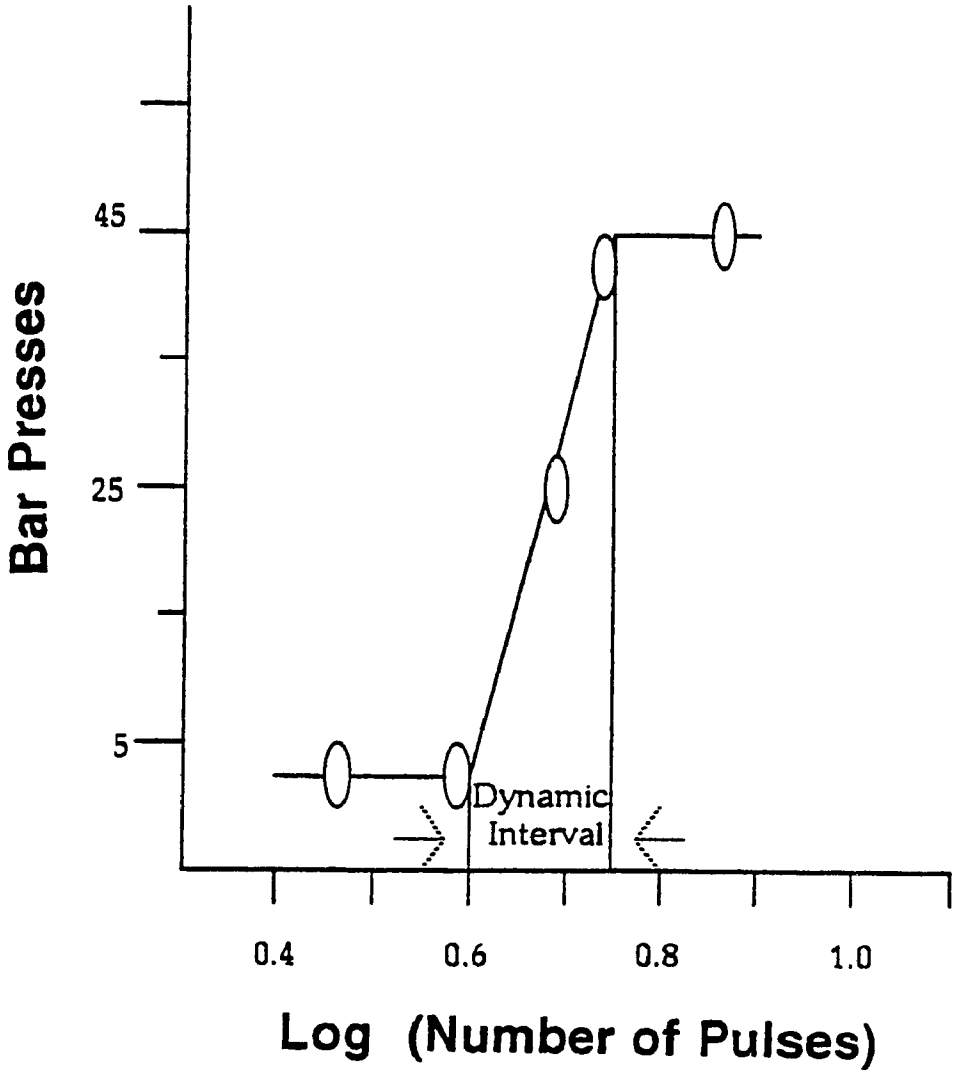
Data Analysis

Rate-number curves were constructed by plotting the response rate as a function of the logarithm of the number of pulses at each current. The first curve at each current was considered a warm-up and was not used in the data analysis.

The number of pulses required to support a half-maximal rate of responding was calculated by interpolation. The mean and standard error of the required number of pulses for each current were calculated for each test session and plotted as a function of pre and postlesion days. Maximum rates for each of the determinations at each current used to interpolate the half-maximal number of pulses were averaged, and standard error of the mean (s.e.m.) was calculated for each pre and postlesion day at each current.

A "broken-line" function, as described by Gallistel and Freyd (1987) was fit to each of the rate-number curves used in the threshold analysis in the case of L20(VTA) and L30(LH). An example of such a function is shown in Figure 5. This function is composed of a straight line that joins lower and upper horizontal asymptotes. The four parameters of the broken line function were chosen so as to minimize the residual sum of squares. The dynamic interval (the range over which the function rises) was obtained by calculating the distance along the abscissa between the upper and lower break points. Dynamic intervals for each of the determinations at each current were averaged and the s.e.m was calculated. These values were subsequently plotted as a function of days. A summary of the average position, on the number axis, of the pre and postlesion curves was also constructed in the cases of rats L20 (VTA) and L30 (LH).

Fig. 5 Example of a broken-line function fit to hypothetical rate-number data (open circles). The dynamic interval was estimated from the difference between the upper and lower break points.


Bar Presses
45
25
5
Dynamic
Interval
0.4
0.6
0.8
1.0
Log (Number of Pulses)

Electrolytic Lesions

Following baseline data collection, an electrolytic lesion was produced in all subjects by passing a direct current of 1.0 mA for ten seconds, with the LPOA electrode serving as the anode and the skull screws serving as the cathode. Testing resumed at least an hour following the lesion, at the same currents tested prior to lesion. Further testing was conducted to determine the stability of any lesion effects.

Statistical analysis

To scale post-lesion changes in terms of the prelesion variability, 95% confidence intervals were constructed around the mean of the required number of pulses, the mean of the maximum response rates, and the mean of the dynamic intervals obtained for the 7 days of prelesion baseline. The standard deviation (S.D.) of the 7 means was used as an estimate of the s.e.m. (Ferguson and Takane, 1989, p.163). Confidence intervals were obtained (as per Waraczynski, 1988) by multiplying the S.D. of the 7 baseline days by the t values associated with the $p = 0.05$ level of significance for 6 degrees of freedom ($t = 2.447$). As a rule of thumb, data obtained on any particular postlesion day could be considered significantly different from baseline if they differed by more than 2.447 standard deviations from the mean.

Histology

At the end of the experiment, the subjects were given an overdose of Sodium Pentobarbitol (Somnotol) and perfused transcardially with 0.9% saline followed by 10% formalin. Prior to the perfusion, marking lesions were made in some subjects to facilitate localization of the electrode sites. A direct current of 0.1 mA was passed for 15 seconds using the LH or VTA electrode as the anode and the skull screws as the cathode. Following perfusion, the brains were removed from the skulls and soaked in 10% formalin. In the case of the animals with the marking lesions, the brains were soaked for 24 hours in a solution of Prussian Blue, which produces a blue iron deposit at the lesion sites. They were then placed in a 10% formalin solution. Twenty-four hours prior to slicing, they were soaked in a 10% sucrose formalin solution. The brains were frozen in dry-ice and sliced in sections 30 um thick using a microtome. The slices were then mounted on gelatin-coated glass slides. After 24 hours, the slices were stained with formol thionine.

The lesions were reconstructed by locating the sections with the most rostral sign of the lesion, the largest cross-section of the lesion and the most caudal sign of the lesion. These were traced on plates from the Paxinos and Watson (1986) atlas of the rat brain in the coronal plane.

Results

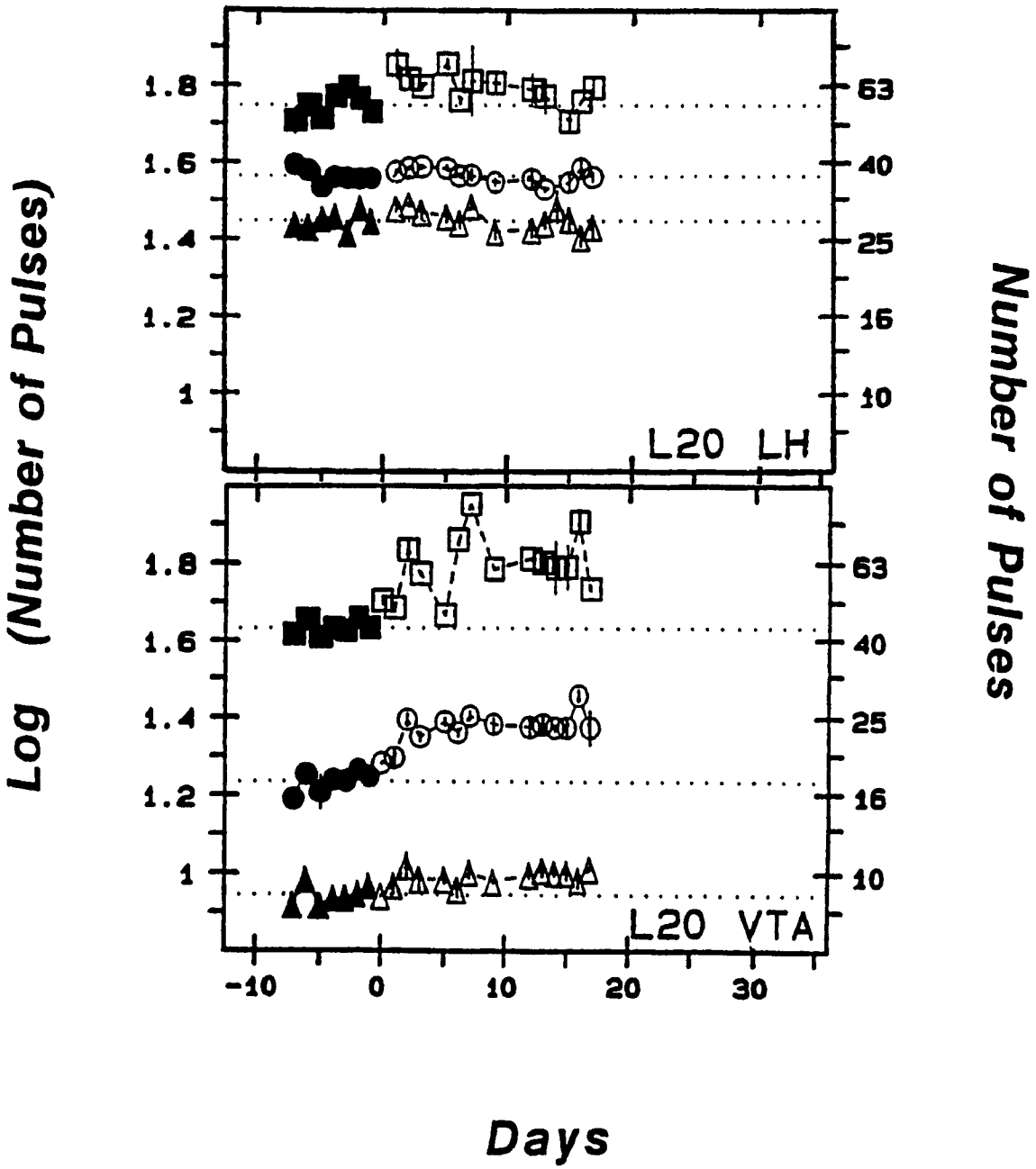
Effects of lesion on rate-number functions

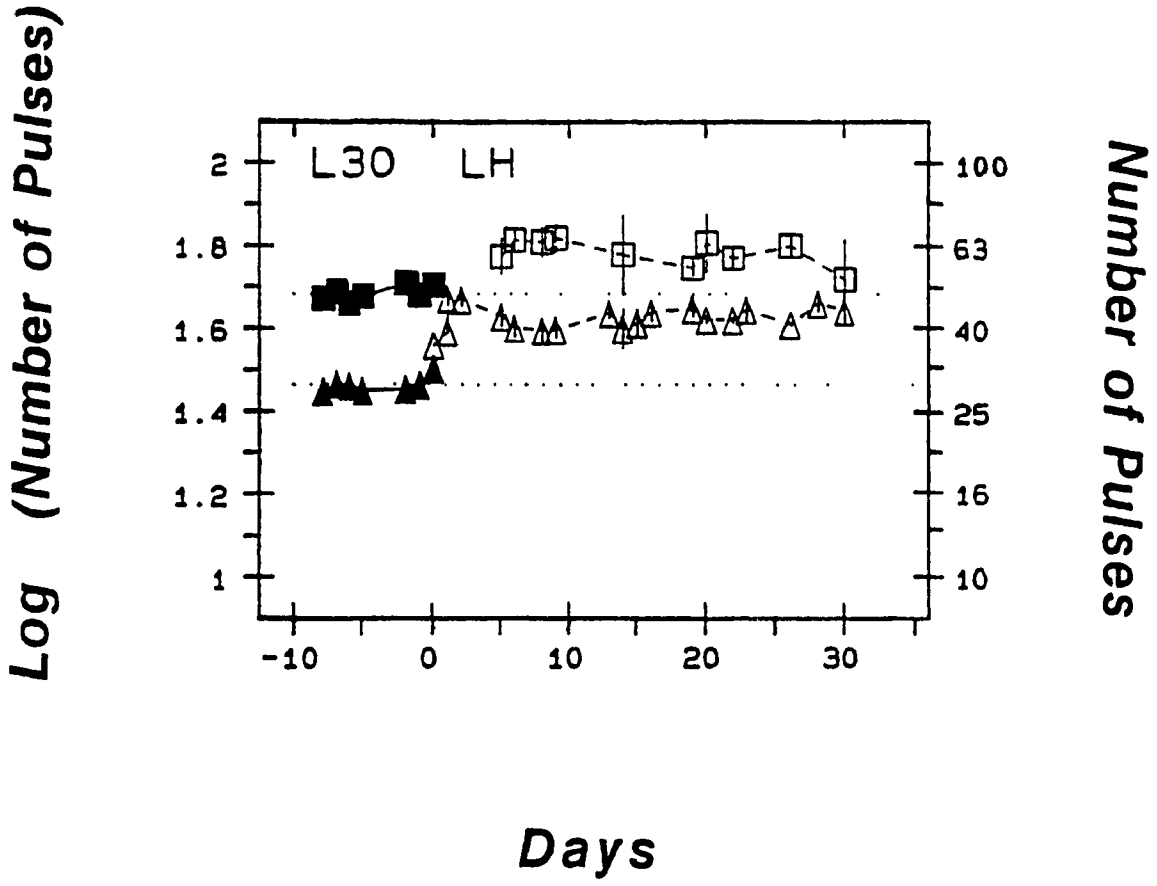
Subjects were divided into three groups depending on the magnitude of the lesion effect. A long-lasting shift from baseline in the required number was defined as an immediate postlesion increase in the required number of at least 0.1 $\log_{10}$ units that lasted for at least 10 days after the lesion. Subjects in the second group had transient effects, defined as an immediate increase in the required number of at least 0.1 $\log_{10}$ units that recovered to within less than 0.1 log10 units by the end of the testing period. In all cases, these criteria exceeded the upper range of the 95% confidence intervals constructed around the baseline mean. The required number of pulses at each current is shown as a function of days pre and postlesion. The three dotted lines extending across the graphs represent the baseline mean of the required number for each of the currents. The range of the y-axis is 1.2 $\log_{10}$ units so that the change from baseline can be directly compared across all graphs.

