# CATECHOLAMINE INVOLVEMENT IN LATERAL HYPOTHALAMIC SELF-STIMULATION: WITH SPECIAL REFERENCE TO THE DORSAL NORADRENERGIC SYSTEM

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# CATECHOLAMINE INVOLVEMENT IN LATERAL HYPOTHALAMIC SELF-STIMULATION: WITH SPECIAL REFERENCE TO THE DORSAL NORADRENERGIC SYSTEM

R. Dale Corbett ©

Thesis submitted to the Department of Psychology, Memorial University of Newfoundland, in partial fulfillment of the requirements for the degree of Master of Science, December, 1974.

ABSTRACT

Six experiments were undertaken to determine the involvement of the nigrostriatal dopamine (DA) pathway and the dorsal noradrenergic (NA) pathway in mediating lateral hypothalamic (LH) self-stimulation. All subjects were implanted with bipolar electrodes in the LH area and were trained to self-stimulate. Once this behavior had stabilized (20-30 days), lesions at various levels of the nigrostriatal DA system and the dorsal NA system were made via intracerebral injections of the catecholamine (CA) neurotoxin, 6-hydroxydopamine (6-OHDA) and/or electrolytic lesions. Testing for self-stimulation was resumed 24 hrs. after the lesions and continued for a period of 5 days. At the conclusion of testing, the animals were sacrificed and prepared for histological examination.

Destruction of several CA systems by injections of 6-OHDA (8µg/4µl) in the cells of origin (A9) of the nigrostriatal DA system resulted in a strong suppression of LH self-stimulation ($p<.025$). Injections of 6-OHDA (8µg/4µl) anterior to the cells of origin (A6) of the dorsal NA system had little or no effect on LH self-stimulation (Experiment 2). 6-OHDA (4µg/µl) and electrolytic lesions of the dorsal NA bundle in combination with electrolytic lesions of A6 also failed to substantially affect LH self-stimulation (Experiments 3 & 5). The fourth experiment attempted to further examine the role of the dorsal NA

system in DA self-stimulation by injections of procaine (a local anesthetic), d-amphetamine and glutamate (a neural excitant) directly in A6. All three treatments resulted in considerable suppression of LH self-stimulation ($p<.001$) but these results were rendered questionable due to possible non-specific effects and methodological considerations. The sixth and final experiment revealed that self-stimulation is rapidly obtained from the region of A6 when a sensitive shaping procedure that maximizes behavioral arousal is employed.

Together, these results suggest that LH self-stimulation is mediated by the nigrostriatal and mesolimbic DA systems alone or in combination with the dorsal NA system but the dorsal NA system itself does not seem necessary for maintenance of LH self-stimulation. Thus, noradrenergic theories of self-stimulation (e.g. Stein & Wise, 1971) must be reconsidered.

## ACKNOWLEDGEMENTS

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TABLE OF CONTENTS

## LIST OF TABLES

# LIST OF FIGURES

LIST OF PLATES

Plate

# CHAPTER I

## INTRODUCTION

The historic discovery of the self-stimulation phenomenon by Olds and Milner (1954) led to a proliferation of studies directed at uncovering the apparent anatomical basis of neural reinforcement. However, interest in this area began to wane in the mid to late 1960's. The main reason for this decline was that over a decade of concentrated effort had failed almost totally in specifying the anatomical loci of self-stimulation. Some researchers argued for a reward center in the lateral hypothalamus (Olds, 1969), while others believed self-stimulation to be diffusely organized in the brain, with no single reward center (Valenstein, 1968).

Despite these difficulties, Stein and his co-workers were providing evidence from their pharmacological studies (Stein, 1964, 1967b; Stein & Wise, 1969) for noradrenergic (NA) involvement in self-stimulation. Stein and Wise (Stein, 1967b; Stein & Wise, 1969) suggested that it was the release of norepinephrine (NE) from pre-synaptic terminals of the medial forebrain bundle (MFB) which produced the rewarding effect underlying self-stimulation. This theory was based on the observation that amphetamine and phenethylamine whose chemical structures are very similar to NE facilitate lateral hypothalamic (LH) self-stimulation (Stein, 1964).

Further support for this theory was that rewarding electrical stimulation of the MFB, as well as systemic pretreatment with amphetamine released NE from specific forebrain areas such as the amygdala and hypothalamus, while aversive stimulation inhibited this release in a few animals (Stein & Wise, 1969).

These data were only suggestive since a direct test of this "noradrenergic hypothesis" was not possible. Conventional lesioning procedures such as electrocoagulation or application of local anesthetics did not discriminate between catecholamines (CA) and other putative neurotransmitters. Also, the locations of the CA in the brain were only beginning to be mapped out (Dahlstrom & Fuxe, 1964; Anden et al., 1966) thus making it nearly impossible to interpret results from lesion or stimulation studies as support for or against a NA hypothesis. Two developments have permitted researchers to precisely evaluate the CA regulation of behavior: (1) The mapping of the major CA pathways in the brain by fluorescent histochemistry (Ungerstedt, 1971a) and (2) The discovery that 6-Hydroxydopamine (6-OHDA) can be deployed as a chemical lesioning tool specific to CA.

6-OHDA does not cross the blood-brain barrier (Ungerstedt, 1968) but when injected intraventricularly, intracerebrally or intracisternally produces complete degeneration of NA and dopaminergic axons and terminals. Dopaminergic cell bodies readily degenerate after treatment

with 6-OHDA while NA cell bodies are highly resistant to the toxic effects of 6-OHDA. Serotonergic neurons are not affected by 6-OHDA. These results are based on histochemical fluorescence studies (Ungerstedt, 1968, 1970, 1971a, 1973; Hokfelt & Ungerstedt, 1973; Uretsky & Iverson, 1969; Evetts et al., 1970), biochemical studies (Bloom, et al., 1969; Jacks et al., 1972; Hedreen & Chalmers, 1972) and by electron microscopy (Bloom et al., 1969; Hokfelt & Ungerstedt, 1973). Thus, the greater specificity of 6-OHDA over conventional lesioning techniques makes it a useful tool in anatomical and functional studies.

The four major CA pathways have been mapped by Ungerstedt (1971a): the nigrostriatal and mesolimbic dopamine (DA) pathways and the dorsal and ventral NA pathways.

The nigrostriatal DA pathway is the major dopaminergic pathway in the brain, containing 70-80% of the total brain DA (Fuxe & Anden, 1966). Its origin is the A9 cell group of Dahlstrom & Fuxe (1964) which is located in the zona compacta of the substantia nigra (SN). This pathway ascends through the ventral tegmental area where it forms a large bundle of fibres which run through the LH area before terminating in the caudate putamen. These projections have been verified by silver staining (Hedreen & Chalmers, 1972; Maler et al., 1973) and by electron microscopic autoradiography (Hattori et al., 1973).

The mesolimbic DA pathway has been much less extensively studied than the nigrostriatal pathway. This pathway originates from cell group A10, just dorsolateral to the interpeduncular nucleus, runs rostrally in the MFB, adjacent to the LH and terminates in the nucleus accumbens and the tuberculum olfactorium.

The dorsal NA pathway arises from the locus coeruleus which corresponds to the A6 cell group. This pathway gives rise to terminals in the hippocampus, septal area, cerebral and cerebellar cortices. The dorsal noradrenergic pathway is of considerable interest since it is able to influence most of the brain in a unique manner (Ungerstedt, 1971a).

The ventral NA pathway has a diffuse origin in the lower brain stem, originating from cell groups A1, A2, A5 and A7 (Ungerstedt, 1971a). The axons ascend in the mid-reticular formation entering rostrally in the MFB and innervate the hypothalamus, preoptic area and the ventral stria terminalis. More recent evidence (Olson & Fuxe, 1972) suggests that the ventral NA pathway can be separated into two distinct components, the subcoeruleus component and the medulla oblongata component. The subcoeruleus component arises from ventral A6, A7 and NA cells connecting these two groups and innervates mainly periventricular areas of the hypothalamus and preoptic area. The medulla oblongata projection originates in A1, A2 and possible A5 and innervates the basolateral hypothalamus, preoptic area

and ventral striae terminalis.

Ungerstedt (1971a, 1973) has described loci where each of these pathways separates from the others to allow specific destruction by 6-OHDA.

Stein and Wise (1971) have reported substantial decreases in LH-MFB self-stimulation after intraventricular injections of 6-OHDA. They attribute these decreases to destruction of NA terminals in the LH-MFB area. However, the four major CA pathways course through or adjacent to the LH-MFB area and subsequent studies (Crow, 1972a, 1972b; Crow et al., 1972; Phillips & Fibiger, 1973) have demonstrated that self-stimulation can be obtained from the nigrostriatal DA pathway, the mesolimbic DA pathway and the dorsal NA pathway. The ventral NA pathway at first was thought to support self-stimulation (Arbuthnott et al., 1971) but more recent evidence suggests the contrary (Clavier & Routtenberg, 1973; Anlezark et al., 1973). Since the intraventricular route of administration is somewhat non-specific in its action, the decreases in LH self-stimulation observed by Stein & Wise (1971) may have resulted from damage to one or several CA pathways which support self-stimulation.

In view of the difficulty in interpreting Stein & Wise's results a series of experiments were conducted to elucidate the involvement of the nigrostratal DA pathway and the dorsal NA pathway in producing the decrements in LH self-stimulation reported by Stein and Wise (1971).

The nigrostriatal DA pathway was selected over the mesolimbic DA pathway for investigation because it is the major DA pathway in the brain and much more is known about its anatomy and function (Ungerstedt, 1971a) than is known about the mesolimbic DA pathway. The dorsal NA pathway was studied since it seems to be the only NA pathway which supports self-stimulation (Crow, 1973).

# CHAPTER II

## THE NIGROSTRIATAL DA PATHWAY

The demonstration that self-stimulation could be obtained from DA areas such as A9 and A10 (Crow, 1972a, 1972b) suggested strongly that self-stimulation was not entirely NA as proposed by Stein (Stein, 1964; Stein & Wise, 1969, 1970, 1971).

Additional support for the DA involvement in self-stimulation is provided from pharmacological studies. Taylor and Snyder (1970) have shown that the levo isomer of amphetamine is approximately ten times less potent in blocking CA uptake into NA neurons than the d- isomer, while both isomers are equipotent in blocking uptake into striatal DA neurons. Farnebo (1971) has suggested that the d- and l- isomers of amphetamine may differ in their ability to increase the release of NE from NA neurons but have similar action on release from DA neurons. Phillips & Fibiger (1973) reported that d-amphetamine produced nearly a ten-fold increase in self-stimulation rates from electrodes in the LH compared to the increase produced by l-amphetamine. Most important was the observation that self-stimulation rates from electrodes in the A9 cell group of the SN which is almost entirely dopaminergic (Ungerstedt, 1971a) were increased as much by d-amphetamine as by l-amphetamine. These results provide considerable evidence for the dopaminergic involvement in self-stimulation.

The present experiment was designed in order to determine if the decreases in LH self-stimulation observed by Stein and Wise (1971) could have been due to destruction of the nigrostriatal DA pathway. 6-OHDA was selected for lesioning this pathway since intracerebral injections of 6-OHDA in A9 are known to produce a selective and complete degeneration of the nigrostriatal system (Ungerstedt, 1971a; Hokfelt & Ungerstedt, 1973).

Subjects

Eight male, Sprague-Dawley rats weighing 300-400 gm at the time of surgery were used in the experiment. Subjects (S's) were housed individually and had unlimited access to food and water.

Surgery

Under sodium pentobarbital anesthesia (50 mg/kg, i.p.), a bipolar, stainless steel electrode was implanted in the LH of each S. The electrodes had a tip diameter of .18 mm and were insulated except at the tip cross-section. In addition, each S was implanted with a 23 gauge stainless steel cannula ipsilateral to the LH electrode. The cannulae were aimed at the zona compacta of the SN. A 00 gauge insect pin, bent in the middle was inserted in the cannula to prevent occlusion.

All implantations were performed using standard stereotaxic procedures with the incisor bar set at 5 mm above the interaural line. The co-ordinates for LH and SN were taken from the Pellegrino and Cushman (1967) atlas

and were +0.4, +2.0, -8.0 and -3.5, +1.8, -6.6 respectively. Each S was allowed at least 3 days to recover from the operation before testing was initiated.

Procedure

Each S was placed in a Skinner box and trained to lever-press. Each press delivered a .5 sec., 60 Hz. alternating current pulse. Once shaping was accomplished, each S was given a daily 15 min. session of bar-pressing during which the total number of responses was recorded.

The response rates were considered stable when on 4 consecutive days, the highest or lowest test day did not differ by more than 15% from the mean number of responses of the 4 day period and the S's response rate did not show a continuous increase or decrease over these 4 days. Stability was achieved after 20-30 days of testing.

On the day following stabilization, 4 S's were injected in SN with 8µg/4µl of 6-OHDA containing 1.0 mg/ml ascorbic acid dissolved in .9% saline. Four control S's were injected with 4µl of the ascorbic acid vehicle solution. Injections were performed under light ether anesthesia via a 30 gauge stainless steel needle inserted in the cannula. The tip of the injection needle protruded from the cannula by approximately .2-.3 mm such that it would be just dorsal to the zona compacta and thus reduce the non-specific damage resulting from the injection itself to an area dorsal to the A9 cell group. To further minimize non-specific damage, all injections were performed at the

slow rate of 1µl/min. The injection needle was left inserted in the cannula for 1.0 min. per each µl of solution injected; in this experiment the needle was left inserted for 4 min. after the injection. This precaution ensured that the solution would be absorbed by the tissue rather than drawn up the cannula shaft.