Effective lesions.

Following the lesions, only two of the subjects showed large, long-lasting increases in the required number. The results for these subjects are shown in Figures 6-7. The

Figs. 6-7 Effects of the lesions on the required number of pulses in L20 and L30; the two subjects in which large, long-lasting increases were seen. Each subject is identified in the top-left corner of the graph. Prelesion data are designated by negative values along the abscissa (filled symbols). The horizontal dotted lines indicate the mean of the baseline data for each current. Lesions were made after the last prelesion test session. Postlesion data are represented by open symbols. The lowest current is indicated by squares, the middle current (where applicable), by circles and the highest current, by triangles. Error bars around some data points represent the standard error of the mean (s.e.m.) for that test day. In cases where error bars are missing, the s.e.m. for that test day was less than half the radius of the symbol.


Log (Number of Pulses)
Number of Pulses
1.8
1.6
1.4
1.2
1
63
40
25
16
10
L20 LH
L20 VTA
-10
0
10
20
30
Days


Log (Number of Pulses)
L30 LH
2
1.8
1.6
1.4
1.2
1
100
63
40
25
16
10
-10
0
10
20
30
Number of Pulses
Days

postlesion increase in the required number was most dramatic in the case of L20 at the VTA site (Figure 6, top-right graph). No effect was observed at the LH electrode for this subject. The largest long-lasting increase, ranging from 0.03 to 0.32 log10 units (x= 0.16 log10 units) at the VTA site was seen at the lowest current. There was a fluctuation in the required number across the first 8 days following the lesion, followed by a stabilization at approximately 0.2 log_{10} units above baseline starting at day 9 and continuing until the end of testing. At the middle current, there was a consistent increase of about 0.2 log_{10} units above baseline that was maintained from postlesion session 3 to the end of testing. At the high current, there was a slight increase which failed to reach the 0.1 log_{10} unit criterion.

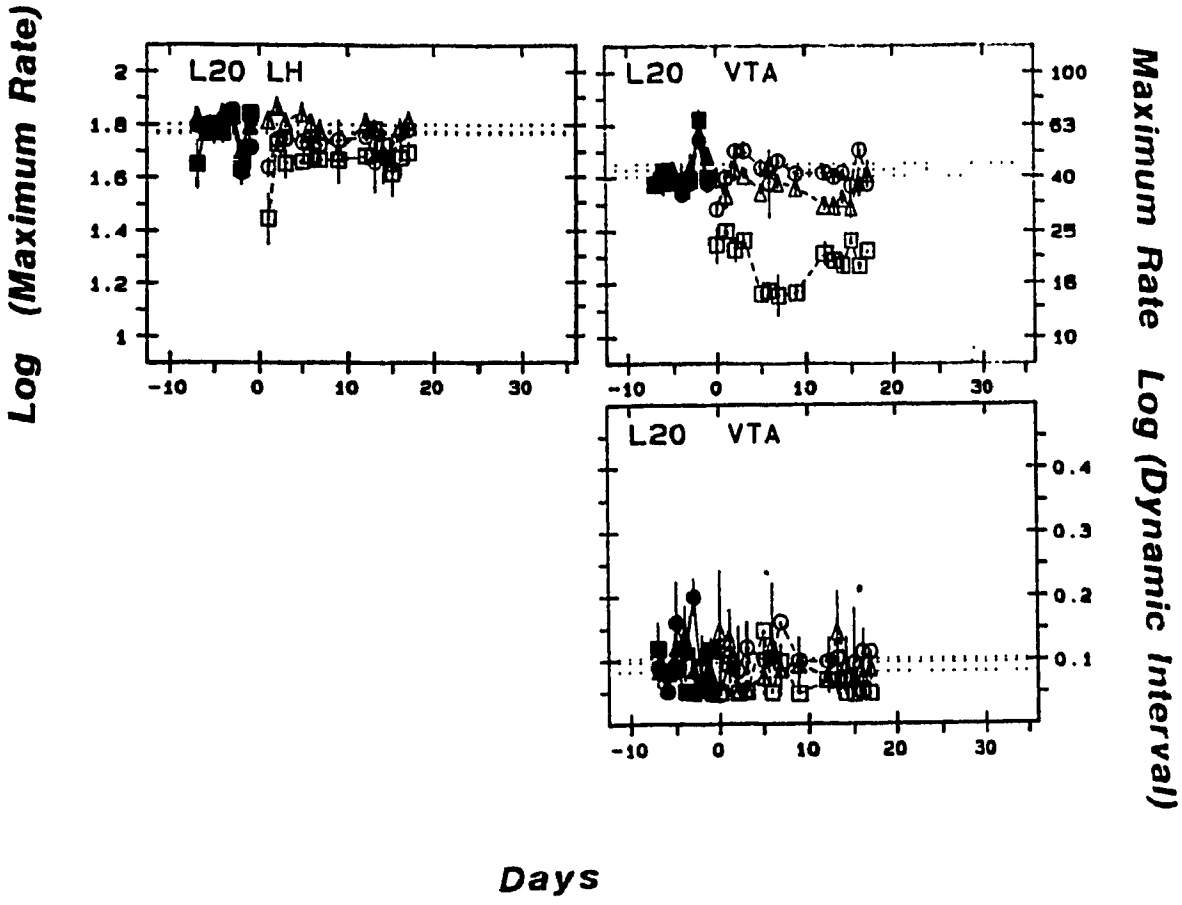
The other long-lasting effect was seen in the case of L30 (Figure 7). This subject did not self-stimulate at the posterior site. In this case, rate number curves were only collected at two currents. This subject was lesioned following the last baseline session and tested one hour later. Thus, two values are represented for the day of the lesion. The largest effect was observed at the highest current, where there was an immediate increase in the required number of approximately 0.1 log_{10} units immediately following the lesion, further increasing to 0.21 log_{10} units over the next two days. This was followed by a

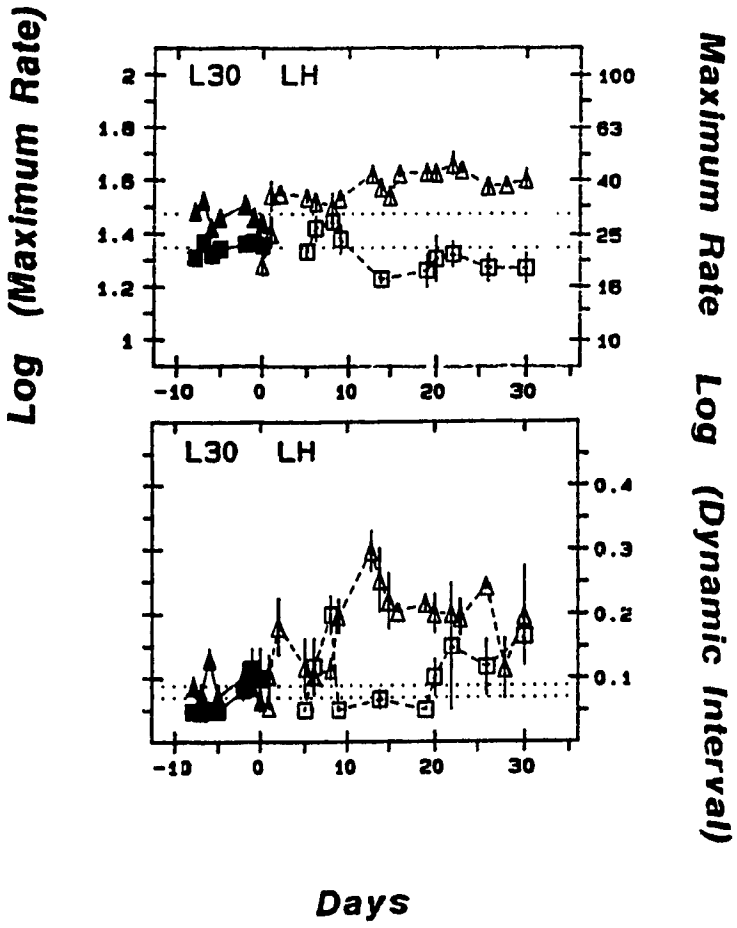
stabilization, at approximately 0.16 $\log_{10}$ units above baseline, for over 30 days of postlesion testing. At the lower current, the subject failed to self-stimulate for 4 days following the lesion. When it resumed pressing, the required number was increased by 0.12 $\log_{10}$ units above baseline, lasting three sessions. The required number then declined, fluctuating below the 0.1 $\log_{10}$ unit criterion until the end of testing.

Maximum rates and dynamic intervals for effective lesions.

The maximum rates and dynamic intervals for L20(LH and VTA) and L30(LH) are shown in Figures 8-9, respectively. In the top graphs, the y-axis represents maximum rates and days are represented on the x-axis. The dotted lines extending across the graphs represent the mean maximum rate for the baseline, at each current. The dynamic intervals, defined as the difference between the upper and lower breakpoints obtained from the broken-line functions, were plotted for L20(VTA) and L30(LH) as a function of pre and postlesion days. The dotted lines represent the mean baseline value at each current. A change was defined as an increase or decrease in the maximum rates beyond the 95% confidence intervals constructed around the baseline mean for at least two consecutive days. Both subjects with substantial increases in the required number exhibited changes in the

Figs. 8-9 Maximum response rates and dynamic intervals for subjects L20 and L30 in which the lesion produced large increases in the required number. Prelesion data (filled symbols) for the seven days preceding the lesion have negative values along the abcissa. Lesions were made at the end of the last prelesion test session. Postlesion data are represented by open symbols. The lowest current is indicated by squares, the middle current (where applicable), by circles and the highest current, by triangles. Error bars around some points represent the standard error of the mean (s.e.m.) for that test day. In cases where error bars are missing, the s.e.m. for that test day was less than half the radius of the symbol.


L20 LH
L20 VTA
L20 VTA
Log (Maximum Rate)
Maximum Rate Log (Dynamic Interval)
Days
2
1.8
1.6
1.4
1.2
1
100
63
40
25
16
10
0.4
0.3
0.2
0.1
-10
0
10
20
30


Log (Maximum Rate)
L30 LH
2
1.8
1.6
1.4
1.2
1
100
63
40
25
16
10
-10
0
10
20
30
L30 LH
0.4
0.3
0.2
0.1
-10
0
10
20
30
Maximum Rate Log (Dynamic Interval)
Days

maximum rates. In L20, at the lowest current tested at the posterior site (squares)(Figure 8, top-right graph), there was an immediate postlesion decrease in maximum rates of approximately 0.30 log10 units, with a subsequent decrease to within 0.50 log10 units below baseline until postlesion day 12. Maximum rates then increased back to within 0.33 log10 units of baseline. At the middle current (circles), maximum rates were initially decreased to within 0.12 log10 units of baseline, with a subsequent increase to 0.10 log10 units at postlesion day 5. Maximum rates remained stable until the end of testing. At the high current, the depression in maximum rates was initially observed at postlesion day 9, further decreasing to within 0.16 log10 units below baseline until the completion of testing.

In the case of the anterior electrode in this subject, there was an immediate decrease in the maximum rates of 0.32 log10 units on day 1 postlesion followed by an increase to within 0.10 log10 units until the end of testing. Note that no effect on the required number was observed at this stimulation site. At the middle current, the only decrease was observed immediately following the lesion (0.13 log10 units below baseline) followed by a stabilization to the baseline level until end of testing. No change was observed at the highest current. No significant change was observed in the dynamic intervals for this subject.

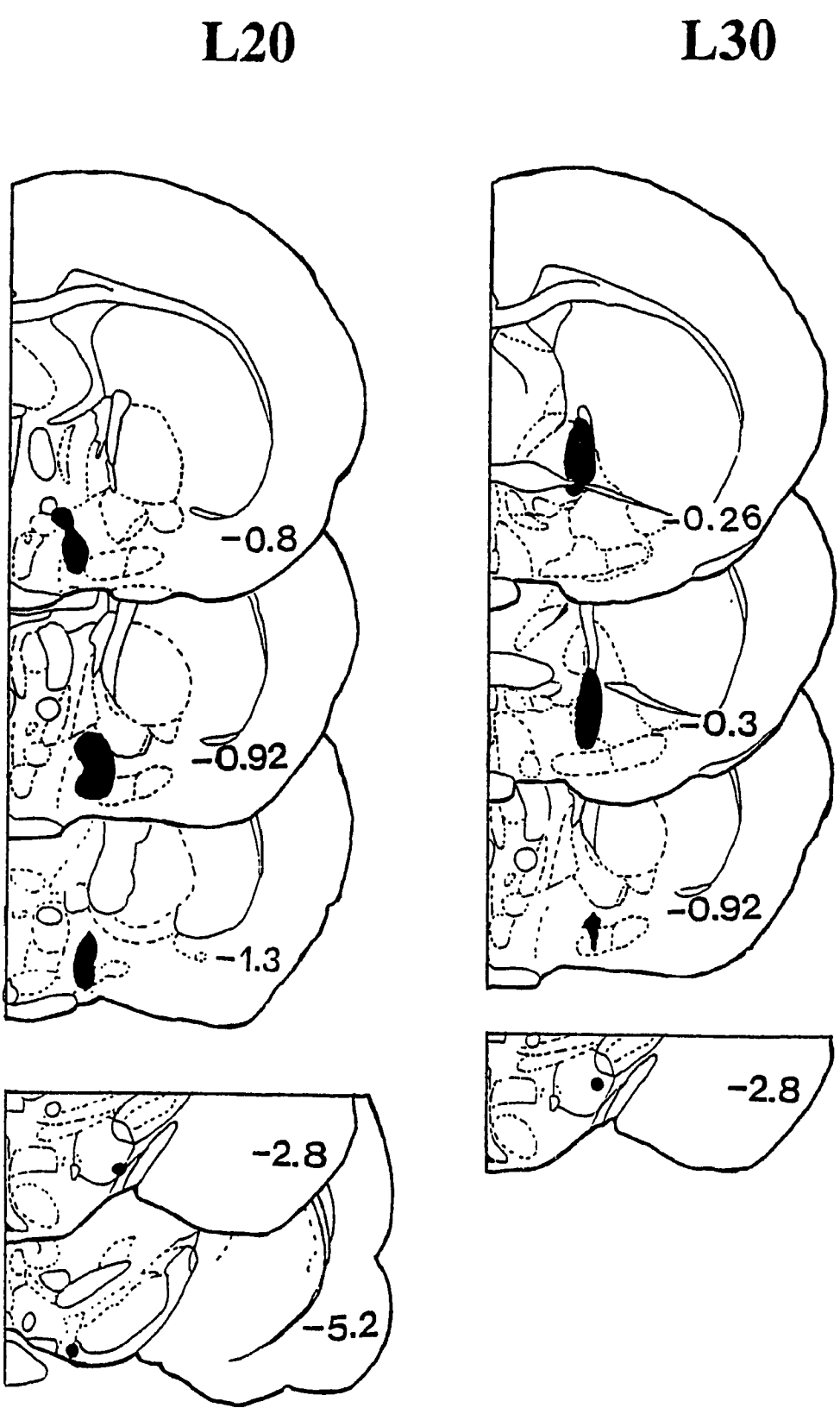
At the lowest current tested, L30 failed to self-stimulate following the lesion until postlesion day 5. A decrease in the maximum rates was observed at postlesion day 8 (0.10 log10 units below baseline) to within 0.06 log10 units of baseline by the end of testing at the low current. It is interesting to note that, at the high current (triangles), there was a 0.19 log10 units increase in the maximum rates, beginning at around day 12 postlesion, and increasing to within 0.13 log10 units by day 20.

An increase in the dynamic intervals was seen at the high current for this subject This increase of approximately 0.10 $\log_{10}$ units above baseline was observed beginning on day 2, with the greatest change occurring at day 13 postlesion.

Lesion and stimulation sites for effective lesions.

Histological reconstructions of the lesions (top three panels of each column) and stimulation sites (bottom panels) for subjects L20 (LH and VTA) and L30 (LH) are shown in Figure 10. The lesions in these two subjects, where increases in the required number were observed, did not overlap. In the case of L20, the largest part of the lesion was located in the LPOA with slight damage to the HDB. The LH electrode tip was located in the LH proper and the posterior electrode, in the mamillary tract, bordering on the

Fig. 10 Lesion and electrode tip locations for subjects with large, long-lasting increases in the required number. Reconstructions were made onto tracings of coronal plates from the Paxinos and Watson atlas (1986). The alphanumeric label at the bottom of each column identifies the subject. The distance of each plate from bregma is given in the bottom corner of each plate. The blackened area in the top three panels of each column represents the first sign, the largest sign, and the last sign of the lesion. Filled circles in the bottom panel(s) show the location of the anterior and/or posterior stimulation sites.


L20
L30
-0.8
-0.92
-1.3
-2.8
-5.2
-0.26
-0.3
-0.92
-2.8

VTA.

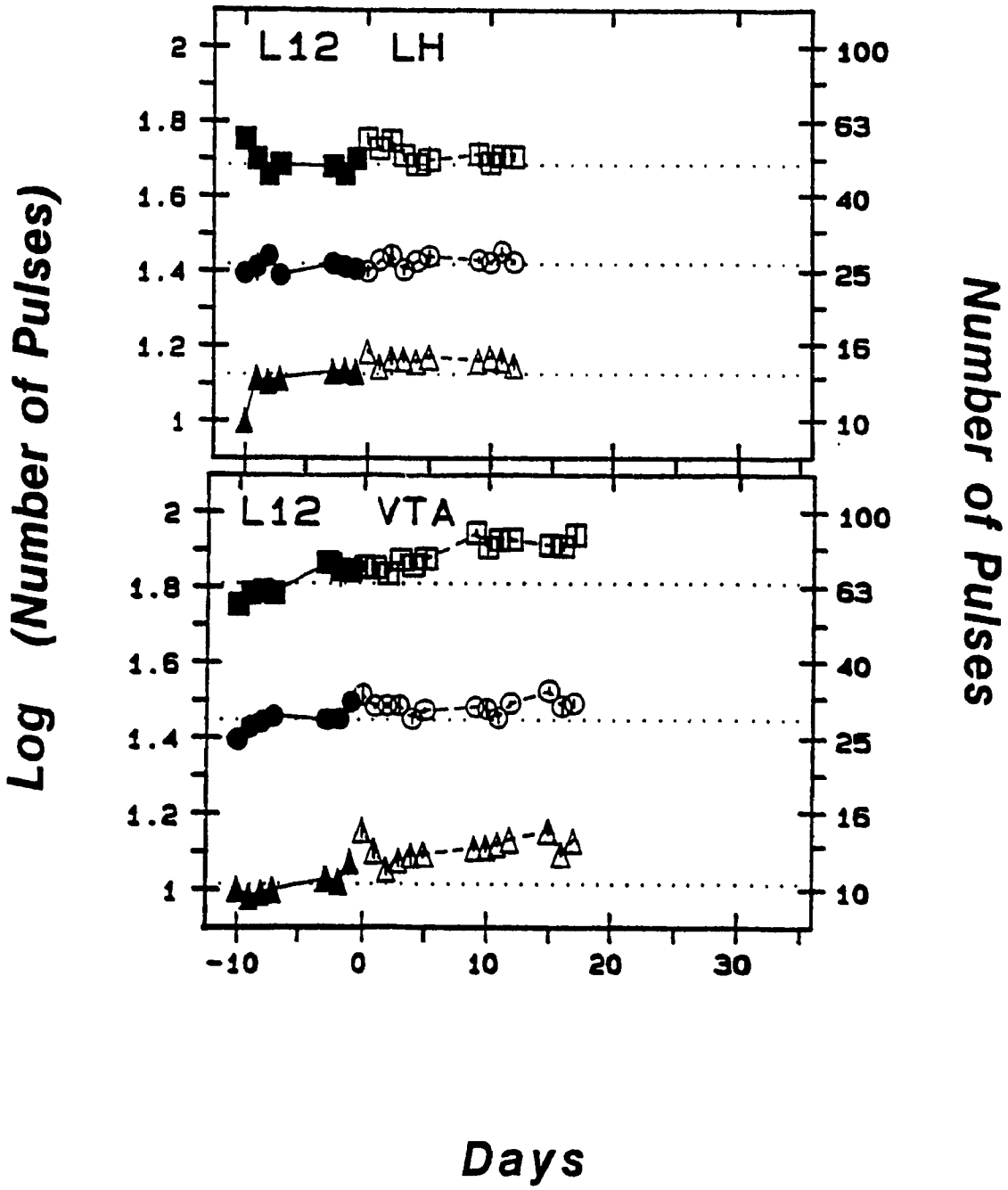
In the case of L30, most of the damage was located in the most medial part of the ventral pallidum (VP), with some damage to the substantia innominata (SI), and bed nucleus of the stria terminalis (BST). The anterior stimulating site in the case of L30 was located in the lateral hypothalamus, 2.8 mm behind bregma.

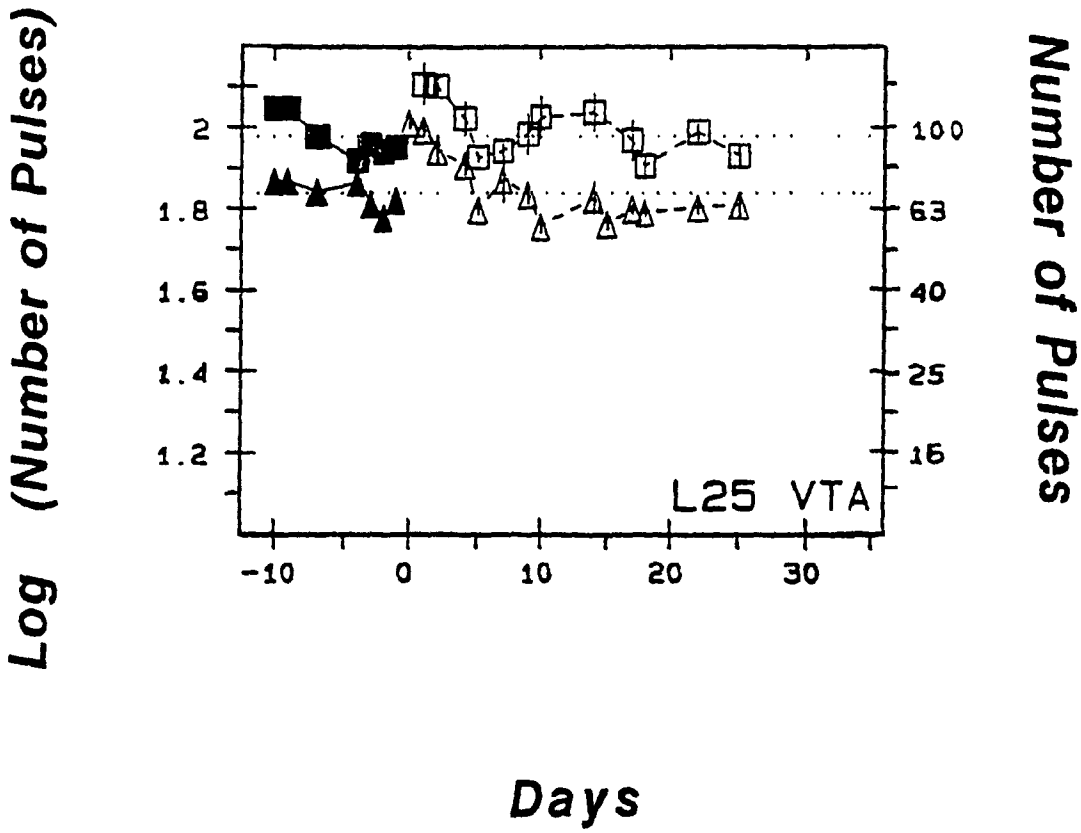
Transient effects.

Two of the subjects, L12 and L25, showed immediate, but transient increases in the required number following the lesion. Both of these were observed at the VTA site. No effect was seen at the anterior site for L12. L25 only self-stimulated at the posterior site. The results for these subjects are shown in Figures 11-12.

In the case of L12(VTA) at the high current, there was an immediate increase of 0.14 $\log_{10}$ units, returning to within 0.10 $\log_{10}$ units of the baseline by postlesion day 2. There was a subsequent trend towards increasing values beginning at postlesion day 4, with an ultimate increase of 0.14 $\log_{10}$ units above baseline by day 15. This animal was included in this group because it does not appear that the increase at day 4 is due to an immediate effect of the lesion. At the low current, the increase (0.14 $\log_{10}$ units)

Figs. 11-12 Effects of the lesions on the required number of pulses in L12 and L25, the two subjects in which transient increases were seen. Each subject is identified in the bottom-right or top-left corner of the graph. Prelesion data have negative values along the abcissa (filled symbols). The horizontal dotted lines indicate the mean of the baseline data for each current. Lesions were made after the last prelesion test session. Postlesion data are represented by open symbols. The lowest current is indicated by squares, the middle current (where applicable), by circles and the highest current, by triangles. Error bars around some data points represent the standard error of the mean (s.e.m.) for that test day. In cases where error bars are missing, the s.e.m. for that test day was less than half the radius of the symbol.