Twenty-four hours after the injections testing was resumed. The total number of responses during daily 15 min. sessions were recorded for a period of 4 days. On completion of the experiment the S's were deeply anesthetized with pentobarbital and perfused with .9% saline followed by 10% formalin. The brains were removed and stored in 10% formalin for at least 3 days, after which 40µ frozen sections were cut and stained with thionin.

The post-injection response levels were expressed in terms of per cent of the mean number of responses during the last two pre-injection sessions and were subjected to a two-factor (Treatment x Days) analysis of variance for repeated measures (Winer, 1971).

Results

The 6-OHDA S's showed a strong suppression of LH self-stimulation in comparison to the vehicle treated controls (see Fig. 1, $F(1,6)=9.59$, $p<.025$). Decreases in responding for the 6-OHDA treated S's were greatest on Day 1 but even at the conclusion of testing the response levels were only approximately 60% of their pre-injection levels. Figure 1 shows that the decreases in the 6-OHDA S's

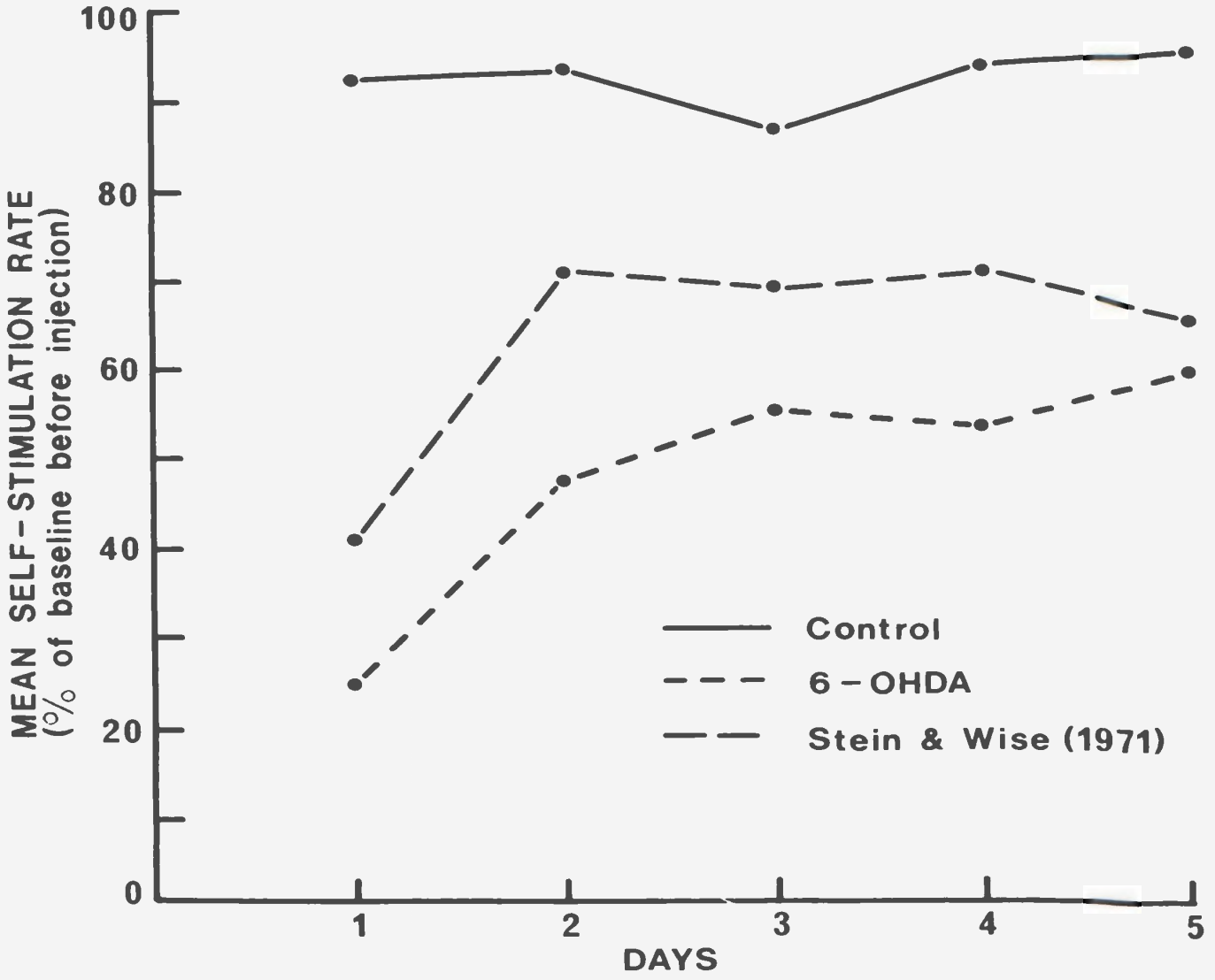



FIG. 1. Suppression of LH self-stimulation after injections of 6-OHDA (8µg/4µl) in SN.

were considerably greater than those reported by Stein and Wise (1971).

Table 1 shows the response rates for each S over the 5 test days following treatment with 6-OHDA or the vehicle solution. Also shown for each S is the stimulus intensity used in testing, the mean number of responses of the last 2 sessions prior to injections and the level of response variability during the 4 consecutive days prior to the injection. Of the 4 6-OHDA treated S's, one S (SN 2) showed nearly total suppression of self-stimulation over the 5 day test period. Two S's (SN 7 & SN 26) had recovered substantially by the last day of testing while the fourth S (SN 28) had returned to pre-treatment levels of responding by the 5th test day. These differences in recovery presumably reflect different degrees of denervation induced by the 6-OHDA.

All LH electrodes were located on the edge of the LH, ventral to the internal capsule and just dorsal to the optic tract at the level of the ventromedial nucleus (see Plate 1). Of the 4 S's injected with 6-OHDA, 2 had cannulae just dorsal and lateral to zona compacta at its caudal level, while the other 2 S's had cannulae just dorsal to zona compacta at its broadest extension (Plate 2). In these last 2 S's a marked cell loss was observed from A9 when compared to the non-injected side (Plate 3). It was difficult to determine if a cell loss was present in the first 2 S's due to slightly improper sectioning and poor quality of staining.

Table 1

Response rates for individual S's after 6-OHDA (8µg/4µl) injections in SN on test days 1-5

| Subject | Stimulus Intensity | Pre-injection Mean | Response Variability | Treatment | Test Days 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|---|---|---|---|
| SN 2 | 14µa | 757.5 | <10% | 6-OHDA | 104 | 35 | 62 | 100 | 30 |
| SN 7 | 12µa | 667.5 | 10% | 6-OHDA | 177 | 436 | 392 | 552 | 412 |
| SN 26 | 26µa | 611.5 | 10% | 6-OHDA | 186 | 480 | 474 | 398 | 442 |
| SN 28 | 18µa | 496.5 | 15% | 6-OHDA | 144 | 208 | 422 | 292 | 493 |
| SN 1 | 24µa | 814.0 | <10% | Control | 722 | 727 | 652 | 629 | 724 |
| SN 3 | 22µa | 723.5 | 15% | Control | 611 | 683 | 520 | 676 | 678 |
| SN 9 | 36µa | 959.0 | 10% | Control | 852 | 891 | 895 | 959 | 865 |
| SN 14 | 16µa | 967.5 | 10% | Control | 1028 | 929 | 941 | 996 | 1038 |

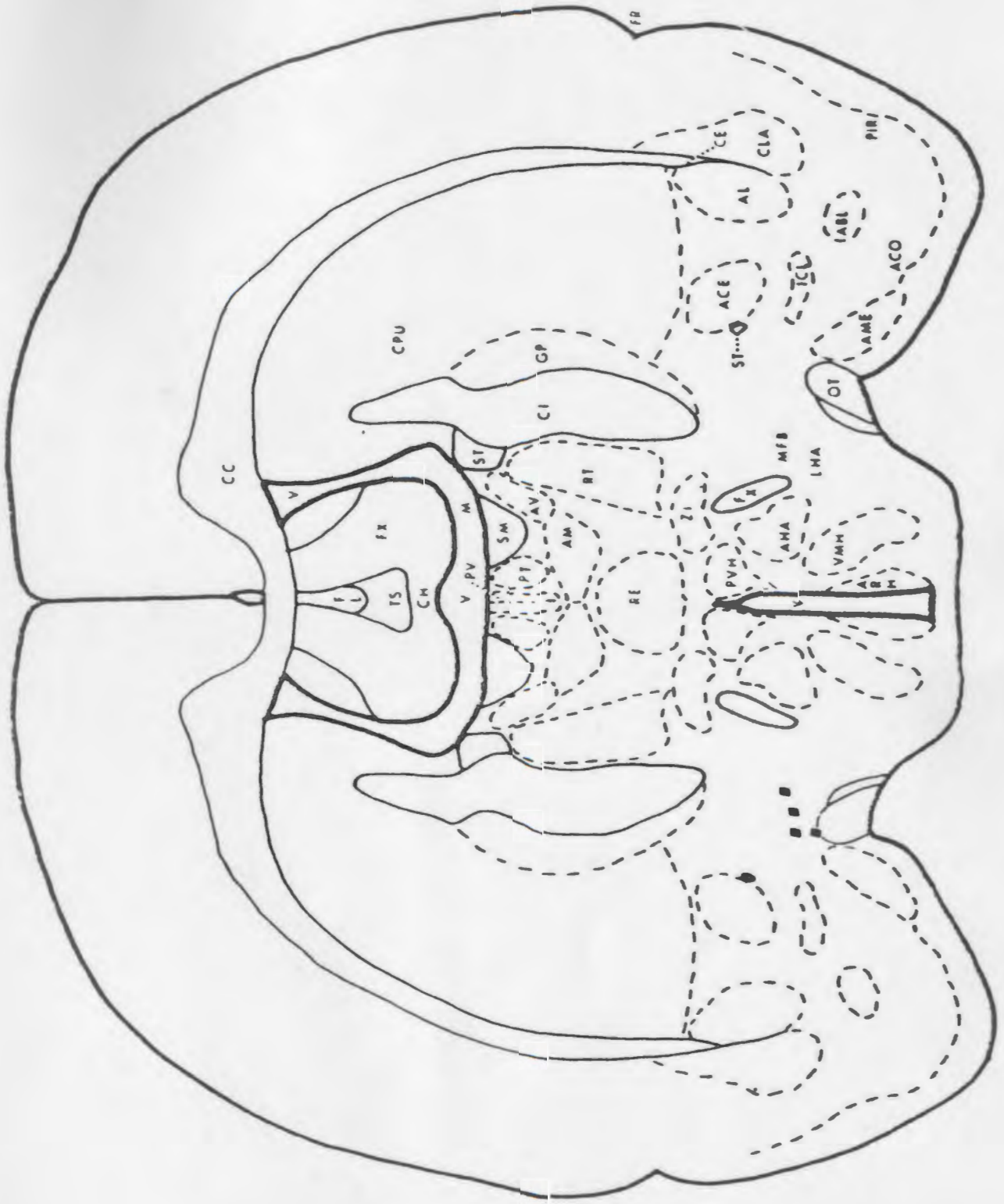
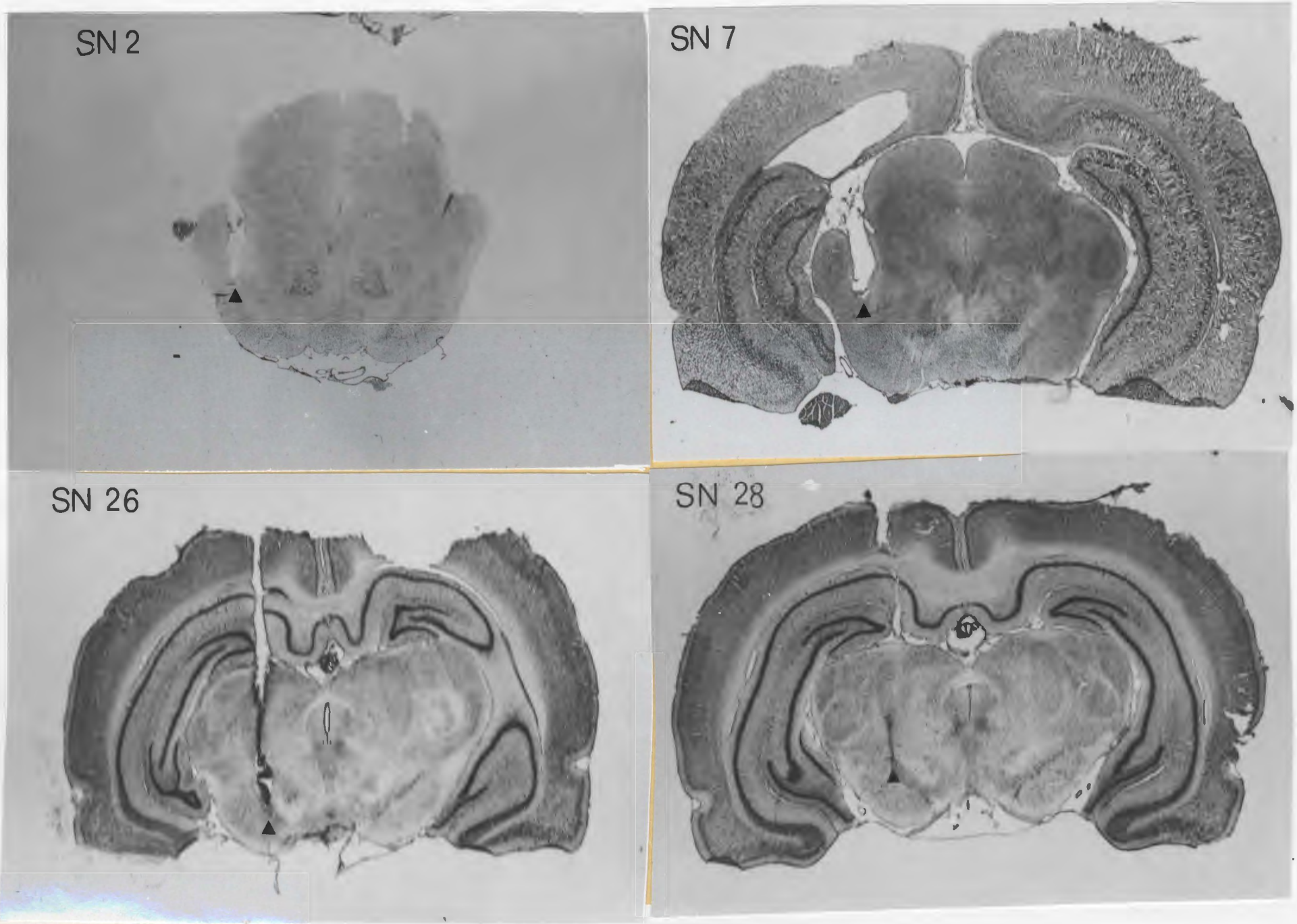



Plate 1. Schematic diagram illustrating placement of LH electrodes in 6-OHDA treated S's (SN 2,7, 26 & 28).