L12 LH
L12 VTA
2
1.8
1.6
1.4
1.2
1
100
63
40
25
16
10
-10
0
10
20
30
Log (Number of Pulses)
Number of Pulses
Days


Log (Number of Pulses)
2
1.8
1.6
1.4
1.2
100
63
40
25
16
Number of Pulses
L25 VTA
-10
0
10
20
30
Days

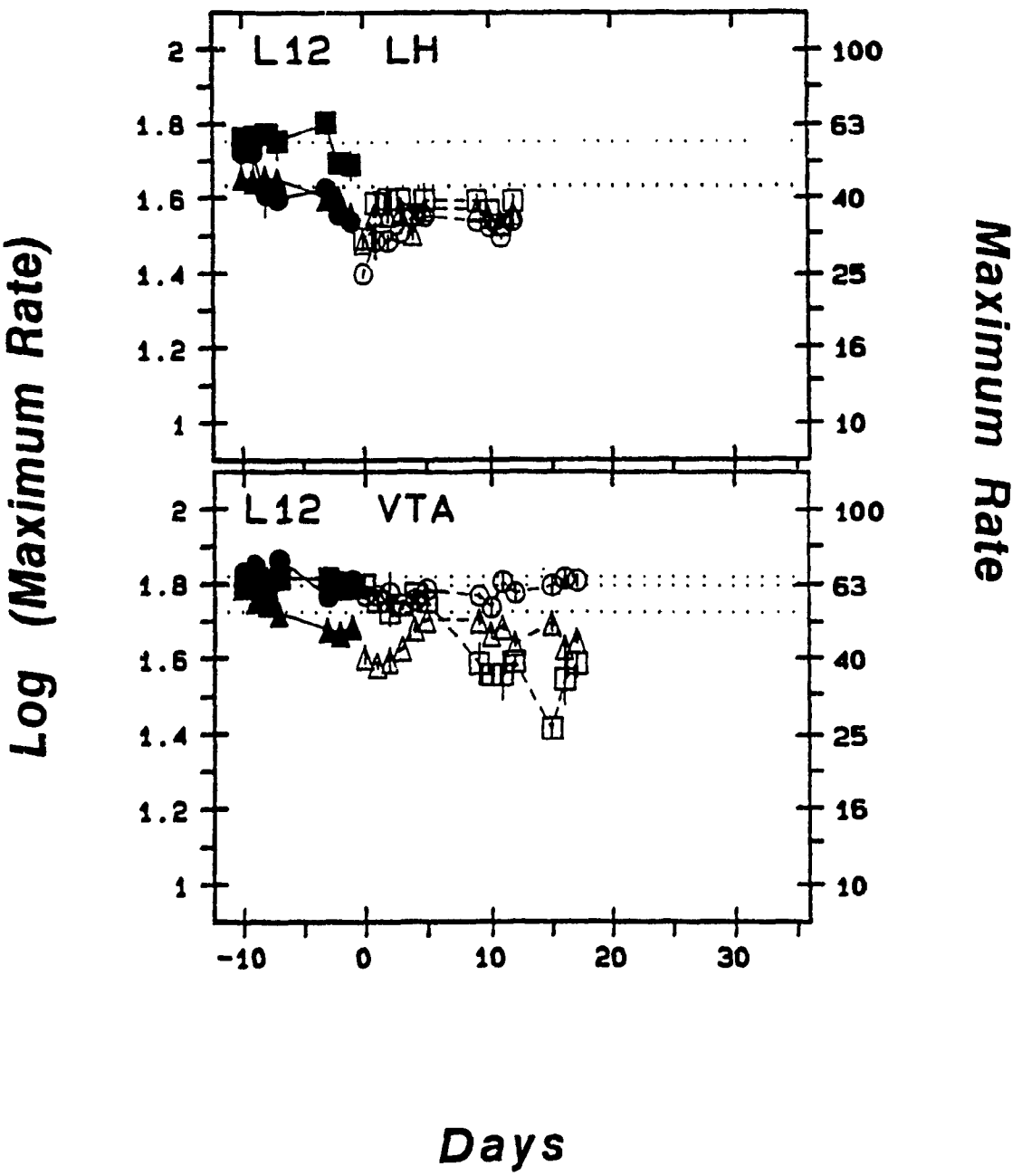
in threshold was observed only at day 7, indicating that the increase was also probably not due directly to the effects of the lesion. Furthermore, no increase was observed at the middle current.

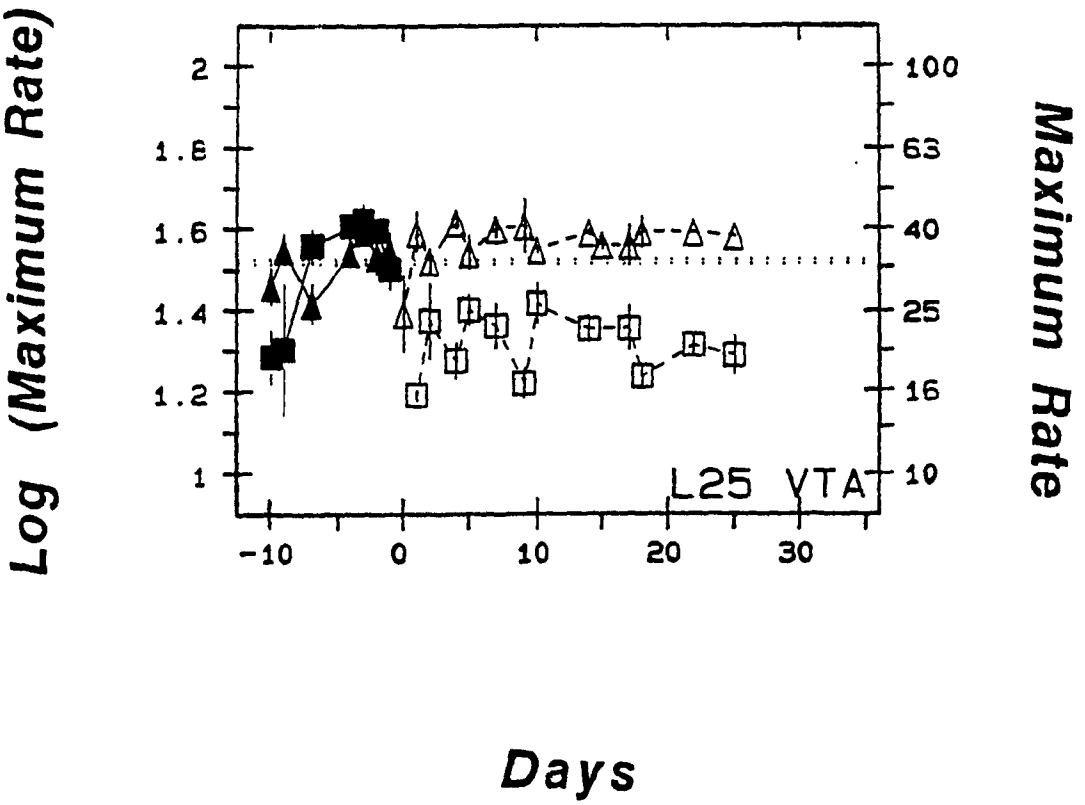
In the case of L25(VTA), only two currents were tested. There was an immediate increase of 0.12 and 0.17 $\log_{10}$ units at the low and high current, respectively. These values returned to within baseline values by postlesion day 5.

Maximum rates for transient effects.

The maximum rates for L12(VTA) and L25(VTA) are show in Figures 13-14. Most of the decreases in the maximum rates were observed at the low current. In the case of L12(VTA), there was a substantial decrease, of approximately 0.20 log10 units, in maximum rates at the low current after postlesion day 6. At the high current, there was a slight decrease of approximately 0.12 log10 units until postlesion day 5. Maximum rates then stabilized at approximately 0.06 log10 units below baseline until the end of testing. For L12(LH), the decrease in maximum rates was approximately 0.19 $\log_{10}$ below baseline units, evident immediately following the lesion and consistent throughout testing at the low current. No decrease was seen at other currents. Again, in the case of L25(VTA), a decrease in maximum rates of approximately 0.20 $\log_{10}$ units at the lower current and was evident

Figs. 13-14 Maximum response rates for subjects L12 and L25 in which the lesion produced transient increases in the required number. Prelesion data (filled symbols) for the seven days preceding the lesion have negative values along the abcissa. Lesions were made at the end of the last prelesion test session. Postlesion data are represented by open symbols. The lowest current is indicated by squares, the middle current (where applicable), by circles and the highest current, by triangles. Error bars around some points represent the standard error of the mean (s.e.m.) for that test day. In cases where error bars are missing, the s.e.m. for that test day was less than half the radius of the symbol.


L12 LH
L12 VTA
Log (Maximum Rate)
Maximum Rate
Days
2
1.8
1.6
1.4
1.2
1
100
63
40
25
16
10
-10
0
10
20
30


Log (Maximum Rate)
2
1.8
1.6
1.4
1.2
1
100
63
40
25
16
10
Maximum Rate
L25 VTA
-10
0
10
20
30
Days

immediately following the lesion. No decrease was observed at the high current.

Lesions and stimulation sites for transient effects.

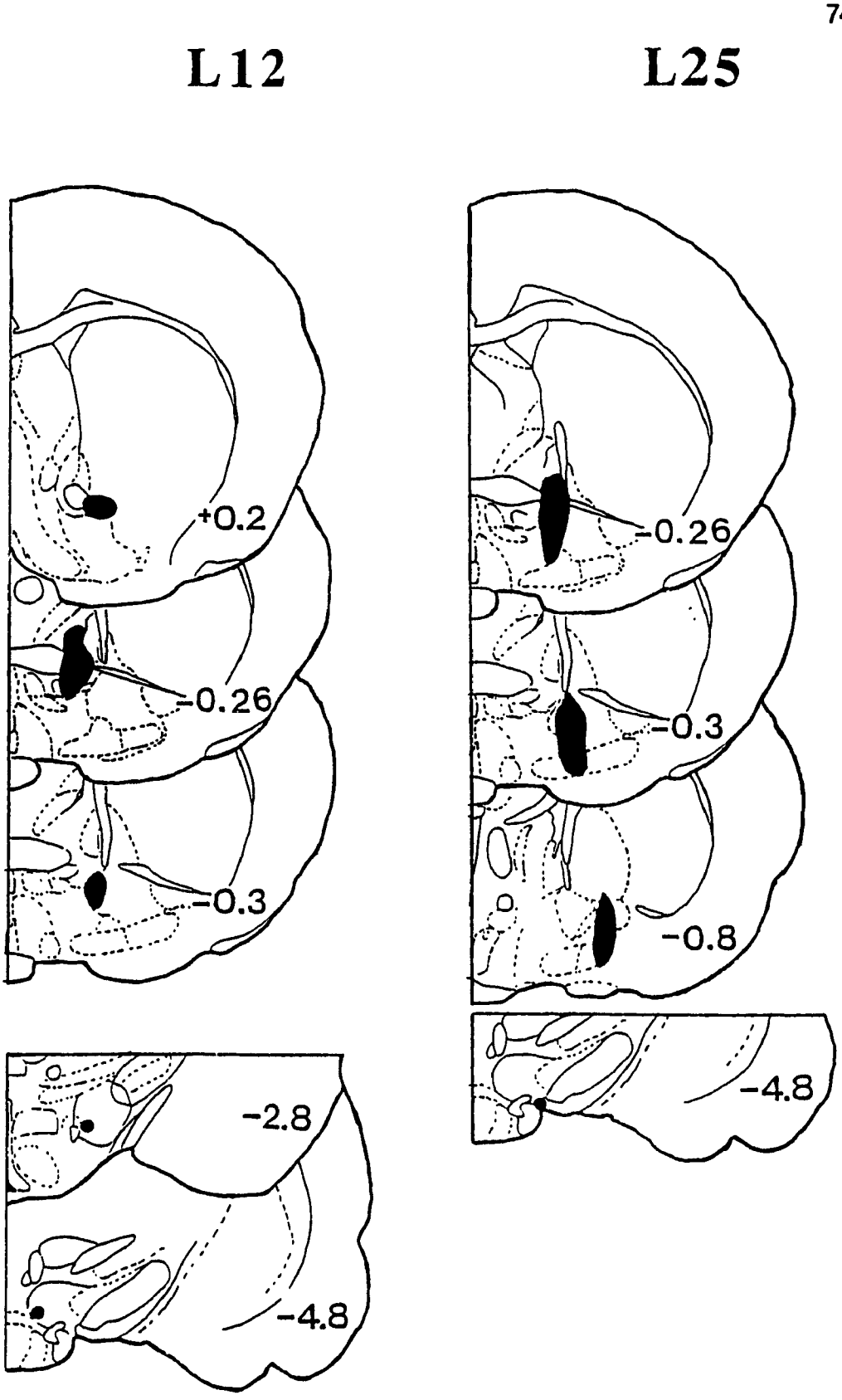
Histological reconstructions of the lesions (top three panels of each column) and stimulation sites (bottom two panels) for subjects L12(LH and VTA) and L25(VTA) are shown in Figure 15. The lesion in the case of L12 was located mostly in the BST, with damage the VP. The anterior electrode was located in the LH, lateral to the fornix. The posterior electrode was located in the medial VTA.

The largest part of the lesion for L25 was located in the medial VP and SI, with some damage to the internal capsule (IC), and part of the HDB. The posterior stimulating electrode was located in the ventral VTA.

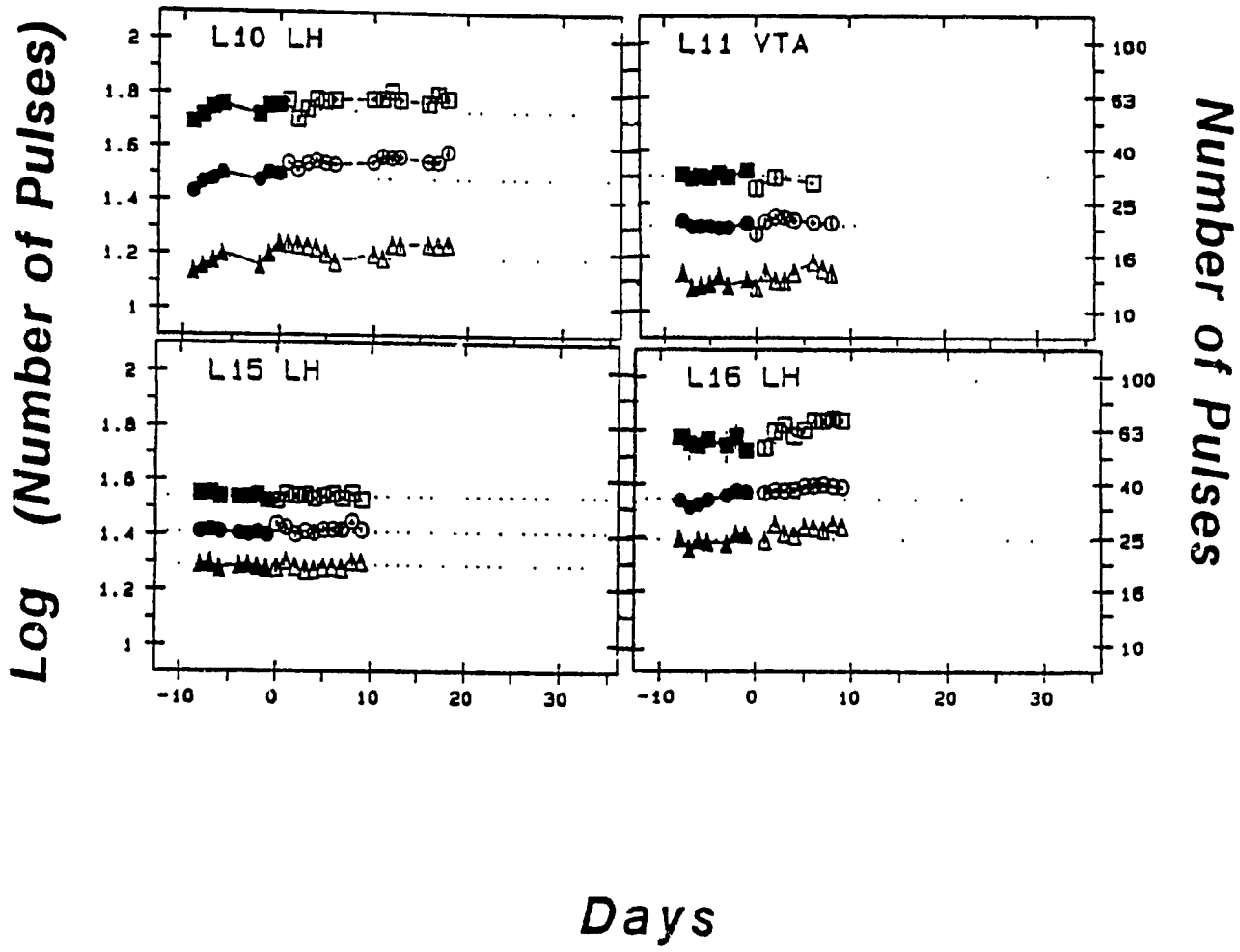
Non-significant effects.

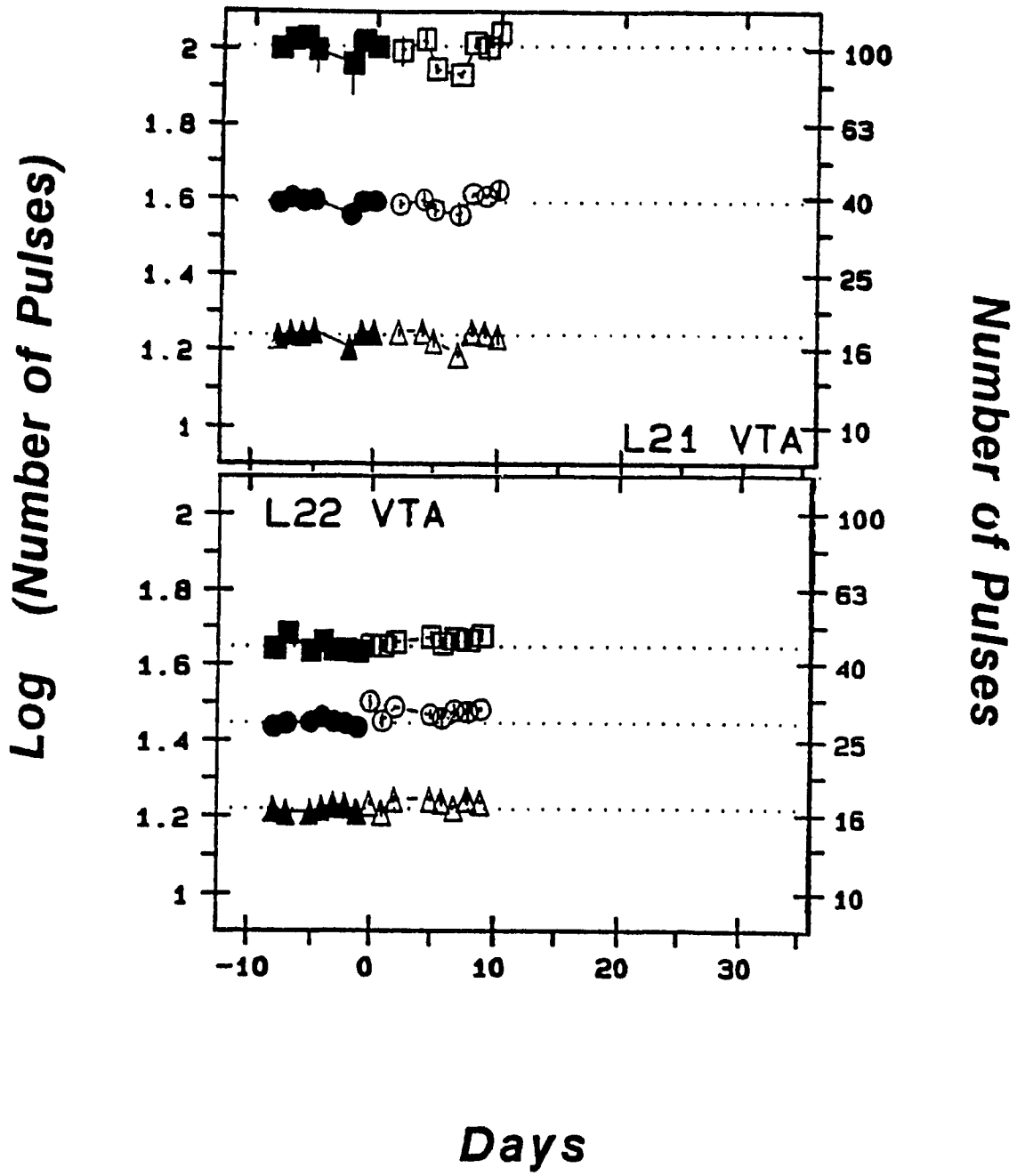
All other subjects failed to show significant shifts in the required number (see Figures 16-19). Some of the subjects' post-lesion data points were above the constructed 95% interval but below the 0.1 log10 unit criterion. In the case of L10(LH) and L16(LH) for example, small increases of approximately 0.03 to 0.09 log10 units of baseline were evident at the middle and low current respectively.

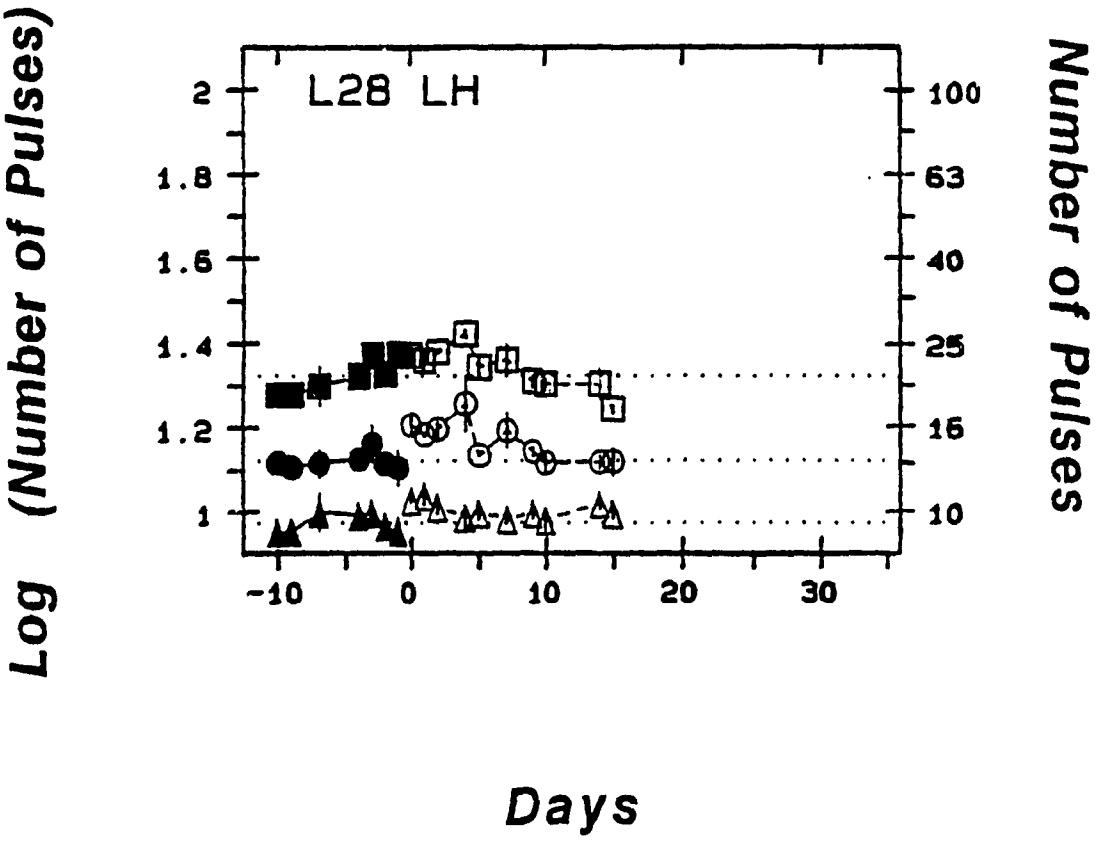
Fig. 15 Lesion and electrode tip locations for subjects with transient increases in the required number. Reconstructions were made onto tracings of coronal plates from the Paxinos and Watson atlas (1986). The alphanumeric label at the bottom of each column identifies the subject. The distance of each plate from bregma is given in the bottom corner of each plate. The blackened area in the top three panels of each column represents the first sign, the largest sign, and the last sign of the lesion. Filled circles in the bottom panel(s) show the location of the anterior and/or posterior stimulation sites.

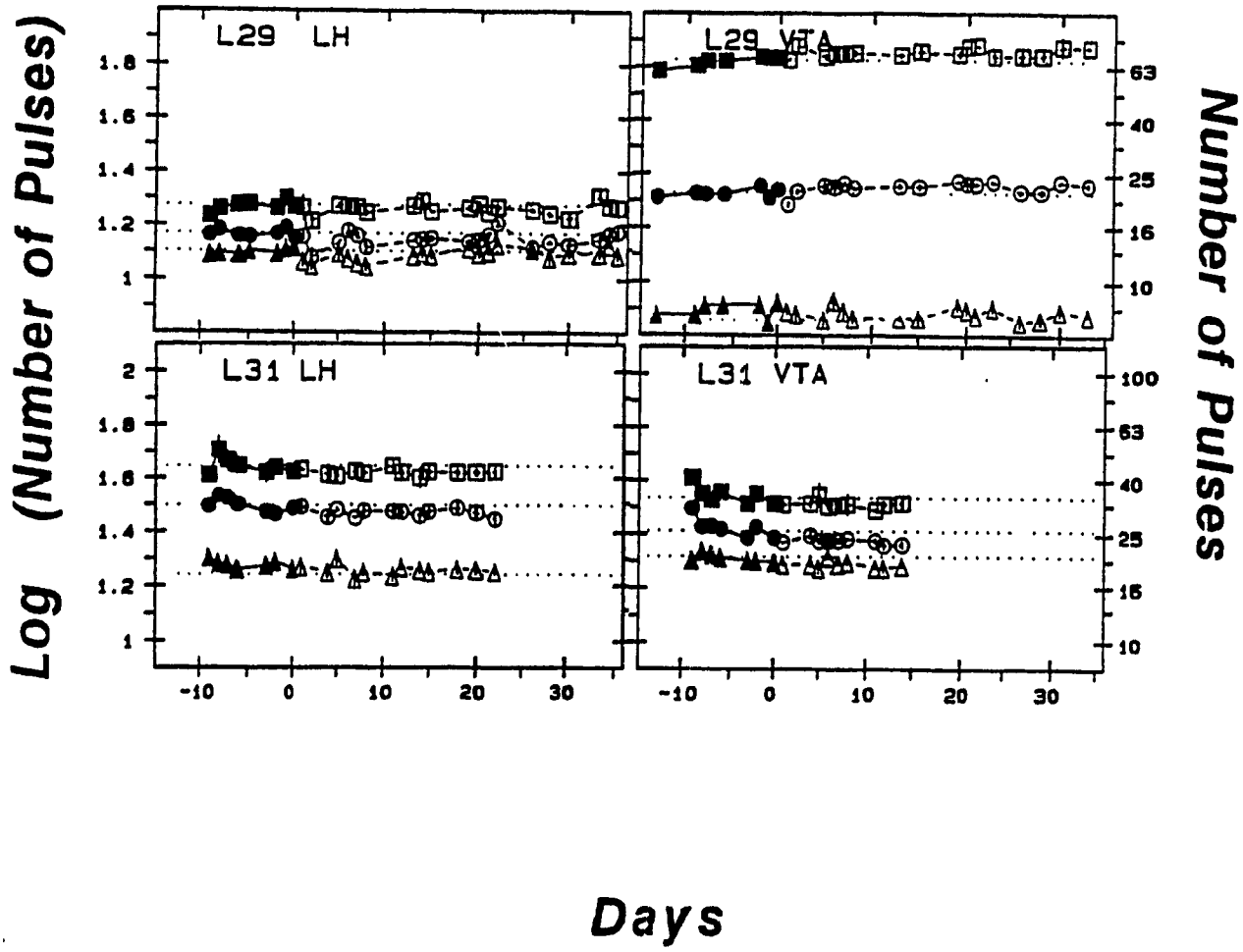

L12
L25
+0.2
-0.26
-0.3
-2.8
-4.8
-0.26
-0.3
-0.8
-4.8

Figs. 16-19 Effects of the lesions on the required number of pulses in L10, L11, L15, L16, L28, L29, and L31, subjects where no meaningful increases were seen. Each subject is identified in the bottom-right or top-left corner of the graph. Prelesion data have negative values along the abcissa (filled symbols). The horizontal dotted lines indicate the mean of the baseline data for each current. Lesions were made after the last prelesion test session. Postlesion data are represented by open symbols. The lowest current is indicated by squares, the middle current (where applicable), by circles and the highest current, by triangles. Error bars around some data points represent the standard error of the mean (s.e.m.) for that test day. In cases where error bars are missing, the s.e.m. for that test day was less than half the radius of the symbol.