SN 2
SN 7
SN 26
SN 28

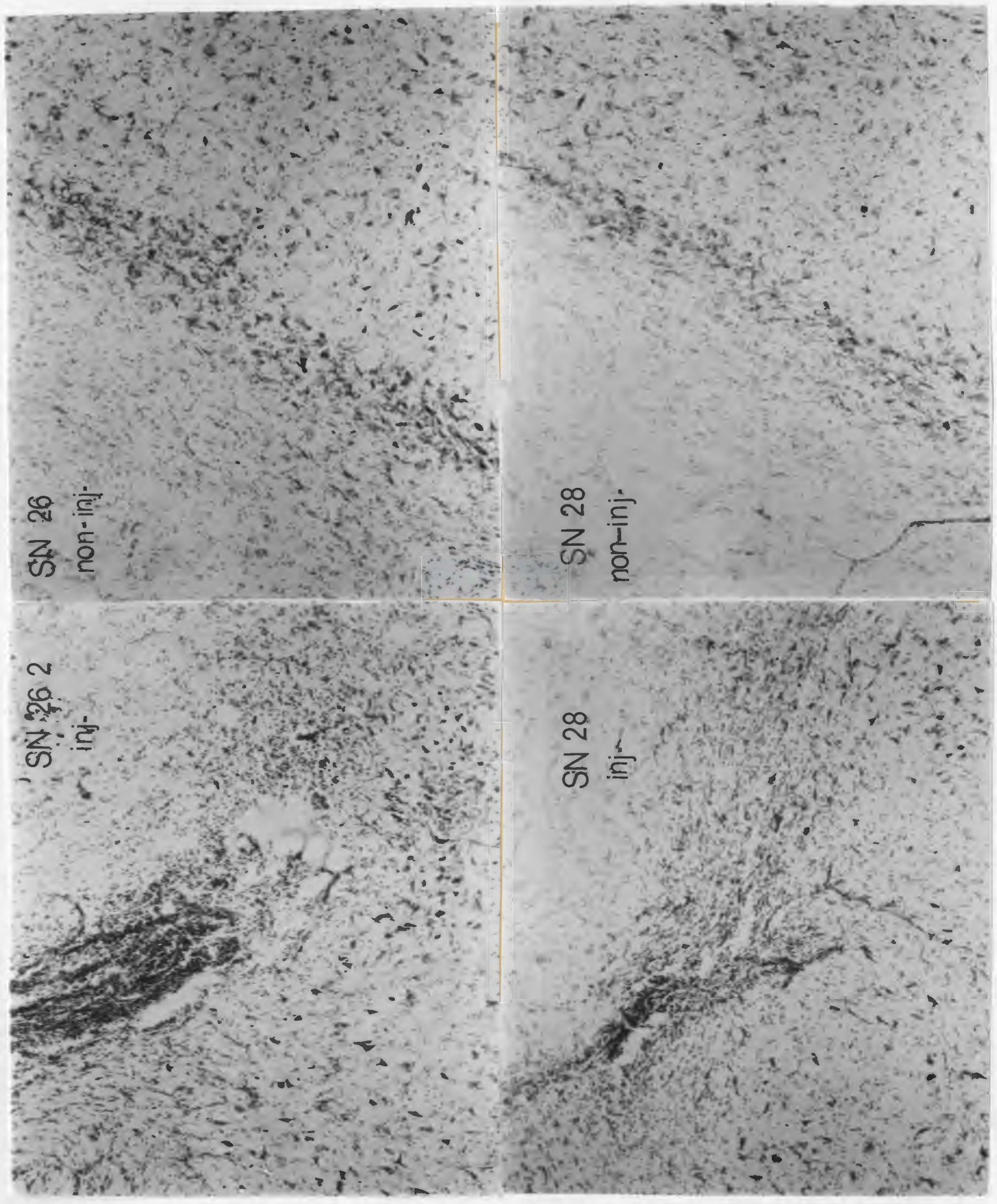



Plate 3. Cell loss in A9 of S's 26 & 28 after 6-OHDA injections in SN compared to non-injected A9 areas of same S's. Magnification X 85.

Discussion

The substantial decreases in LH self-stimulation after injections of 6-OHDA in SN initially suggested that these decreases were due to damage of the nigrostriatal DA pathway rather than NA systems as suggested by Stein and Wise (1971). However, recent evidence concerning the diffusion and specificity of 6-OHDA (Sotelo et al., 1973; Agid et al., 1973; Hokfelt & Ungerstedt, 1973) indicates that the 4µl injections of 6-OHDA used in the present study, in addition to destroying the nigrostriatal DA pathway, would also have affected the mesolimbic DA pathway and possibly the dorsal NA pathway. Thus, the possibility that the mesolimbic DA pathway and the dorsal NA pathway were involved in producing these decreases cannot be excluded.

Phillips (1973) has reported little or no change in hypothalamic self-stimulation as measured in a shuttle-box apparatus after 2µl injections of 6-OHDA in SN or with small electrolytic lesions of SN. These treatments did, however, disrupt object carrying which was also elicited by the hypothalamic stimulation.

The discrepancy in results between the present experiment and that of Phillips (1973) may be resolved in terms of the different procedural and experimental manipulations employed in these studies. First of all, the placement of the LH electrodes in the present study (see Plate 1) is in an area traversed by the nigrostriatal and mesolimbic DA pathways (Ungerstedt, 1971a). Phillips (1973)

does not report the exact location of his "hypothalamic electrodes" but if they were located more medially than those in the present study then they may have been primarily activating NA fibres (Ungerstedt, 1971a). A DA biased electrode or a NA biased electrode placement may influence in part any subsequent change in LH self-stimulation after destruction of a particular CA pathway(s).

Phillips' (1973) failure to observe any change in hypothalamic self-stimulation after destruction of the nigrostriatal DA pathway may also have been due to his use of shuttlebox crossing as the dependent measure. Shuttlebox crossing may not be as sensitive in reflecting changes in the "reward value" of electrical stimulation as is bar-pressing.

Finally, Phillips' (1973) 2µl injections of 6-OHDA would be primarily confined to the SN and may not have affected the mesolimbic DA pathway and it is unlikely that his 6-OHDA injections or electrolytic lesions would have damaged the dorsal NA pathway. The 4µl injections of 6-OHDA in this experiment, however, would have affected both DA pathways and possibly the dorsal NA pathway.

Together, the results of the present experiment and those of Phillips (1973) suggest that LH self-stimulation may be dependent on as many as 3 CA pathways: the nigrostriatal and mesolimbic DA pathways and the dorsal NA pathway.

# CHAPTER III

## THE DORSAL NA PATHWAY

The dorsal NA pathway has its origin in the A6 cell group or LC of the dorsolateral pontine tegmentum. This pathway turns medially after leaving LC, ascends through the midbrain and enters the MFB where it joins the DA and ventral NA pathways. The dorsal NA pathway innervates the septum, hippocampus and the cerebral and cerebellar cortices (Ungerstedt, 1971a). A complex function is suggested for the dorsal NA pathway since it innervates "higher" cortical structures and is the only neuronal system in the brain known to show collateral innervation of all cortices (Ungerstedt, 1971a).

It is known that self-stimulation can be obtained from the LC (Crow, 1972a; Ritter & Stein, 1973). Crow (1972b, 1973) has suggested that this pathway together with the nigrostriatal and mesolimbic DA pathways form the neural basis for self-stimulation.

### Experiment 1

The present experiment is the first in a series of experiments designed to determine the involvement of the dorsal NA pathway in LH self-stimulation.

Subjects

Seven male, Sprague-Dawley rats weighing 300-400 gm at the time of surgery were used in Experiment 1.

Surgery

All 7 S's were implanted with bipolar LH electrodes as described in Chapter II. Each S was also implanted with a cannula ipsilateral to the LH electrode in the LC (-7.4, +0.5, -5.7).

Procedure

As described in Chapter II. The 4 experimental S's were injected with 6-OHDA (8µg/4µl) in LC and the 3 control S's received 4µl injections of the ascorbic acid vehicle.

Results

Analysis of variance for repeated measures showed no significant differences in response levels between the 6-OHDA treated S's and the ascorbic acid control S's (see Fig. 1, $F(1,5)=1.24$, $p>.05$).

Two of the 6-OHDA treated S's showed a slight tendency (mean increases of 11.78% and 18.74%) towards increased responding, one S showed little or no change while the final S exhibited decreased responding (mean decrease of 18.3%) after the 6-OHDA injections. Table 1 shows each S's individual response levels over the 5 test days, the pre-injection mean and response variability over 4 consecutive days prior to the injections.

Plate 1 illustrates the placement of the LH electrodes in the 4 experimental S's. All electrodes were located in the region of the projections of the DA pathways, lateral to the LH. One of the 6-OHDA injected S's had a cannula located in the most rostral part of A6, one just

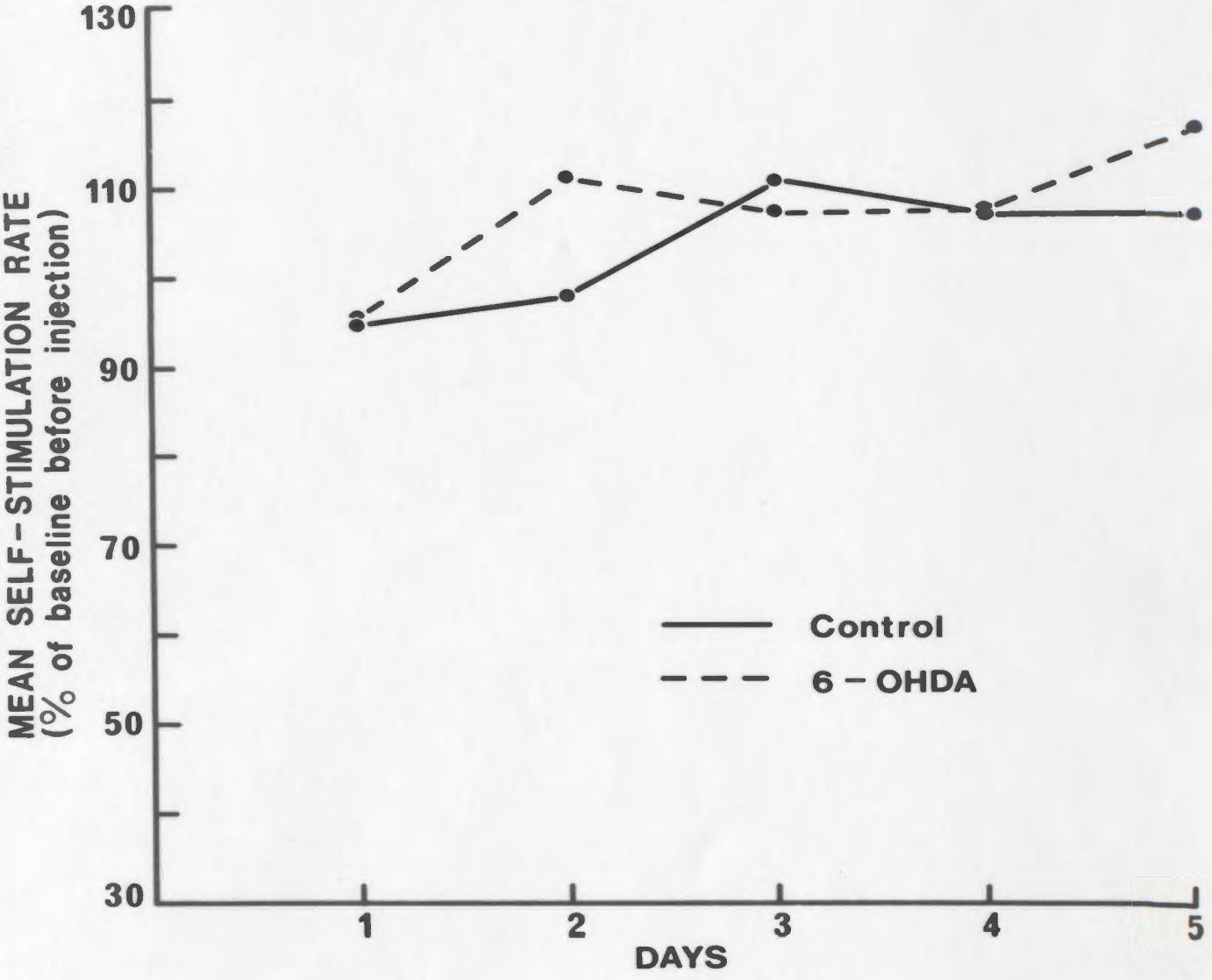



Fig. 1. Effect of LC injections of 6-OHDA (8µg/4µl) on LH self-stimulation in S's LC 1, 7, 11 & 14.

Table 1

Response rates for individual S's after 6-OHDA injections in the region of LC on test days 1-5

| Subject | Stimulus Intensity | Pre-injection Mean | Response Variability | Treatment | Test Days 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|---|---|---|---|
| LC 1 | 28µa | 664.5 | <10% | 6-OHDA | 680 | 706 | 671 | 903 | 755 |
| LC 7 | 40µa | 1104.5 | 10% | 6-OHDA | 1123 | 1279 | 1050 | 1124 | 1160 |
| LC 11 | 36µa | 702.0 | 15% | 6-OHDA | 753 | 809 | 874 | 746 | 986 |
| LC 14 | 30µa | 672.0 | 10% | 6-OHDA | 479 | 675 | 582 | 774 | 727 |
| LC 10 | 24µa | 695.0 | <10% | Control | 701 | 688 | 779 | 740 | 662 |
| LC 13 | 12µa | 665.0 | 10% | Control | 315 | 539 | 725 | 648 | 735 |
| LC 15 | 36µa | 671.0 | 15% | Control | 681 | 675 | 729 | 721 | 674 |

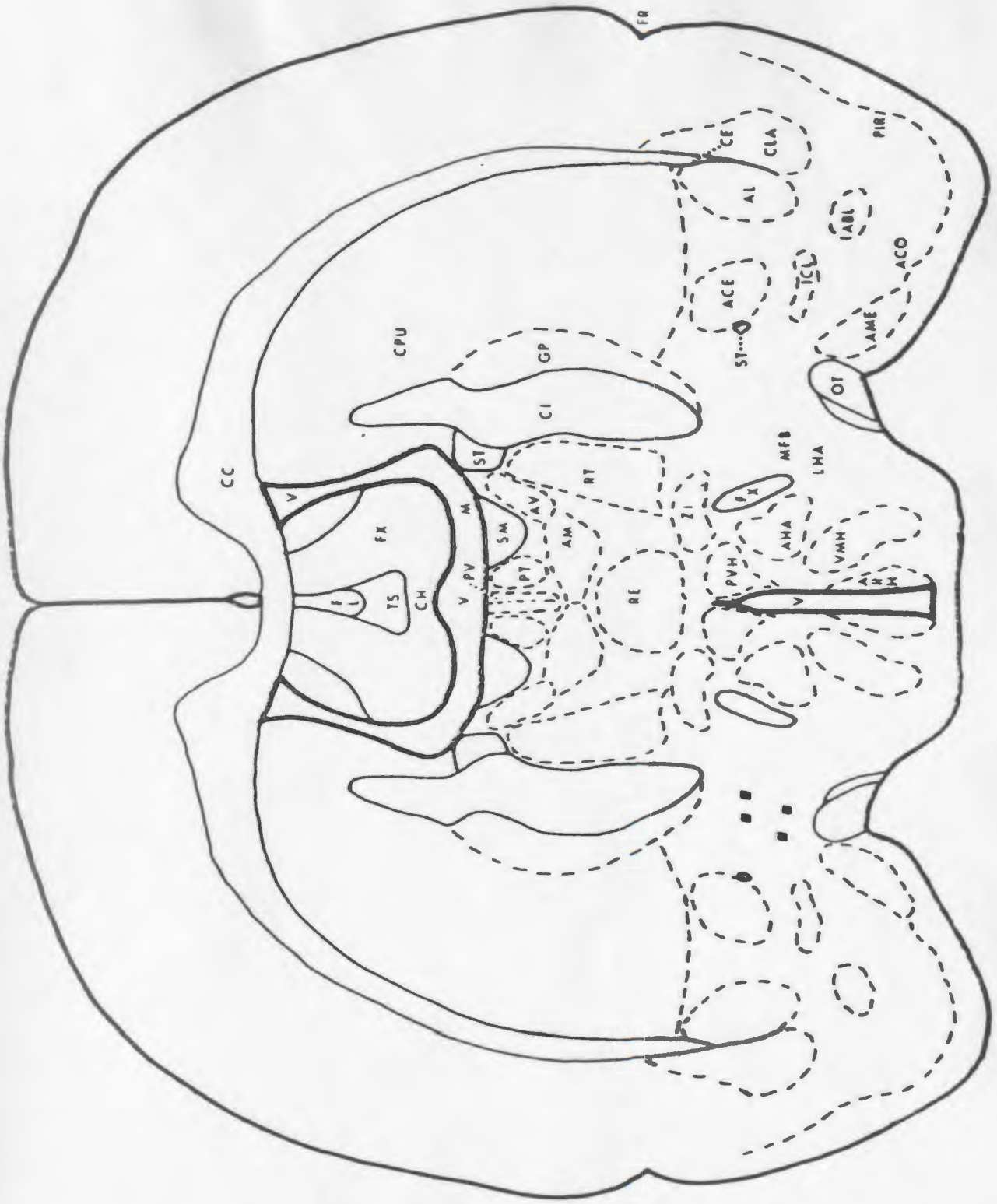



Plate 1. Schematic diagram illustrating placement of LH electrodes in 6-OHDA (8µg/4µl) treated (LC) S's (LC 1, 7, 11 & 14).

dorsal or in the brachium conjunctivum (BC) and the other considerably dorsal to the BC on the edge of the periventricular grey substance. The fourth S's cannula was located at the level of the dorsal tegmental nucleus at the floor of vermian lobule II (see Plate 2). The S having a cannula in anterior A6 and the S with its cannula on the floor of vermian lobule II showed a slight increase in responding while the S having a cannula dorsal to BC on the edge of the periventricular grey showed decreased responding after the 6-OHDA injections.

Discussion

Without biochemical assays, it is difficult to ascertain from standard histological data if the injections of 6-OHDA in LC produced any significant amount of degeneration since the cannula placements were rather variable. Precise placement would be crucial for degeneration to occur since the dorsal NA bundle forms a tight group of axons after leaving A6 (Ungerstedt, 1971a). Even if the 6-OHDA diffused to A6 no degeneration may have taken place since NA cell bodies are relatively resistant to 6-OHDA (Ungerstedt, 1971a, 1973).

The above considerations do not allow a resolution of the involvement of the dorsal NA pathway in LH self-stimulation.

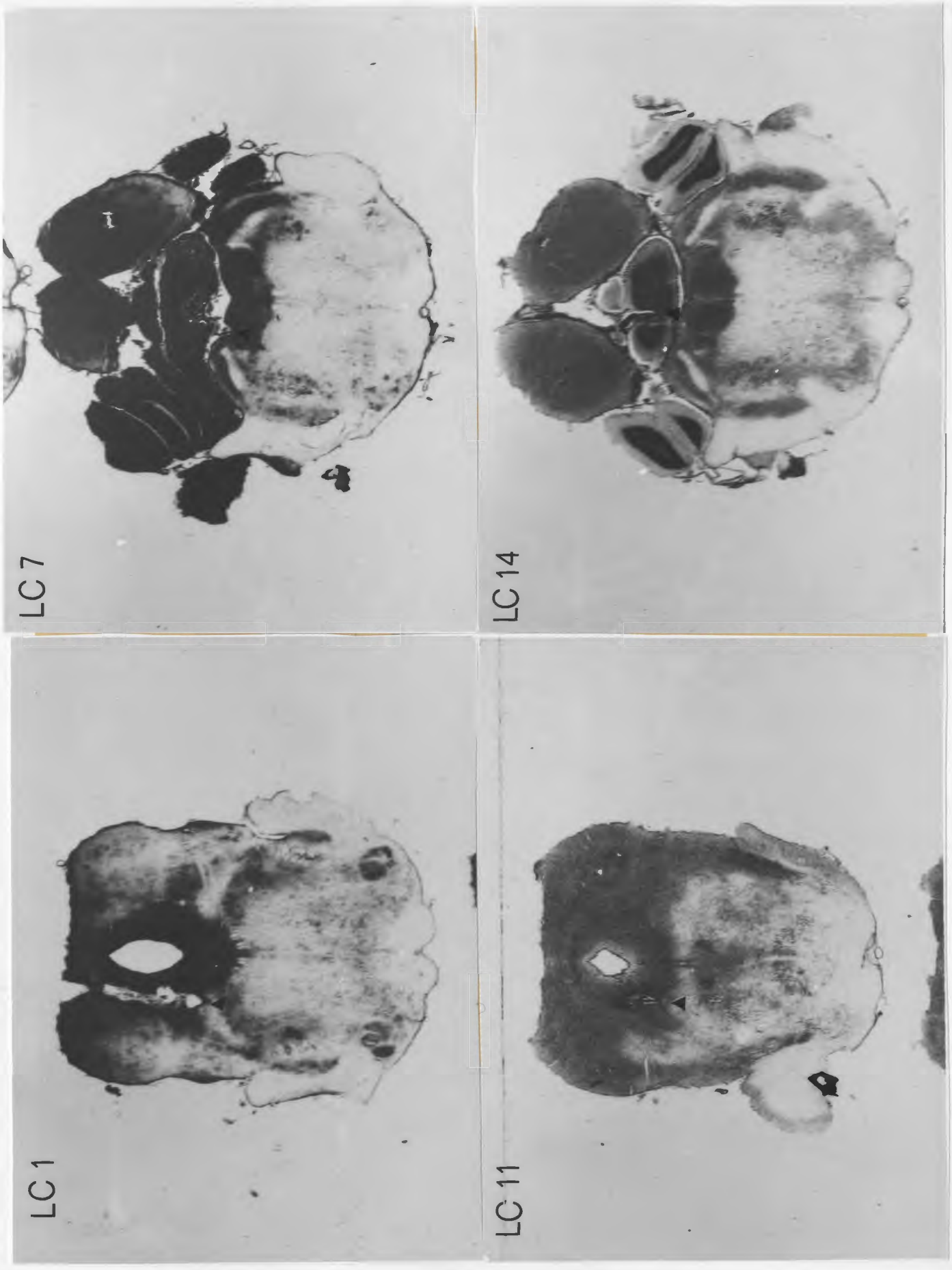



Plate 2. Placement of cannulae in LC 1, 7, 11 & 14 who were injected with 6-OHDA (8µg/4µl) in the region of LC.

## Experiment 2

Since 6-OHDA lesions just anterior to A6 produce less severe degeneration than electrolytic lesions (Chu & Bloom, 1974) the finding in Experiment 1 that 6-OHDA injected anterior to LC had no effect on LH self-stimulation would be strengthened if similarily placed electrolytic lesions also failed to alter LH self-stimulation. Damage to the A6 cell group or its projections could be confirmed without histochemical fluorescence or bioassay evaluation by making the LC lesions via electrodes that supported self-stimulation since the dorsal NA system seems to be the only NA system that supports this behavior (Clavier & Routtenberg, 1974; German & Bowden, 1974) and at the level of the LC, the dorsal NA system is well separated from the DA systems which also support self-stimulation (Ungerstedt, 1971a). Prior to the electrolytic lesions of LC, it was decided to inject 6-OHDA in the dorsal NA bundle at the level of the medial longitudinal fasciculus (FLM). By lesioning the dorsal NA system at two different levels, that is, the fibre bundle and the cells of origin (A6) the probability of extensive damage to the dorsal NA system would be increased over a single lesion of this system.

Subjects

Seven male, Sprague-Dawley rats weighing 300-400 gm at the time of surgery were used in this experiment.

Surgery

As described in Experiment 1. All S's were implanted in the LH with a bipolar electrode. Ipsilateral to the LH electrode, each S was implanted with a cannula in the dorsal NA pathway (-4.4, +0.8, -6.6) and a bipolar electrode in LC (-7.7, +0.9, -5.6).

Procedure

Twenty-four hrs. after responding had stabilized, 3 S's were injected with 6-OHDA (4µg/µl) in the dorsal NA pathway. Twenty-four hrs. after the injection testing was resumed and the total number of responses during daily, 15 min. sessions was recorded for both the LH and LC electrodes. Twenty-four hrs. after completion of this 5-day testing period these S's sustained electrolytic lesions of LC (2.5 ma - 10 sec.) via the LC electrode. Twenty-four hrs. after the lesion testing was resumed and continued for 5 days in the usual manner.

Three additional S's were injected in the dorsal NA bundle with 1.0µl of the ascorbic acid vehicle and were tested for 5 days, then lesioned in LC and tested for 5 more days. The last S in this group was lesioned in LC after completion of the 5 day testing period following the injection of the vehicle solution in the dorsal NA bundle.

As in preceding experiments, data except where noted were analyzed by a Two Factor (TxD) analysis of variance for repeated measures.