L10 LH
L11 VTA
L15 LH
L16 LH
Log (Number of Pulses)
Number of Pulses
Days
2
1.8
1.6
1.4
1.2
1
100
63
40
25
16
10
-10
0
10
20
30


Log (Number of Pulses)
Number of Pulses
L21 VTA
L22 VTA
2
1.8
1.6
1.4
1.2
1
100
63
40
25
16
10
-10
0
10
20
30
Days


L28 LH
Log (Number of Pulses)
Number of Pulses
Days
2
1.8
1.6
1.4
1.2
1
100
63
40
25
16
10
-10
0
10
20
30


L29 LH
L29 VTA
L31 LH
L31 VTA
Log (Number of Pulses)
Number of Pulses
Days
1.8
1.6
1.4
1.2
1
2
63
40
25
16
10
100
-10
0
10
20
30

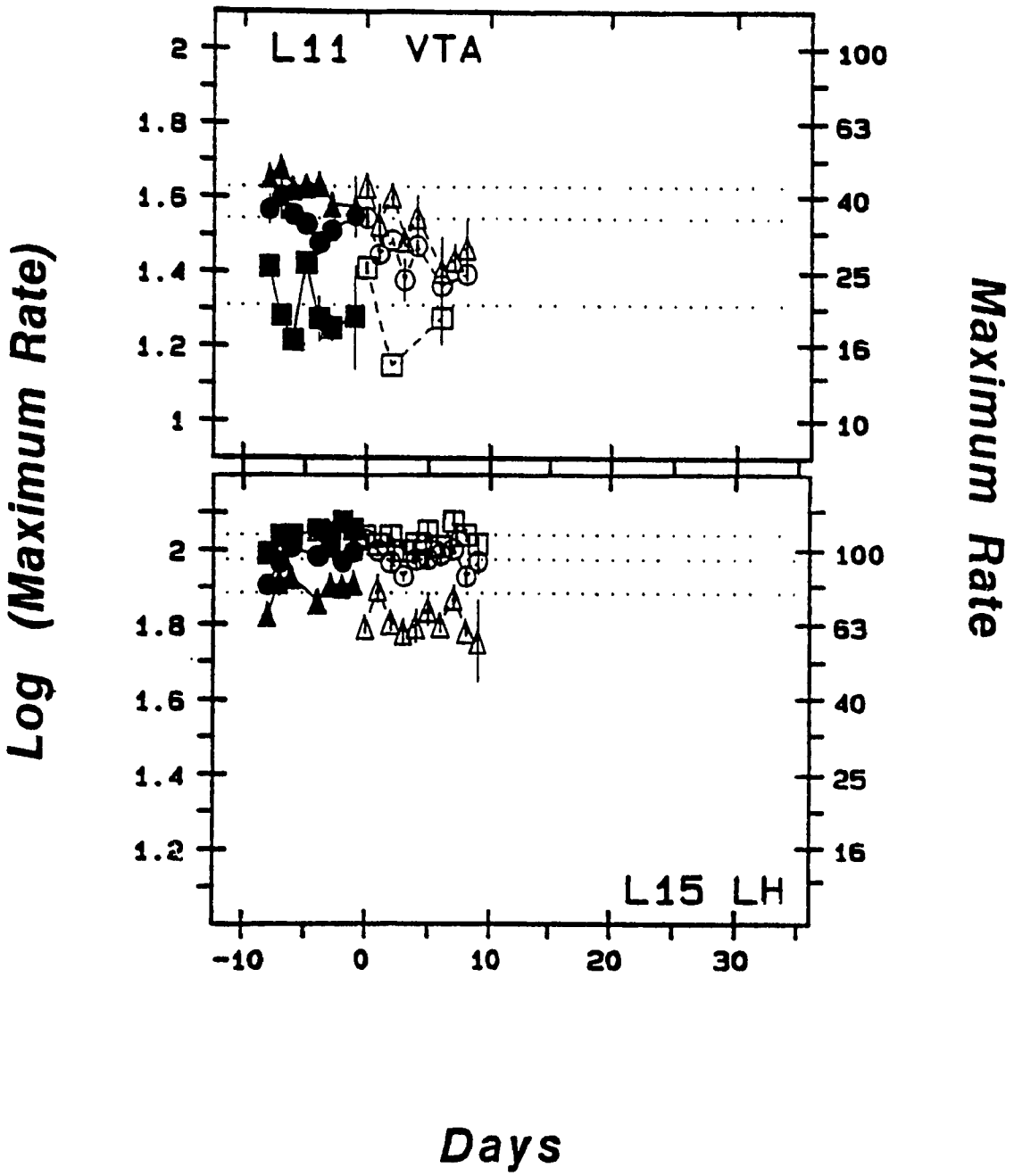
Maximum rates for ineffective lesions

The maximum rates for subjects with ineffective lesions are shown in Figure 20. It is interesting to note that decreases in the maximum rates were observed in the cases of L11(VTA, low and middle current) and L15(LH, high current) where absolutely no change was observed in the required number. Decreases ranged from 0.06 to 0.22 log10 units.

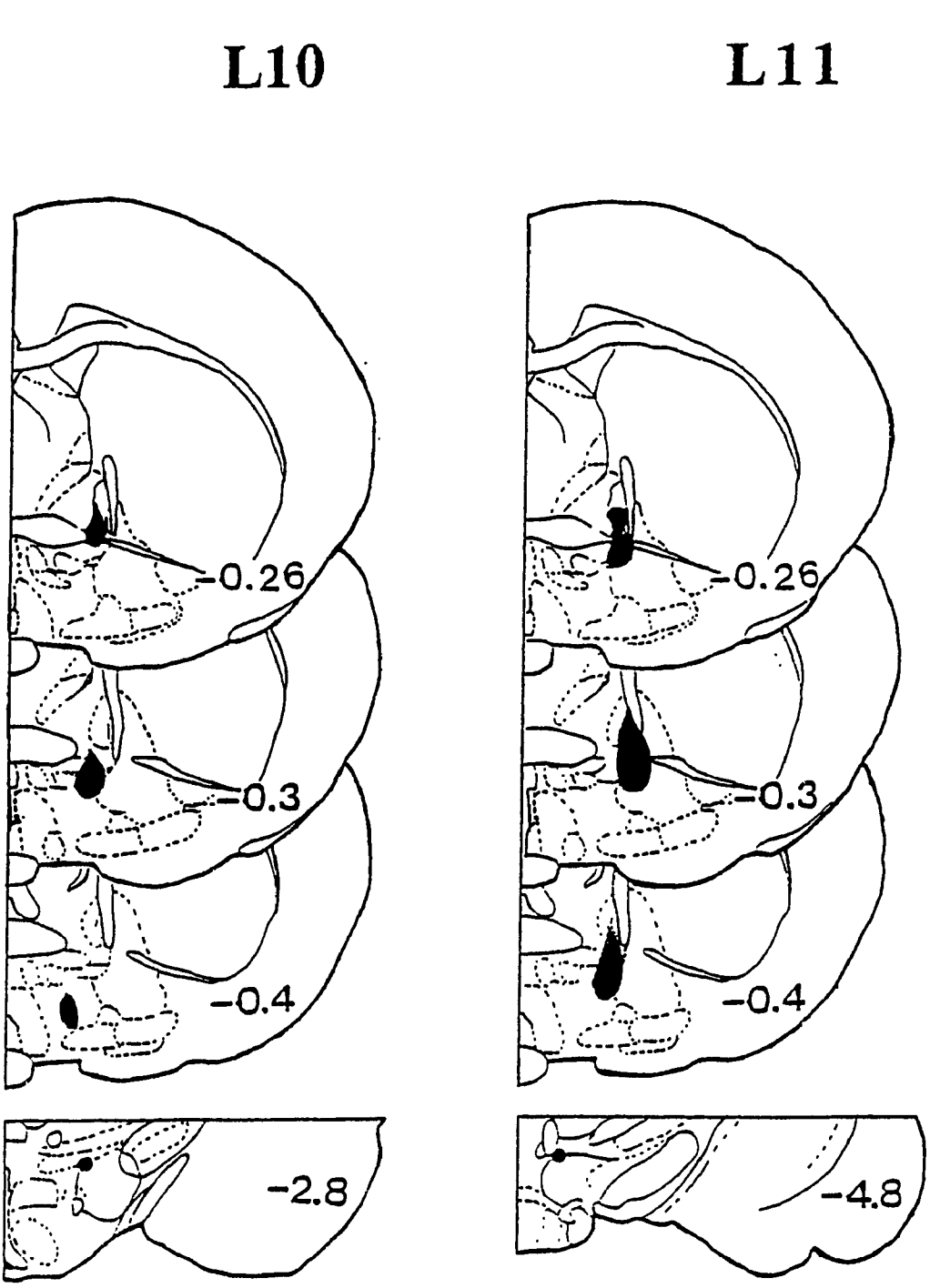
Lesions and stimulation sites for ineffective lesions

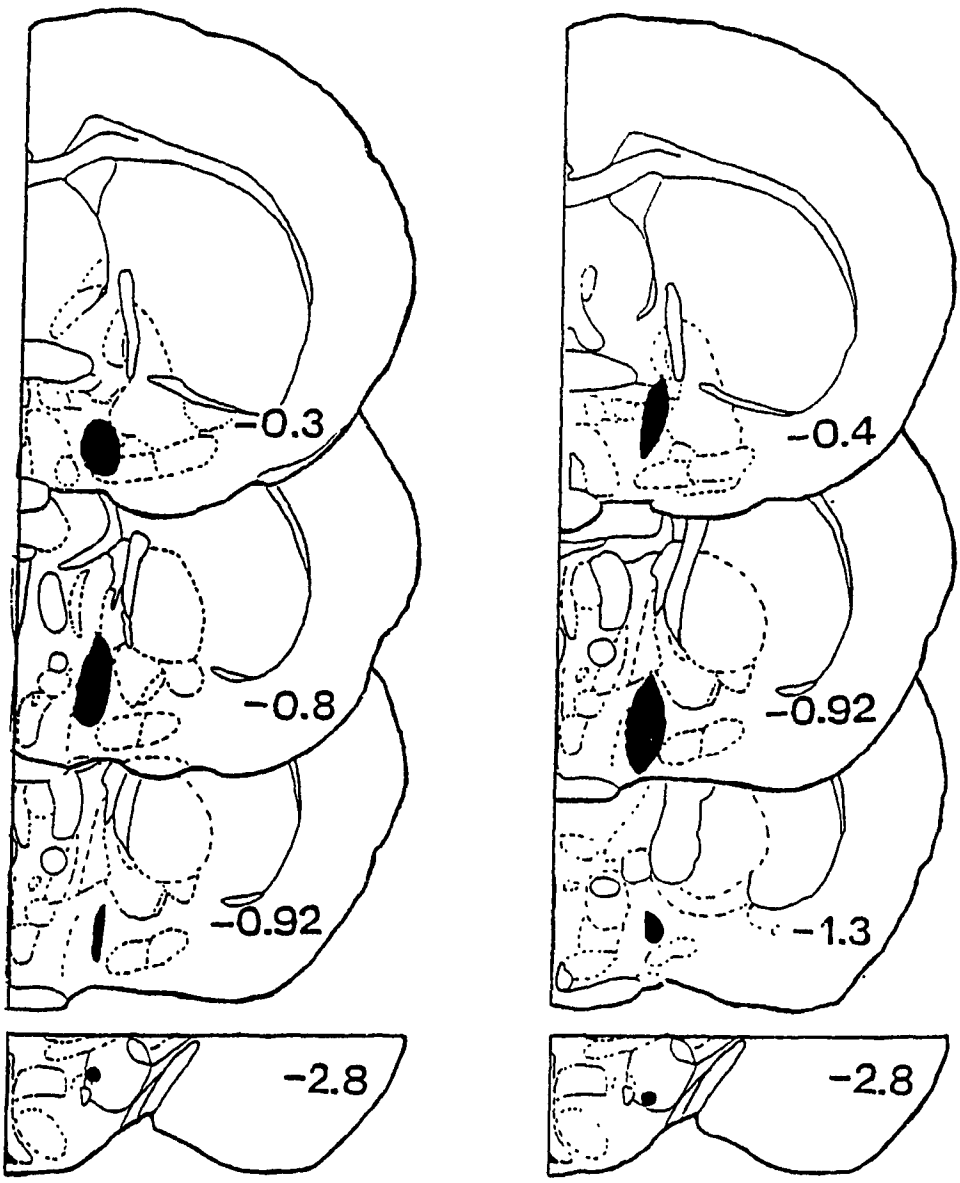
Histological reconstructions of the lesions (top three panels of each column) and stimulation sites (bottom two panels) for subjects that failed to show any significant changes in the required number are shown in Figures 21-24. No histology is shown for L29. Large islands of non-specific damage were evident throughout the brain slices and it was impossible to distinguish any damage that may have been caused by the lesion. Furthermore, tissue tear rendered the localization of the stimulating electrode tips impossible. Some of the lesions overlapped with the lesions that produced large effects in L20(VTA) and L30(LH). Most of the ineffective lesions were located in the area of BST and dorsal LPOA. Damage was also evident in the IC, HDB, VP, MPOA and in the case of L28, in the caudate putamen (CP). All of the anterior stimulating electrodes were located within the LH, while all the posterior stimulating electrodes

Fig. 20 Maximum response rates for subjects L11 and L15 in which the lesion produced no increases in the required number. Prelesion data (filled symbols) for the seven days preceding the lesion have negative values along the abcissa. Lesions were made at the end of the last prelesion test session. Postlesion data are represented by open symbols. The lowest current is indicated by squares, the middle current (where applicable), by circles and the highest current, by triangles. Error bars around some points represent the standard error of the mean (s.e.m.) for that test day. In cases where error bars are missing, the s.e.m. for that test day was less than half the radius of the symbol.