Results

Figure 2 shows that the 6-OHDA injections had no significant effect on LH self-stimulation when compared to the vehicle injected controls ($F(1,7)=1.24$, $p>.05$). The 6-OHDA injections also did not significantly affect LC self-stimulation ($F(1,2)=.18$, $p>.05$). Included for comparison in Figure 2 are the results of electrolytic LC lesions on LH self-stimulation. A comparison of the pre-lesion mean response rate with the post-lesion response rate of these S's by means of a correlated t-test (Ferguson, 1971) indicated no significant differences ($T(4)=-.52$, $p>.05$).

Table 2 presents individual response rates of the LH self-stimulating S's after treatment with 6-OHDA, saline and electrolytic LC lesions. Table 3 shows the effects of 6-OHDA and saline on LC self-stimulation.

Histology

The LH electrodes were located just lateral to the LH at the level of the VMH and extended in a rostral caudal distribution of -0.2 mm - +0.2 mm from the plane represented in Plate 3. The FLM cannulae were located lateral to the dorsal NA bundle at the mid-caudal level of the interpeduncular nucleus and in several cases were too ventral, extending into the red nucleus (see Plates 4 & 5). The LC lesions extended in a rostral caudal direction of approximately .6 mm - .8 mm, the most anterior lesion being at the level of the dorsal tegmental nucleus (DT). In

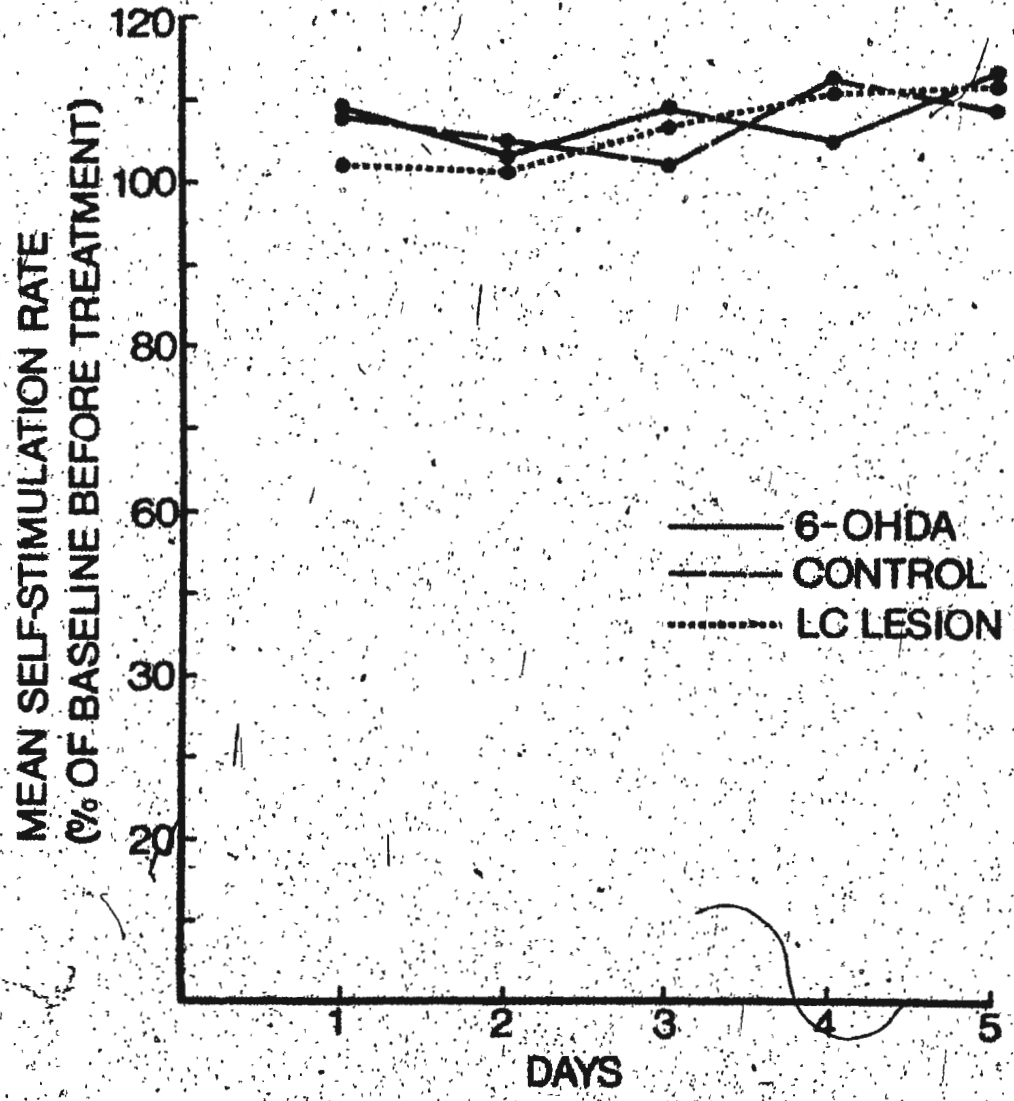



Fig. 2. Effects of 6-OHDA (4µg/µl) injections in dorsal NA bundle and LC lesions on LH self-stimulation.

Table 2

LH self-stimulation rates after 6-OHDA (4µg/µl) injections in the dorsal NA bundle and electrolytic lesions of LC

| Subject | Stimulus Intensity | Response Variability | Pre-injection Mean | Treatment | Test Days 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|---|---|---|---|
| FLM 14 | 18µa | <10% | 1006.5 | 6-OHDA | 1104.0 | 1104.0 | 1165.0 | 933.0 | 1026.0 |
| 15 | 16µa | 15% | 799.0 | | 967.0 | 870.0 | 868.0 | 936.0 | 1000.0 |
| 21 | 35µa | 15% | 280.5 | | 309.0 | 268.0 | 320.0 | 317.0 | 372.0 |
| 23 | 24µa | 10% | 521.5 | | 559.0 | 546.0 | 578.0 | 565.0 | 661.0 |
| 24 | 24µa | 5% | 1295.0 | | 1377.0 | 1420.0 | 1368.0 | 1390.0 | 1209.0 |
| FLM 14 | | | 979.5 | LC Lesion | 1218.0 | 1087.0 | 1165.0 | 1049.0 | 1161.0 |
| 15 | | | 1006.0 | | 918.0 | 880.0 | 950.0 | 1015.0 | 991.0 |
| 16 | 38µa | <10% | 467.0 | | 559.0 | 514.0 | 543.0 | 544.0 | 509.0 |
| 21 | | | 344.5 | | 378.0 | 380.0 | 415.0 | 465.0 | 501.0 |
| 23 | | | 613.0 | | 559.0 | 606.0 | 614.0 | 670.0 | 622.0 |
| 24 | | | 1299.5 | | 994.0 | 1195.0 | 1202.0 | 1264.0 | 1287.0 |
| FLM 16 | | | 393.0 | Saline | 436.0 | 438.0 | 452.0 | 459.0 | 475.0 |
| 21 | | | 272.5 | | 288.0 | 257.0 | 245.0 | 285.0 | 276.0 |
| 23 | | | 1239.0 | | 1340.0 | 1350.0 | 1252.0 | 1313.0 | 1277.0 |

Table 3

LC self-stimulation after injections of 6-OHDA (4µg/µl) in the dorsal NA bundle

| Subject | Response Variability | Stimulus Intensity | Pre-injection Mean | Treatment | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| FLM 14 | 5% | 36µa | 1322.5 | 6-OHDA | 981.0 | 1281.0 | 1212.0 | 1312.0 | 1422.0 |
| 15 | 10% | 40µa | 379.5 | 6-OHDA | 727.0 | 767.0 | 751.0 | 645.0 | 625.0 |
| 23 | 5% | 36µa | 347.0 | 6-OHDA | 398.0 | 370.0 | 369.0 | 366.0 | 374.0 |
| 23 | | | 332.0 | Control | 334.0 | 364.0 | 349.0 | 343.0 | 351.0 |

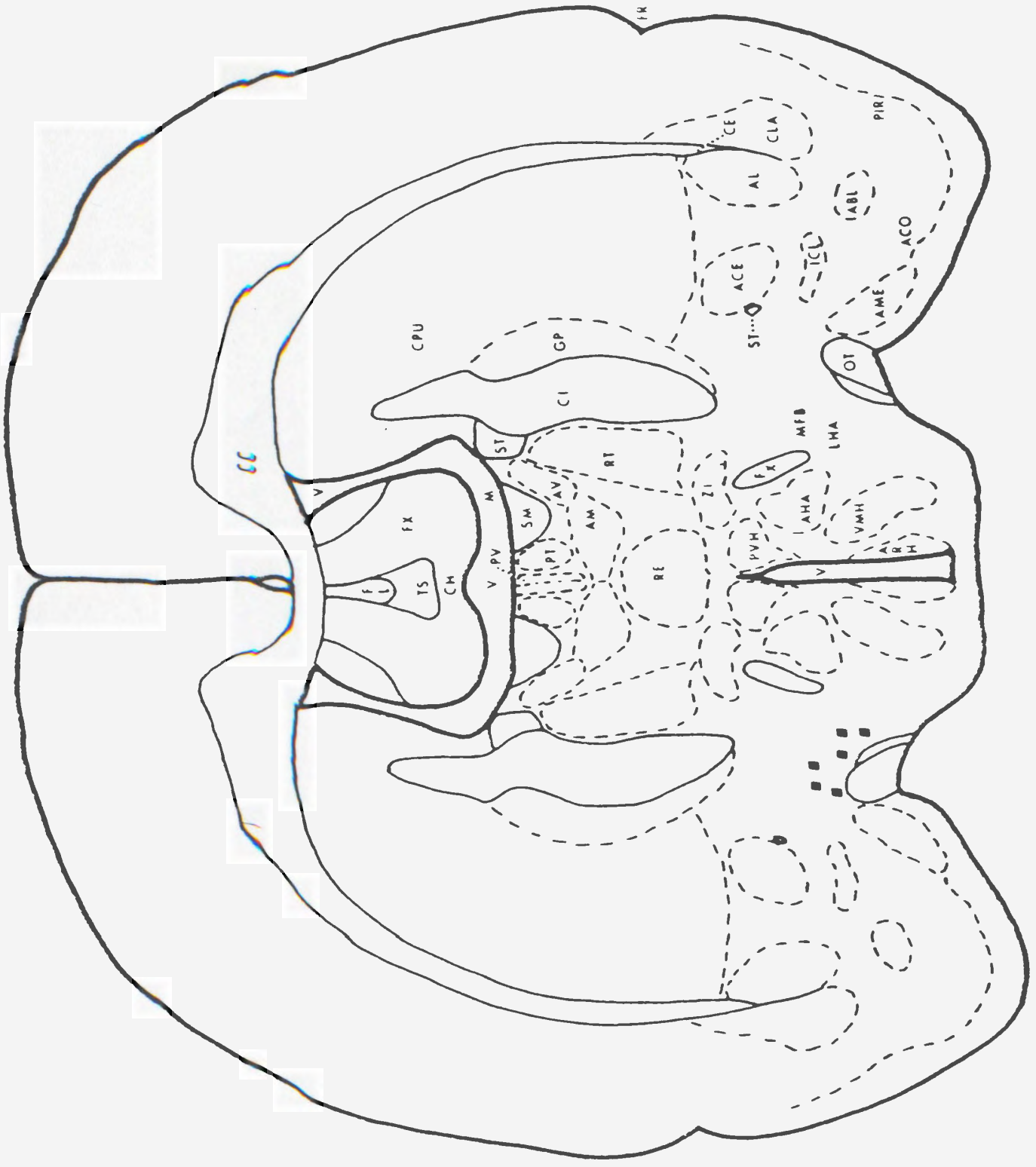



Plate 3. Schematic diagram illustrating placement of LH electrodes in rats of Experiment 2.

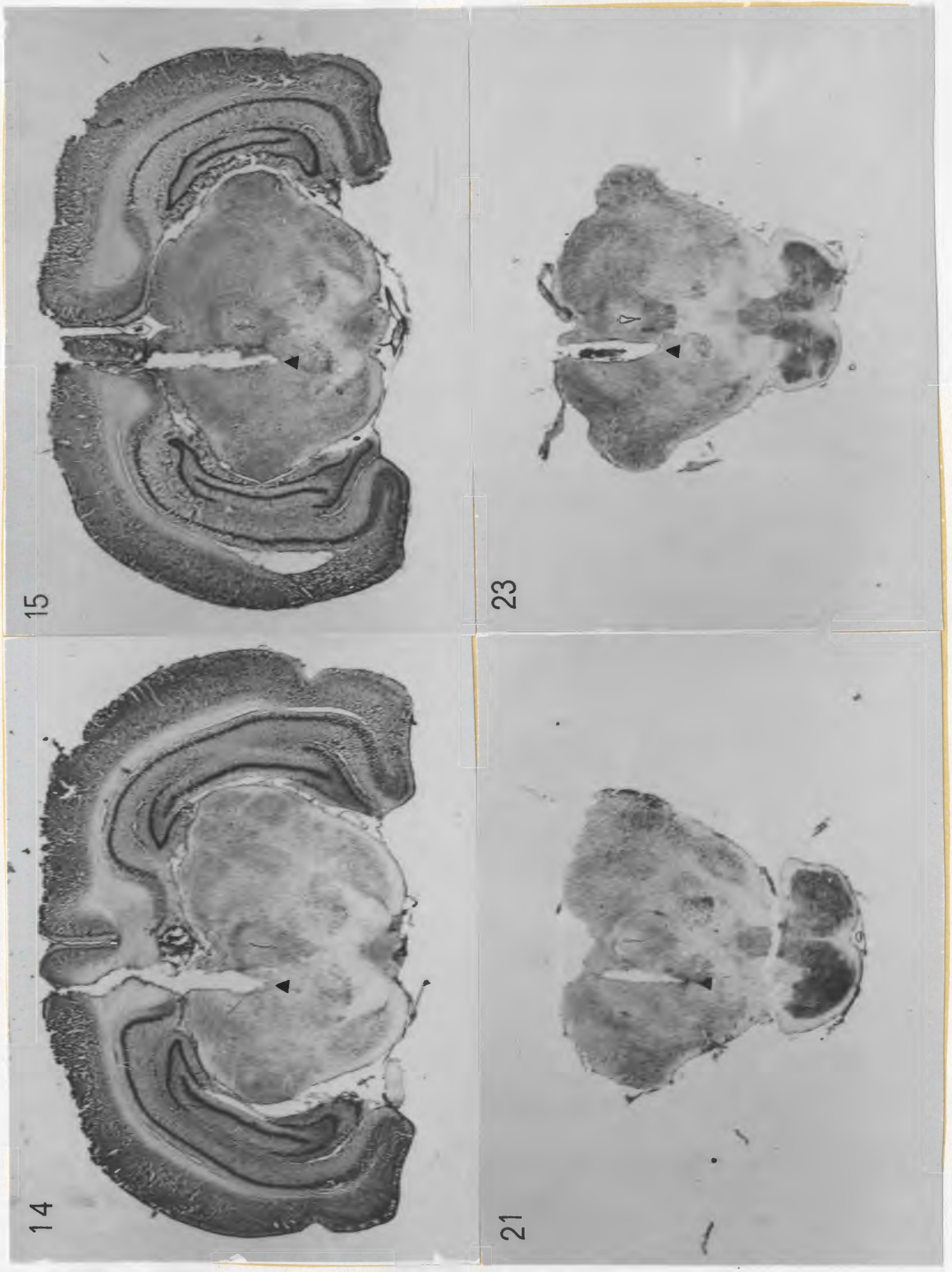



Plate 4. Placement of FLM cannulae in S's 14, 15, 21 and 23.

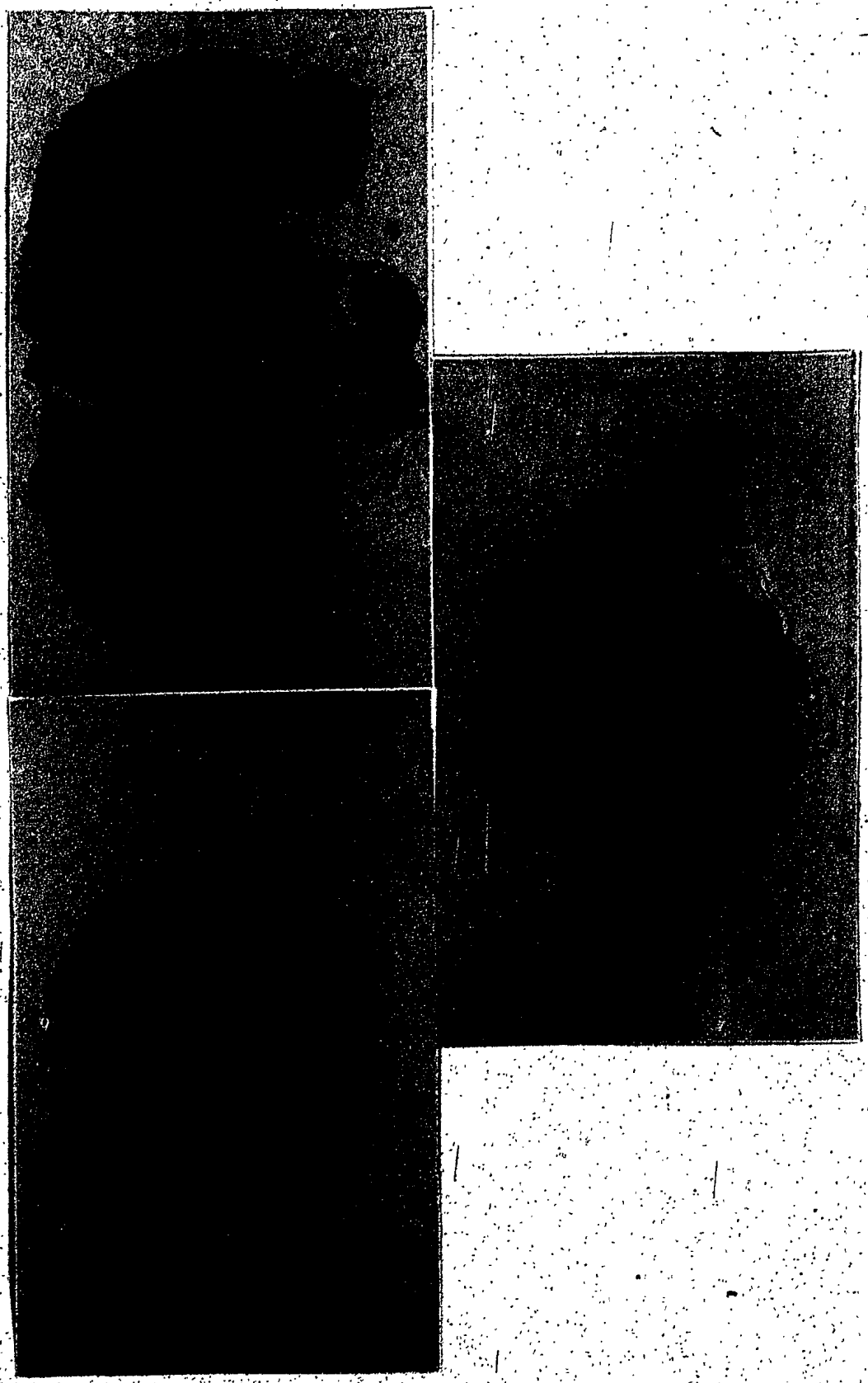


Plate 5. Placement of FLM cannula in S 24 and LC lesions in S's 23 & 24. Note the extensive damage to the rostral aspect of the A6 cell group in S 24.

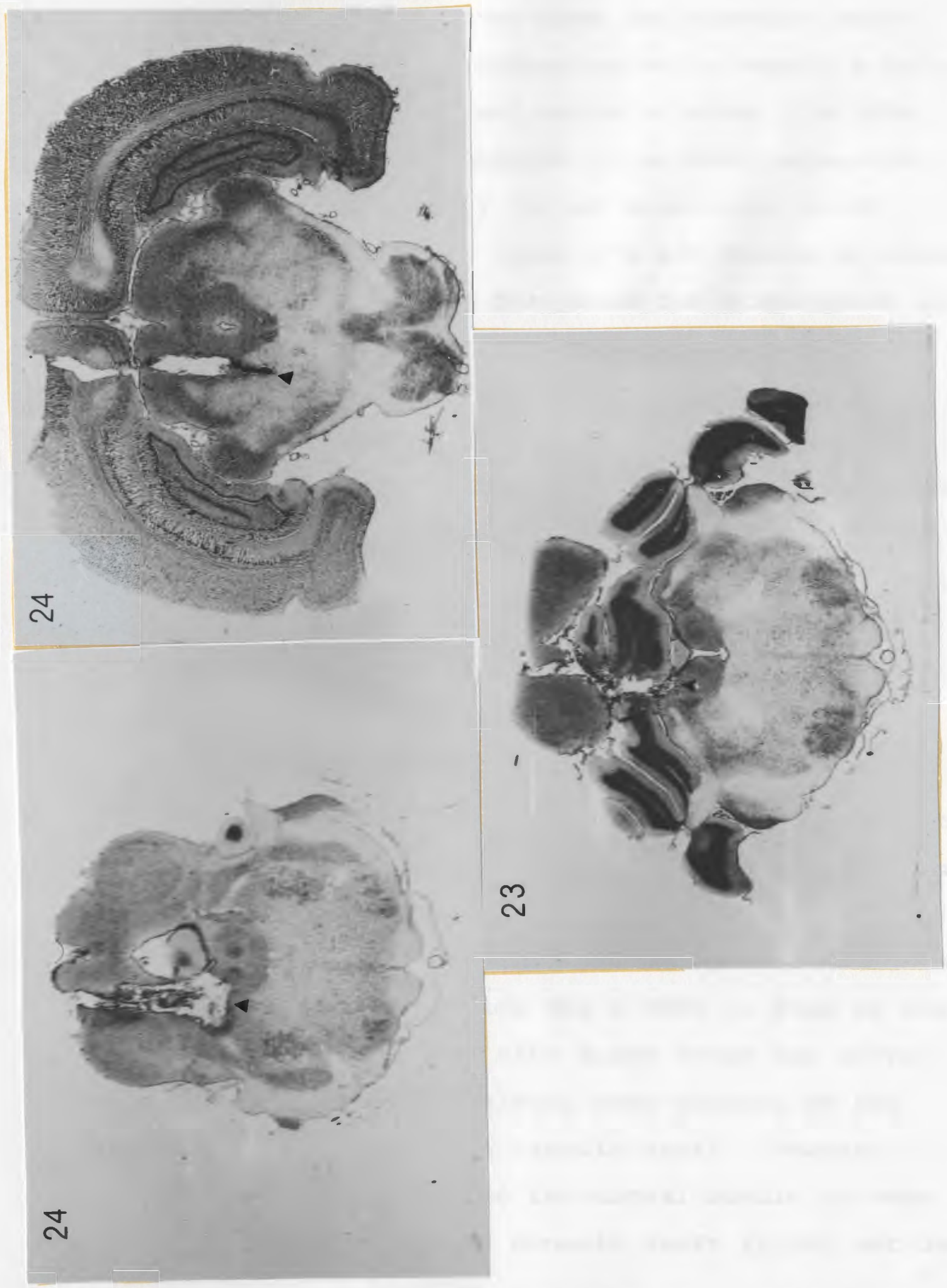



Plate 5. Placement of FLM cannula in S 24 and LC lesions in S's 23 & 24. Note the extensive damage to the rostral aspect of the A6 cell group in S 24.

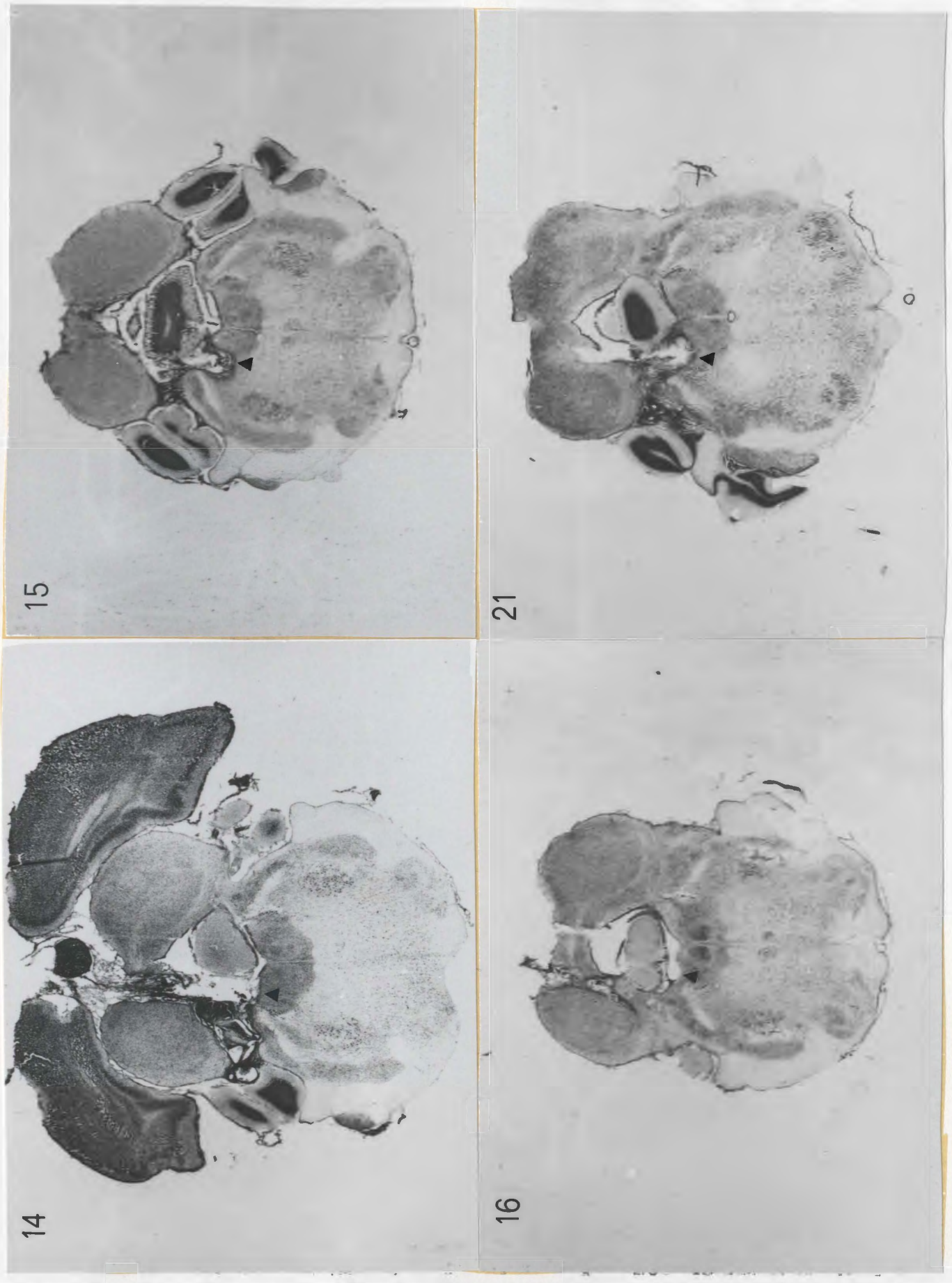



Plate 6. LC lesions in S's 14, 15, 16 & 21. The lesions in S's 14 & 16 seem to have resulted in only partial destruction of A6 while the lesions in S's 15 & 21 extensively damaged A6.

reached the dorsal bundle to produce any significant degeneration.