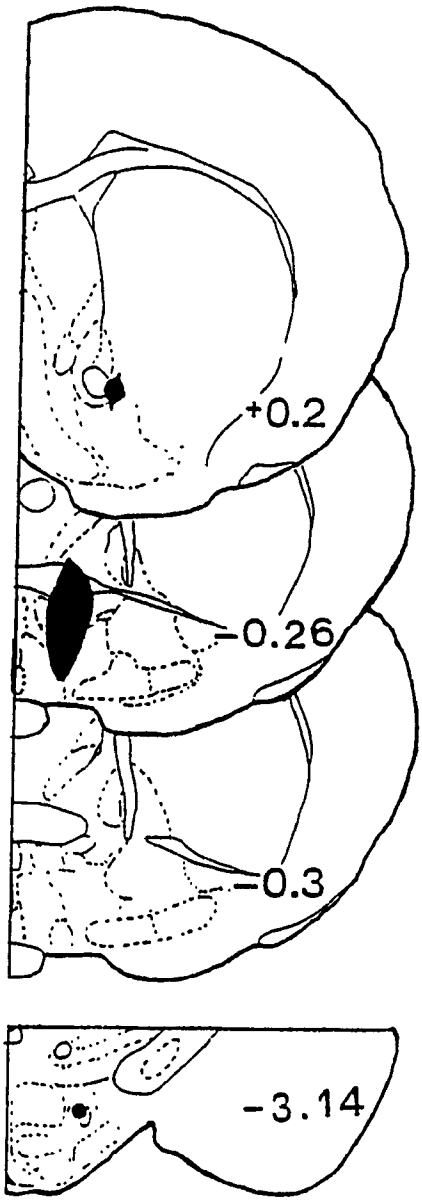

L11 VTA
L15 LH
Log (Maximum Rate)
Maximum Rate
Days
2
1.8
1.6
1.4
1.2
1
100
63
40
25
16
10
-10
0
10
20
30

Figs. 21-24 Lesion and electrode tip locations for subjects with no significant increases in the required number. Reconstructions were made onto tracings of coronal plates from the Paxinos and Watson atlas (1986). The alphanumeric label at the bottom of each column identifies the subject. The distance of each plate from bregma is given in the bottom corner of each plate. The blackened area in the top three panels of each column represents the first sign, the largest sign, and the last sign of the lesion. Filled circles in the bottom panel(s) show the location of the anterior and/or posterior stimulation sites.

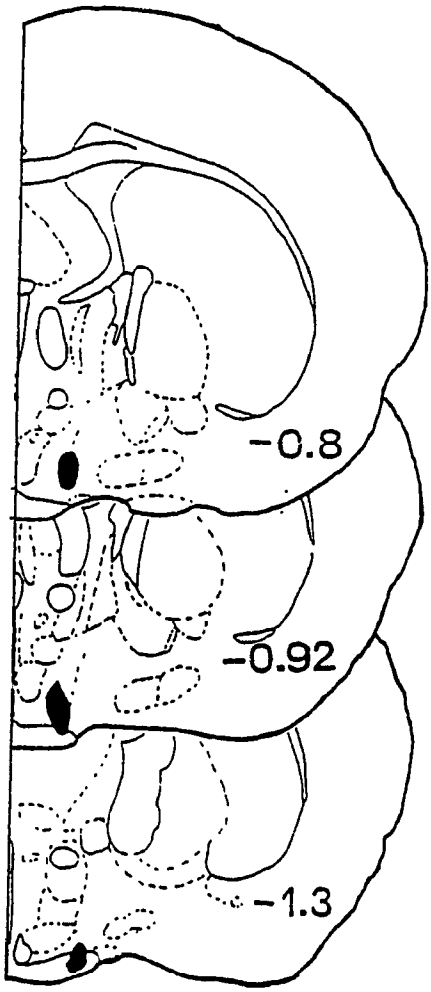

L10
L11
-0.26
-0.3
-0.4
-2.8
-0.26
-0.3
-0.4
-4.8

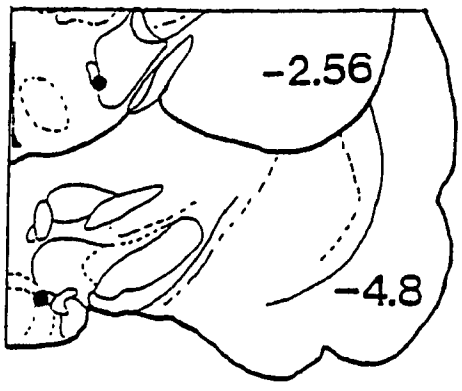

L15
L16
-0.3
-0.8
-0.92
-2.8
-0.4
-0.92
-1.3
-2.8

L21

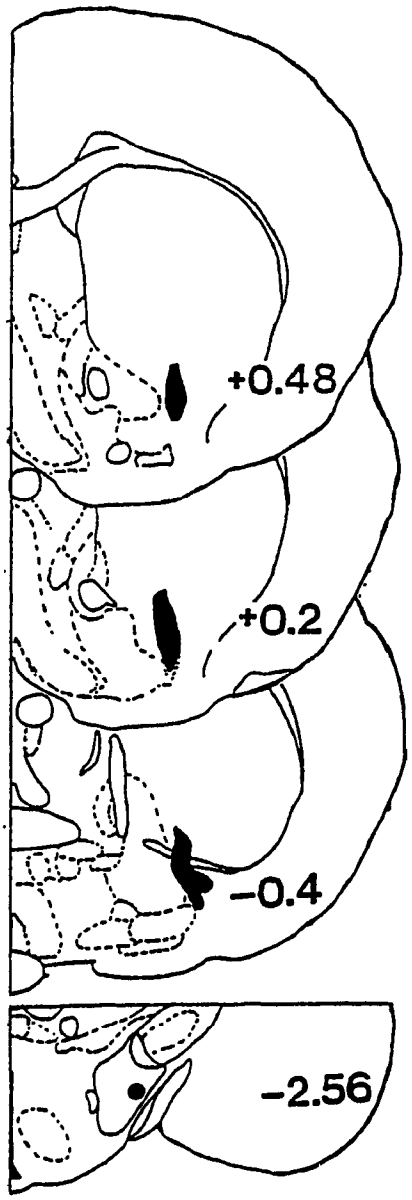


L22

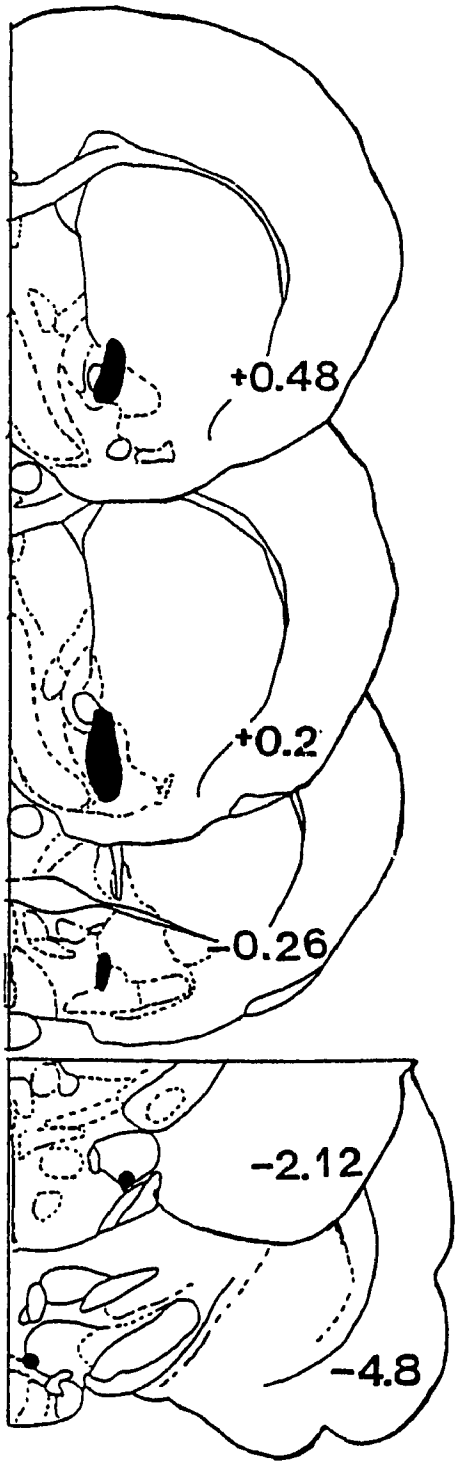




L28



L31



were found in the VTA. The only exception was in the case of L21, whose VTA electrode was placed quite anterior, -3.2 mm from bregma.

Discussion

Lesion Effects

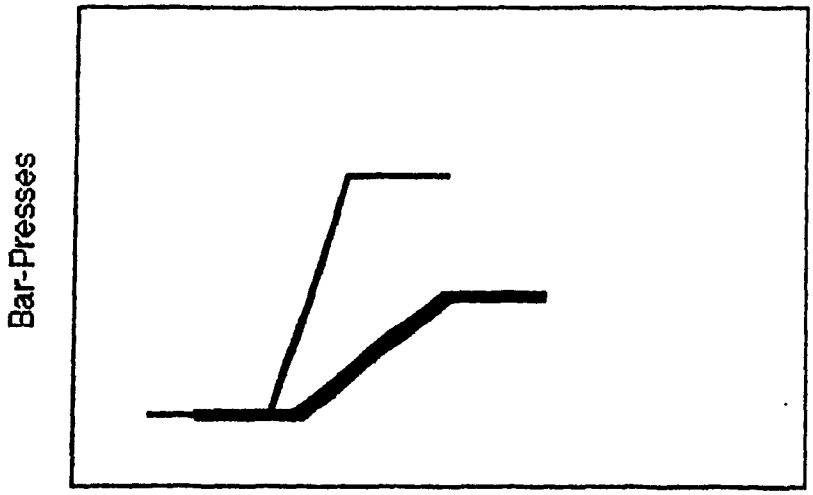
Refractory period estimates for the LPOA sites overlap those obtained at more caudal sites suggesting that a common population of reward-relevant neurons might be activated by stimulation of the LPOA and the MFB. Earlier lesion studies have hinted that the LPOA might be important in MFB self-stimulation, suggesting that this site is worthy of further investigation (Janas & Stellar, 1987; Murray & Shizgal, 1991; Waraczynski, 1988). In the present study, lesions were aimed at the LPOA to assess the importance of this area in self-stimulation of the LH and VTA. Long-term increases, ranging from 0.1 to 0.32 $\log_{10}$ units, in the required number of pulses was seen at either LH or VTA sites in two subjects. Transient increases in the required number were seen in the case of two rats. Lesions failed to produce any effects in the case of nine rats. It is argued below that these data implicate neurons in anterior MFB sites in the rewarding effect produced by stimulating more posterior MFB sites.

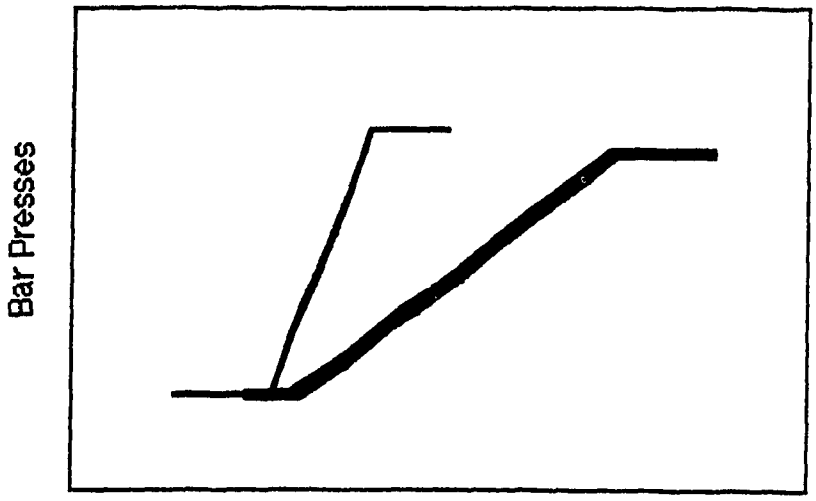
Effective Lesions

The required number data must be interpreted in relation to measures of maximum rate and dynamic interval, since a change in these last two variables alone can produce a curve-

shift without necessarily reflecting changes in reward. The two subjects with substantial, long-lasting increases in the required number also exhibited decreases in the maximum rates. In the case of L30(LH), maximum rate analysis revealed no systematic relation between the magnitude and sign of the changes in the maximum rate and the required number. For this rat, the largest increase in required number was seen at the high current, with a relatively smaller increase observed at the low current. In contrast, maximum rates were substantially increased at the high current, whereas at the low current, there was an initial increase, followed by a large decrease in the maximum rates. Given this difference, it is tempting to state that the change in required number reflects a real decrease in reward effectiveness, at least at the high current. However, the assessment of the magnitude of the increases in the required number are rendered more complicated by the fact that, at the high current, there was an increase in the dynamic interval whereas no substantial change in the dynamic interval was observed at the low current. These results demonstrate the importance of carrying out the analysis of the dynamic intervals along with that of the maximum rates. Even if the maximum rates remain unchanged, there is still a possibility that the dynamic interval has increased, thus artificially altering the position of the rate-frequency curves along the number axis (See Figure 25).

Fig. 25 Effect of increases in the dynamic interval on the required number. The thin line represents a hypothetical rate-number curve prior to a lesion. The thick line represents the rate-number curve following the lesion. In the top graph, the maximum rate is depressed and the dynamic interval is increased in the curve following the lesion, thus "artificially" increasing the required number. In the bottom graph, however, the maximum rates have not changed, but the dynamic interval is nonetheless increased, again "artificially" increasing the required number. This stresses the importance of examining both changes in maximum rates and conducting dynamic interval analysis when examining the effect of a lesion on the required number.


Bar-Presses
Number of Pulses


Bar Presses
Number of Pulses

Given the observed increase in the dynamic interval for L30(LH), one may question whether the increase seen in the required number reflects a decrease in reward effectiveness. However, it is important to note that the change in the required number was stable during postlesion testing, whereas the dynamic interval was variable, increasing appreciably only on some of the postlesion sessions. Furthermore, variability was also seen for the dynamic interval at the low current, whereas the increase in the required number was stable throughout postlesion testing. That there is only a weak relationship between changes in dynamic interval and required number reinforces the view that the lesion reduced reward effectiveness.

In the case of L20(VTA), increases in the required number were seen at both the low and middle currents, with the increase at the low current exhibiting more variability. A substantial decrease in the maximum rates was seen at the low current, whereas no marked change from baseline was evident at the other currents. The dynamic intervals remained unchanged at all currents. The independence of the changes in asymptotic performance and increases in the required number is striking in this case. Most likely, changes in performance capacity cannot account for changes in reward. The decrease observed at the low current could not be a result of a generalized performance-deficit because no decrease in performance was observed at the higher currents.

This might have been due to the fact that at very low currents, where only high frequencies can support self-stimulation, some of the action potentials generated by the stimulation might fail to propagate due to the inability of the first-order neurons to follow at such high frequencies (Simmons & Gallistel, submitted). Malette and Miliaressis (1990) and Waraczynski and Kaplan (1990) have observed that rate-frequency curves collected at very low currents, the asymptotic level of pressing is lower than at high currents. A lesion, impinging on reward-relevant neurons activated by the stimulation electrode, which is equivalent to a lowering the current, could well push the curves to a level where asymptotic response is lowered. There is still another reason to believe that the current dependent change in asymptotic rate is not responsible for the changes in the reward efficacy. That is, the postlesion dynamic intervals did not vary appreciably from baseline. This argues for a parallel curve-shift, indicative of a change in the reward effect.

Transient Effects

Two rats, L12 and L25, showed transient increases in the required number at the VTA site. The most peculiar effect is that in L12, at the low current, where an increase in the required number was observed about one week following the lesion, whereas at the high current, an immediate postlesion

increase in required number was seen but then dissipated following a few days, only to turn to an increased level after a few more days. It is difficult to ascribe the delayed changes in reward effectiveness to any effects of the lesion. This delayed increase might be due to a movement of the electrode relative to the substrate as a result of a loosening electrode assembly. The transient increase in required number observed at the high current in the case of L12 (VTA) and at each of the tested currents for L25(VTA) can be only partially explained by the postlesion changes in maximum rates. That is, as in the case of the previously mentioned effective lesions, the required number seems to be independent of the response rates. For example, although transient effects were observed at both currents tested for L25(VTA), a change in maximum rate from baseline was only observed at the low current. Furthermore, the lowering of the response asymptote at the low current persisted throughout testing, unlike the required number changes which only lasted for about a week.

One hypothesis that might account for the transience of the effects in the required number is the spontaneous regeneration of axons. Foerster (1982) has demonstrated that cut axons regenerate following lesions produced by mechanical destruction in many different areas of the brain. Her results show that massive detours of populations of fibers eventually develop around the edges of lesions, and

that these detours around the lesioned area seemed to reconnect to severed tracts. Her data suggest that the swelling and edema that occurs after a lesion are unlikely to affect this regeneration. In the histological material she examined, swelling and shrinkage were not pronounced, and even as early as three days following the lesion, new fibers started to be apparent. She concluded that the developing fibers are not the result of simple passive mechanical deformation or of a deformation that developed with time as a consequence of shrinking. The most striking aspect of her data is that evidence of axonal regeneration occurred in the first days following a lesion. According to Foerster, "Perhaps axonal regeneration should be considered as one of the mechanisms contributing to the recovery of function after brain lesions, particularly the early reversibility of deficits." One could argue that electrolytic lesions are different from cuts because they do not only sever axons but also cauterize them, thus not allowing the release of growth factors. Furthermore, electrolytic lesions are three-dimensional creating a hole in the brain. Nevertheless, electrolytic lesions in this study were rather small, possibly as a result of shrinkage such as reported by Wolfe and DiCara (1969). The inward compression of the initial lesion could be the result of regeneration around the lesion (Foerster, 1982). Foerster (1982) also found that when a severed tract did not show regeneration, the cut usually extended beyond the boundary of the tract by a distance of as

much as 400 um or more. This could explain why regeneration might not occur in all cases. It should also be pointed out that if axonal regeneration is responsible for the transient effects, then fibers of passage transversing the lesion area, and not cell bodies within that area are responsible for the decrease in reward effectiveness.

Another hypothesis that addresses the transience of the effects is that the reward system is plastic, and neuronal mechanisms might come into play to return the system to a more normal degree of functioning. Zigmond & Stricker (1974) have observed recovery of function after damage to central catecholamine-containing neurons after lesions of the lateral hypothalamus. Depending on the size and the symmetry of the lesions, the periods of recovery varied. Damage to such neurons might diminish the initial activity of the neural pathway and cause proportional increases in compensatory activity in the synaptic area. Evidence for this is that there is an increase in catecholamine synthesis rate and turnover in fibers spared by a lesion. There is also a decrease in reuptake probably due to a decrease in affinity for uptake or loss of uptake sites. Functional recovery may also be due to the supersensitivity that develops after the disruption of presynaptic fibers. It has already been mentioned in the introduction, that the dopamine system plays a role in MFB reward. The LPOA receives important projections from the Acb, which is the major output of the

mesolimbic dopamine system originating in the VTA (Mogenson, Swanson, & Wu, 1983; Williams, Crossman, & Slater, 1977). In return, the LPOA send descending projections to the VTA (Swanson, 1976). This system therefore could form a loop between the ascending dopaminergic projections and the descending path from areas in the anterior forebrain down to the VTA. Therefore, the partial destruction of the LPOA, for example, could produce changes in the dopamine system, such as to restore integrity of function.

Current Dependency of Lesion Effects

Increases in required number were observed in this study at different currents, both low and high. In the case of the largest effect was seen at the low current (L20VTA), it can be argued that the lesion lay in close alignment with the electrode tip and thus damaged reward-relevant fibers recruited by low currents. In this case, the effects at the higher currents would be expected to be smaller since the expansion in the field of stimulation might have led to the recruitment of intact reward-relevant fibers. This additional recruitment is likely to dilute any effect due to the lesion. In the case of L30(LH), L12(VTA) and L25(VTA), the largest effect was observed at the highest current. This is consistent with the notion that the lesion impinged on reward-relevant fibers that passed through the outer shell of the stimulation field produced by the highest current. In

the context of future studies, the results obtained in this study warrant retaining the three current design, so as to increase the likelihood of observing lesion effects.

Ineffective Lesions

Lesions were ineffective in the case of 9 subjects. In the case of L11(LH) and L15(VTA), decreases in maximum rate were observed following the lesion despite the fact the required number remained at baseline levels. It is noteworthy, that if the maximum rates were used as a measure of the reward effect, instead of the required number, decreases in reward effectiveness would have been erroneously attributed to these two lesions.

The location of the lesions in the case of the effective, transient, or non-effective groups overlapped to some extent. Furthermore, although some of the subjects self-stimulated through the lesioning electrode prior to the lesion, no effect on LH or VTA self-stimulation was observed. One might expect that if the lesioning site and the stimulation sites are part of a common reward substrate, then lesioning a part of this substrate would affect another part. The issue of alignment between the tip of the stimulating electrode and the lesioned axons offers a potential explanation for the inconsistent effects of lesions that overlapped to some extent. It might also provide an

explanation for the fact that in some cases, no effect of the lesion was observed even though the animal self-stimulated through that electrode. Despite the three current design, perhaps the lesion was placed so that even at the highest current there was no overlap between the field of excitation and the damaged area. In support of this notion, recall that one rat (eg. L12) bar-pressing for stimulation delivered to both the LH and the VTA electrodes showed a postlesion increase in the required number solely for stimulation through one of the electrodes, presumably the one better aligned with the lesion. In order to validate the above hypothesis it will be worthwhile to test for collision, using the stimulating and the lesioning electrodes, before proceeding with the lesion. In addition, movable electrodes (Miliaressis & Philippe, 1983) could be used so as to ensure collision and therefore alignment between the tips of the stimulating and lesioning electrodes.

The assumption underlying the argument concerning alignment is that the reward substrate forms a homogeneous bundle, inside which the lesion and the stimulating electrode can occupy varying locations. Alternatively, it is possible that the MFB comprises multiple bundles of reward fibers whose outputs are integrated separately. Based on work by Gallistel & Leon (1991), Conover and Shizgal (submitted) have proposed a way of combining the outputs of such separate integrators such that only a modest increase in the input to

one integrator could compensate for the elimination of the input to a second integrator. In that respect, larger lesions are likely to be more effective.

The small area of tissue damage produced by the lesions in this study could have contributed to the paucity of effects. The goal of this study was to localize accurately an area whose removal degrades the reward effect. In that light, small, well defined lesions were made, in the hope of differentiating important from less important areas subserving MFB self-stimulation. Nevertheless, there is some evidence to suggest that small lesions might not be as effective as larger ones. Murray and Shizgal (1991) increased their yield of positive effects by performing multi-stage lesions. In several of their subjects, a second or even a third lesion in the ALH was required to increase the threshold for self-stimulation. Murray (unpublished doctoral dissertation, 1992) tried to extend these findings, but in order to preserve histological accuracy she lesioned only once, regardless of the outcome of the lesion. In this second study, the proportion of subjects with increases in the required number following the lesion was considerably smaller. It is possible that multi-stage lesions in the LPOA might have been more effective in producing threshold increases. Additional evidence in favor of large lesions comes from a study by Arvanitogiannis and Shizgal (1993), who found that NMDA lesions of the basal forebrain, affecting

structures such as the LPOA, ALH, and substantia innominata (SI), were more successful in producing large, long-lasting or transient increases in threshold than the smaller lesions made by Murray (Unpublished doctoral dissertation, 1992).

General Discussion

The evidence from this study shows that neurons in the LPOA and the VP have physiological characteristics that overlap those of reward neurons activated at other basal forebrain as well as more posterior MFB self-stimulation sites. Furthermore, some lesions in these areas produced increases in the required number for self-stimulation of the LH and the VTA. These results are consistent with the notion that somata located in the basal forebrain or fibers of passage projecting through these regions are involved in MFB self-stimulation. Evidence from immunocytochemistry and electrophysiological experiments suggest that neurons in the LPOA and the VP are activated by rewarding stimulation.

Shizgal, Arvanitogiannis, Conover, and Pfaus (1993) have demonstrated that ipsilateral c-fos expression in anterior forebrain sites, including the LPOA and the substantia innominata, is increased following rewarding stimulation of the LH. These data suggest that neurons in these nuclei are activated during LH self-stimulation. Murray (1993) has shown that cells in the basal forebrain antidromically activated by LH stimulation have excitability characteristics that overlap those obtained from behavioral psychophysical experiments. Some of the cells that she recorded from were located in the VP.

Shizgal, et al. (1989) carried out electrophysiological recordings from neurons in the anterior forebrain that were antidromically activated by an LH stimulation electrode which supported self-stimulation. The strength of this study stems from the fact that the rats were behaviorally tested prior to the recording session. Psychophysical estimates of the refractory periods were obtained using the same stimulation and sites as in the recording session. Thus, refractory periods obtained from the activated cells in the basal forebrain could then be compared to those derived behaviorally using the same electrical stimuli. Some of the antidromically activated neurons in areas such as the VP and the LPOA had refractory periods that matched those of the behaviorally obtained ones. In all, the results of this study, together with the immunocytochemistry and electrophysiological data, are consistent with the notion that neurons with cell bodies in the LPOA and/or the VP comprise part of the directly-stimulated substrate for MFB reward. Indeed, Arvanitogiannis, et al. (1993) used a soma-selective excitotxin, NMDA to lesion the basal forebrain and found some large increases in the required number for self-stimulation of the LH and the VTA. In these subjects, damage was located in areas such as the LPOA, lending further support that somata in these areas give rise to at least some of the directly-activated fibers subserving MFB reward.

Summary

The goal of the present study was to assess the contribution of the LPOA to the reward effectiveness of MFB stimulation. The excitability properties of neurons in this area were found to partially overlap those obtained at more caudal sites along the MFB. Furthermore, a small proportion of lesions damaging the LPOA and neighbouring areas were effective in attenuating the rewarding impact of the stimulation. Overall, these findings are consistent with the notion that cell bodies residing in or fibers of passage coursing through the LPOA play a role in MFB self-stimulation. Several reasons were given explaining the failure of most lesions to change the required number, accompanied by suggestions for methodological improvements. It is hoped that these improvements will increase the ratio of effective to ineffective lesions, strengthening the conclusion of this thesis. The histological examination for some of the subjects revealed that the lesion damaged the VP. These results suggest that a follow-up study is warranted to examine the contribution of the VP to BSR.

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