The 6-OHDA injections in the dorsal NA bundle also did not affect LH self-stimulation but again the question arises as to whether these injections actually damaged the dorsal bundle. However, electrolytic lesions in or adjacent to A6 did not have any significant effect on LH self-stimulation and thus are in agreement with the results of Experiment 1 in which 6-OHDA injections anterior to A6 were without effect on LH self-stimulation.

The failure to disrupt LH self-stimulation by 6-OHDA injections anterior to A6 (Experiment 1) or by electrolytic lesions of LC in the present experiment via electrodes that supported self-stimulation (FLM 14, 15 and 23) strongly suggests that although the dorsal NA system supports self-stimulation it does not seem to play an integral role in LH self-stimulation.

## Experiment 3

The results of the previous experiments suggested that the dorsal NA system does not play an important role in LH self-stimulation, yet this system supports high rates of self-stimulation and seems to be the only NA system which supports this behavior (Clavier & Routtenberg; German & Bowden, 1974). It thus seemed of interest to further assess the role of the dorsal NA system in LH self-stimulation by

methods other than permanent lesions of A6. One method of investigating the function of this system is to inject various psychoactive agents directly in LC. Any effects of a particular drug are of course temporary, usually lasting only a few minutes and since the effects are reversible different drugs may be injected over a period of days (Nakajima, 1972). It was decided to attempt to replicate the 6-OHDA and electrolytic lesion results of Experiments 1 and 2 by injecting procaine hydrochloride in LC. Procaine (a local anesthetic) would create a temporary lesion by reducing the transport of $NA^+$ ions across neuronal membranes and therefore prevent the generation of nerve impulses (DeJong & Wagman, 1963). The temporary lesion method has been used by others to investigate the neural substrates of self-stimulation (Madryga & Albert, 1971; Nakajima, 1972; Nakajima & Iwasaki, 1973).

Iontophoretic application of d-amphetamine to LC (Hoffer et al., 1973) and the systemic administration of d-amphetamine (Walters et al., 1974) both inhibit the spontaneous firing of A6 cells. Injections of d-amphetamine in LC would presumably also inactivate the dorsal NA system temporarily, which on the basis of results from lesions of this system should not affect LH self-stimulation. Since no treatment of the dorsal NA system had produced a deficit in LH self-stimulation, it was of interest to see if excitation of LC with a neural excitant such as glutamic acid (Krnjevic, 1964) would result in an increase in self-stimulation.

Subjects

Three male, Sprague-Dawley rats weighing 300-400 gm at the time of surgery were used in this experiment.

Surgery

All 3 S's were implanted with LH electrodes and ipsilateral LC cannulae as described previously.

Procedure

After shaping for self-stimulation was accomplished each S was given a daily 20 min. session of bar-pressing. The number of responses were recorded for each minute of the test session. When the total number of responses during 6 min. blocks (1-6, 8-13 & 14-19) of a particular test session did not significantly deviate from a rectangular distribution ($X^2 < 11.07$, df=5, $p > .05$) the S's response rate was considered stable (Nakajima, 1972).

On the day following stabilization, the cannula plug was removed from the cannula and the injection needle inserted. The injection needle had been previously inserted in a short piece of 23ga PE tubing, prefilled with the solution to be injected. This tubing was in turn connected to a long piece of 23ga PE tubing filled with distilled water and was attached to a microsyringe. The S was then placed in the Skinner box and allowed to self-stimulate. The response rate was recorded for each minute and during the 7th minute 1.0µl of solution was injected. The following solutions were injected in LC: procaine hydrochloride (2%), glutamic acid (4.4mg/ml), d-amphetamine sulfate (20µg/µl)

and .9% saline as a control. Thus, each S received a single injection of each solution, one injection per day over the 4 day period following response stabilization.

The number of responses during the post-injection 8-13 min. block was compared to the pre-injection 1-6 min. block using the following chi-square test (Nakajima, 1972).

$$X^2=(A-B)^2/(A+B)$$

Where A=total number of responses during 1-6 min. block,

B=total number of responses during 8-13 min. block

On completion of the experiment S's were sacrificed by an overdose of sodium pentobarbital and prepared for histological examination.

Results

Table 4 shows that 1.0µl injections of procaine hydrochloride, glutamic acid and d-amphetamine sulfate all strongly suppressed LH self-stimulation.

The behavior of the S's after the injections was different for each of the drugs. After injection of procaine all 3 S's showed circling ipsilateral to the injected side, pressing the level occasionally and sniffing about the box. No S showed complete cessation of responding during any minute after the injection of procaine. D-amphetamine produced quite different results than procaine. Two S's (A6:3 + A6:4) crouched low to the floor of the Skinner box, extended fore- and hind-limbs and moved slowly about showing

Table 4

Effects of procaine, d-amphetamine and glutamic acid injections in the region of LC on LH self-stimulation

| Subject | Procaine | | D-amphetamine | | Glutamate | | Saline | |
|---|---|---|---|---|---|---|---|---|
| Minutes | 1-6 | 8-13 | 1-6 | 8-13 | 1-6 | 8-13 | 1-6 | 8-13 |
| A6:1 | 388 | **249 (64.2%) | 287 | **198 (69.0%) | 266 | **74 (27.8%) | 311 | +293 (94.2%) |
| A6:3 | 137 | ** 74 (54.0%) | 179 | ** 19 (10.6%) | 135 | ** 0 (0.0%) | 161 | +165 (102.5%) |
| A6:4 | 142 | * 97 (68.3%) | 154 | ** 76 (49.4%) | 140 | ** 4 (2.9%) | 159 | +153 (96.2%) |
| Mean Response | | 62.2% | | 43.0% | | 10.2% | | 97.6% |

+$x^2 > .05$, df=1

*$x^2 < .01$, df=1

**$x^2 < .001$, df=1

Numbers in parenthesis indicate per cent of pre-injection response rate. Injections performed during the 7th min.

considerable loss of motor coordination. A6:1 did not exhibit any obvious change in motor coordination but during several minutes of the post-injection period began gnawing at the cage floor and various protrusions from the Skinner box walls. The injections of glutamic acid totally suppressed LH self-stimulation in all 3 S's. All S's displayed some excitation and agitation after the injections, ears were back flat against the head. A6:4 and A6:3 moved to the left and then back to the right in quick, jerky movements while A6:1 remained immobile.

Histology

The LH electrodes were located on the edge of the LH at the level illustrated in Plate 7. The LC cannulae (Plate 8) were all located at the level A6. A6:1 had his cannula medial to A6, A6:3's cannula was just dorsal and medial to A6 while A6:4's cannula was considerably dorsal to A6, just above the roof of the IVth ventricle.

Discussion

The finding that procaine, d-amphetamine and glutamic acid injections in LC all strongly suppressed LH self-stimulation suggested that the suppression of neural activity (excluding glutamate) by these drugs is not analogous to the inactivation of neural systems by permanent 6-OHDA or electrolytic lesions.

The changes in self-stimulation induced by injections of these drugs typically has a duration of 4-6 min. The conclusion from the effects of procaine and d-amphetamine

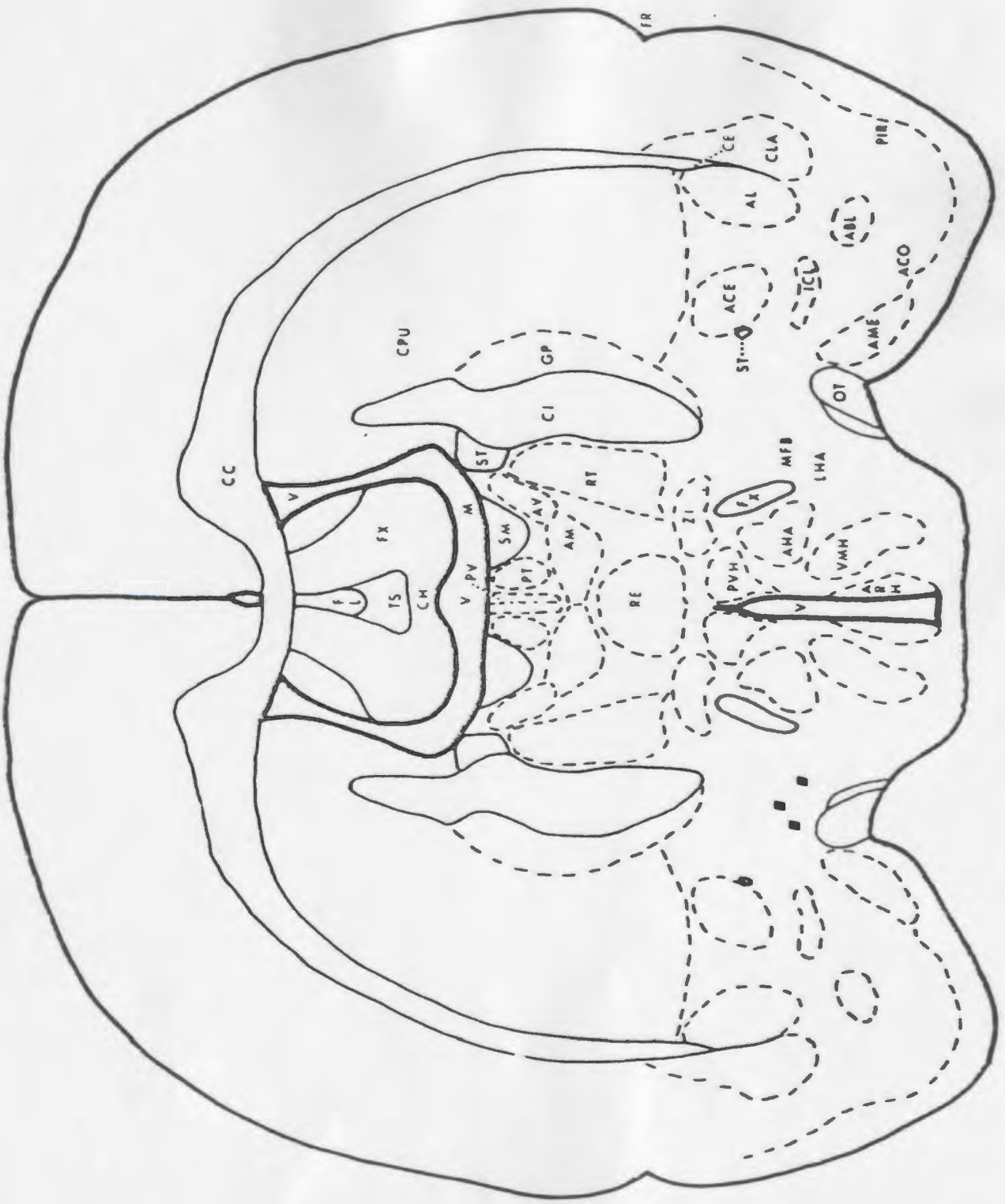



Plate 7. Schematic diagram illustrating placement of LH electrodes in S's AG:1, 3 & 4.

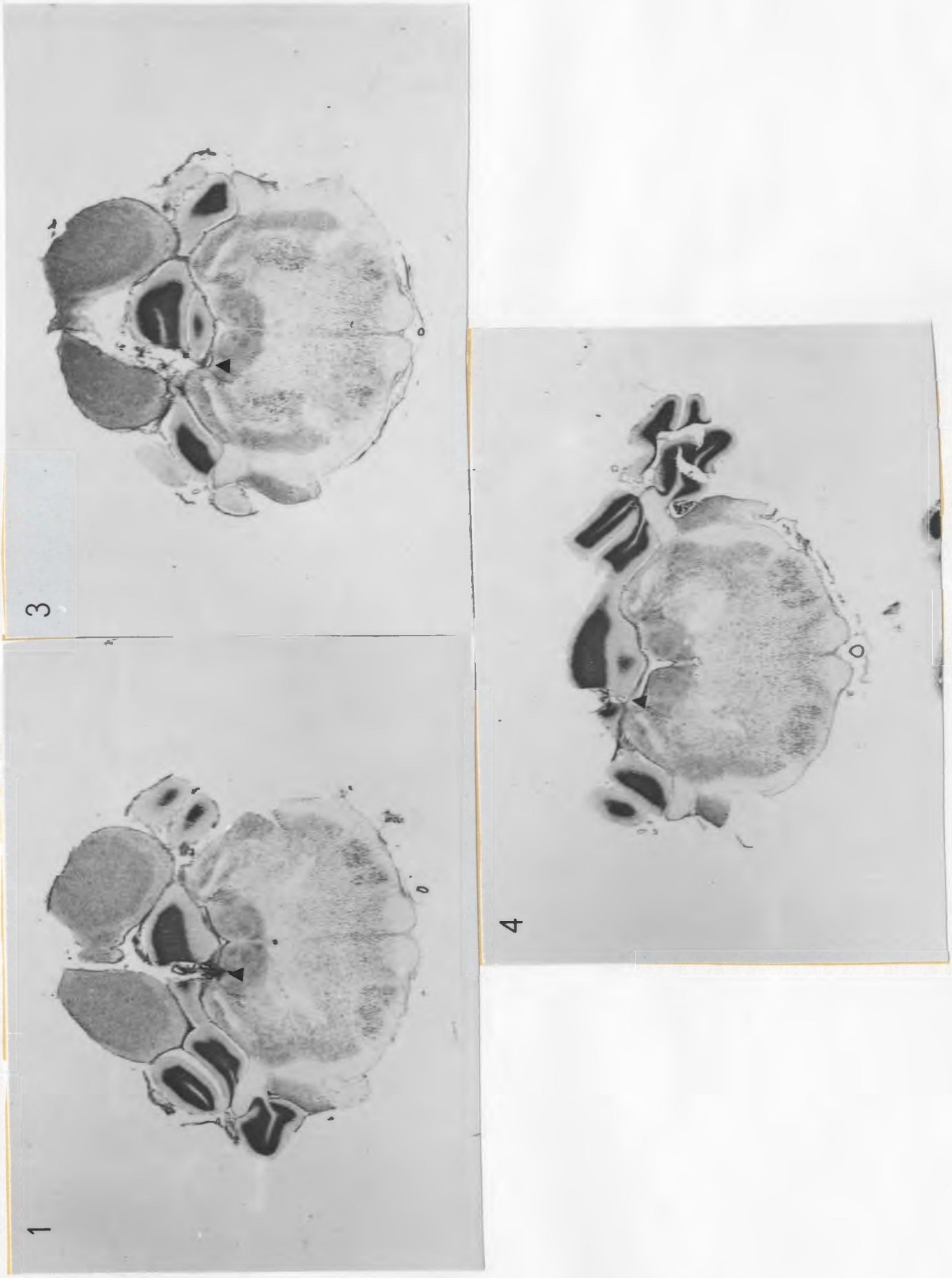



Plate 8. Placement of LC cannulae in S's A6: 1, 3 & 4.

on self-stimulation would be that the dorsal NA system is necessary for maintenance of LH self-stimulation. However, 6-OHDA or electrolytic lesions of the dorsal NA system do not support this conclusion.

The suppression of LH self-stimulation after injections of glutamate may be due to a non-specific inhibition of LC. The neural actions of glutamic acid, although frequently used as a neural excitant are not well known (Salmoiraghi & Sletanis, 1967). Glutamic acid does not always result in excitation of cells, inhibition of spontaneous firing is often encountered (Salmoiraghi & Sletanis, 1967; Gellar & Woodward, 1974). Similarly, glutamic acid has produced inconsistent results on self-stimulation. Glutamic acid injected in the ventral tegmental area strongly suppressed LH self-stimulation in 3 animals while not affecting self-stimulation in 2 other animals (Nakajima, 1972). Similar discrepancies have been reported after injections of glutamic acid in the anterior olfactory area (Nakajima & Iwasaki, 1973). If glutamic acid sometimes acts non-specifically by inhibiting the spontaneous firing rate of neurons rather than exciting them, then its action may be compared to amphetamine which inhibits firing of A6 cells (Walters et al., 1974; Hoffer et al., 1973). Thus, it may be that procaine, amphetamine and glutamate injected in A6 all suppressed spontaneous firing by slightly different pharmacological actions. The failure to disrupt LH self-stimulation after electrolytic or 6-OHDA lesions

of the dorsal NA system suggested that inhibition of this system by injections of procaine, d-amphetamine and glutamate would also have failed to alter LH self-stimulation.

Thus, the suppression of LH self-stimulation after injections of these drugs in LC must be due to factors other than the inhibition of A6. A 1.0µl injection of any solution has an approximate spread of 1.0 mm (Myers, 1971). Assuming that the injections of procaine, d-amphetamine and glutamate spread 1.0 mm from the injection site then the neural tissue affected by these drugs would be similar to the tissue destroyed by the electrolytic lesions of LC in Experiment 2. In addition to affecting similar tissue as the electrolytic lesions, these solutions may have diffused into the IVth ventricle since by examining Plate 8 it is apparent that all 3 LC cannulae were within 1.0 mm from the ventricle. Thus, the suppression of LH self-stimulation by injections of procaine, d-amphetamine and glutamate may be the result of pharmacological actions distal to the injection locus. It is not clear from this experiment what neural mechanisms could have been altered by diffusion of these drugs through the ventricular system, perhaps DA systems were being affected or the suppression may be due to non-specific interference with cerebral motor systems. Whatever the actual mechanism(s) underlying the disruption of LH self-stimulation in the present study, it would seem that the electrolytic lesioning method rather than the temporary lesioning method is the more precise means of investigating

the neural substrates of self-stimulation.

## Experiment 4

Perhaps the most interesting experiment designed to test the involvement of the dorsal NA pathway in LH self-stimulation would be lesions of the pathway through electrodes in the dorsal bundle that support self-stimulation. This preparation could assess the effects of dorsal NA bundle lesions on LH as well as LC self-stimulation. It was of special interest to see if the dorsal bundle lesions would disrupt LC self-stimulation since 6-OHDA injections in the dorsal bundle were without effect on LC self-stimulation (Experiment 2). However, the 6-OHDA injections seemed to be slightly off target and may not have damaged the dorsal NA bundle to any significant extent.

### Subjects

Five male, Sprague-Dawley rats weighing 300-400 gm at the time of surgery were used in this experiment.

### Surgery

Each S was implanted as described previously with a bipolar electrode in LH and LC and an additional electrode aimed at the dorsal NA bundle at the level of the FLM (-4.4, +0.8, -6.6).

### Procedure

Each S was shaped as described previously and after response stabilization electrolytic lesions (2.5ma-10 sec)

were made via the FLM or LC electrodes (see Results). Upon completion of the experiment the S's were sacrificed with an overdose of sodium pentobarbital (50mg/kg) and prepared for histological examination. No statistical analysis of the data was performed due to the small sample size.

Results

Of the 5 S's implanted for this experiment all 15 electrodes supported self-stimulation. One S's LC electrode (DNB2) would only support self-stimulation at high current intensities (80-90μa) and thus testing on this electrode was discontinued. Also, this S developed seizures upon stimulation of the LH so a lesion was made via the LC electrode to assess the effects on dorsal NA bundle self-stimulation.

The dorsal NA bundle lesions strongly suppressed LH self-stimulation in S's DNB4 and DNB5. Subject DNB3 developed convulsions upon LH stimulation on the first post-lesion test day so no quantitative effects of the lesion on LH self-stimulation are possible, except to mention that upon termination of the seizure the S would self-stimulate so it may be that the dorsal NA bundle lesion was without effect. The dorsal bundle lesions had no effect on LC self-stimulation in DNB3 and DNB4 while DNB5 showed an initial suppression of LC self-stimulation on the first 2 test days followed by subsequent recovery on the remaining test days.

The LC lesion in DNB2 had no effect on dorsal NA bundle self-stimulation. The results of this experiment are presented in Table 5.

The LH electrodes were located on the edge of the LH-MFB area extending in a rostral-caudal plane of approximately ±0.5 mm as illustrated in Plate 9. Plates 10 and 11 show the location of the dorsal NA bundle lesions in DNB 3, 4 and 5 and their LC electrode placements. Plate 10 shows the location of the LC lesion in DNB2 and the FLM electrode placement. The dorsal NA bundle lesion in DNB3 (Plate 10) was lateral and ventral to the dorsal bundle in a region similar to the loci of the cannulae in Experiment 2. It is interesting to note that in this location neither electrolytic or 6-OHDA lesions had any effect on LH or LC self-stimulation. The lesions in DNB4 and DNB5 clearly encompassed the dorsal NA bundle and terminated in the dorsomedial portion of the medial lemniscus. DNB2's FLM electrode was located in the vicinity of the dorsal NA bundle at the mid-anterior level of A9. The LC lesion in this S whose LC electrode supported self-stimulation at only high current intensities was anterior to LC and dorsomedial to the PCS.

Discussion

Two S's (DNB4 & DNB5) who clearly had damage to the dorsal NA bundle exhibited a strong suppression of LH self-stimulation. At the conclusion of testing DNB4's response rate had recovered to 75% of the pre-lesion level while

Table 5

Effects of dorsal NA bundle lesions on LH and LC self-stimulation

| Subject | Stimulus Intensity | Pre-Lesion Mean | Response Variability | Treatment | Test Days 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|---|---|---|---|
| DNB2 | 22µa (FLM) | 295.0 | <10% | LC Lesion | 379 | 358 | 293 | 308 | 279 | - |
| DNB3 | 40µa (LH) | 362.5 | 10% | FLM Lesion | 321* | - | - | - | - | - |
| DNB4 | 25µa (LH) | 1090.0 | 10% | FLM Lesion | 424 | 466 | 581 | 778 | 830 | - |
| DNB5 | 10µa (LH) | 1036.0 | 5% | FLM Lesion | 14 | 177 | 410 | 588 | 808 | 705 |
| DMB3 | 20µa (LC) | 558.5 | <10% | FLM Lesion | 582 | 547 | 665 | 619 | 628 | - |
| DNB4 | 20µa (LC) | 466.0 | 10% | FLM Lesion | 511 | 479 | 396 | 387 | 437 | - |
| DNB5 | 16µa (LC) | 610.0 | 10% | FLM Lesion | 19 | 236 | 577 | 588 | 428 | 564 |

Mean response rate per 15 min. session for dorsal NA bundle self-stimulation

| | | |
|---|---|---|
| DNB2 | 22µa (FLM) | 295.0 |
| DNB3 | 20µa (FLM) | 268.0 |
| DNB4 | 20µa (FLM) | 111.0 |
| DNB5 | 16µa (FLM) | 107.5 |

*Animal developed convulsions after LH stimulation on 1st post-lesion test day. Response rate indicated is for 15 min. period immediately following termination of seizure.

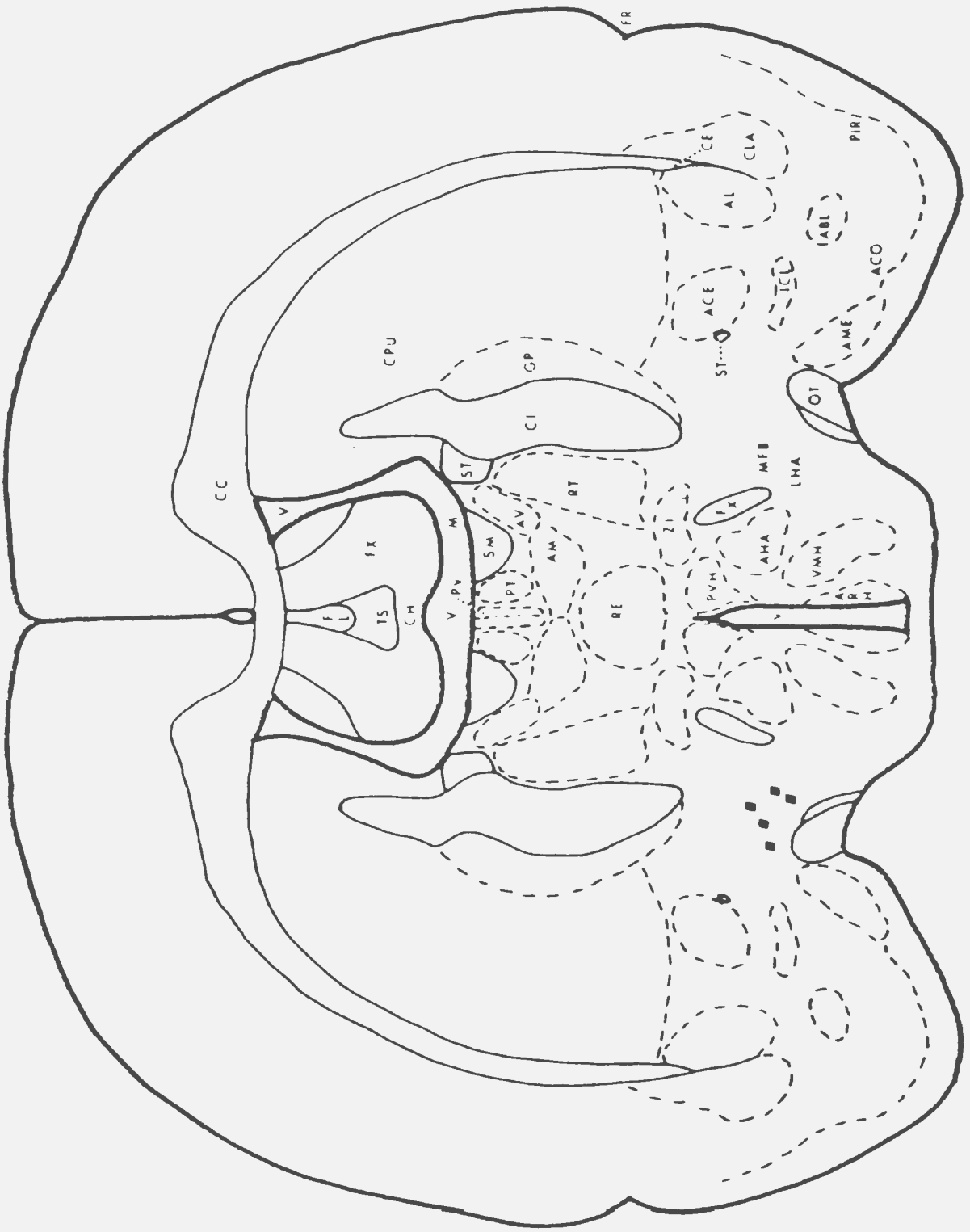



Plate 9. Schematic illustration of LH electrode placements for all S's of Experiment 4.

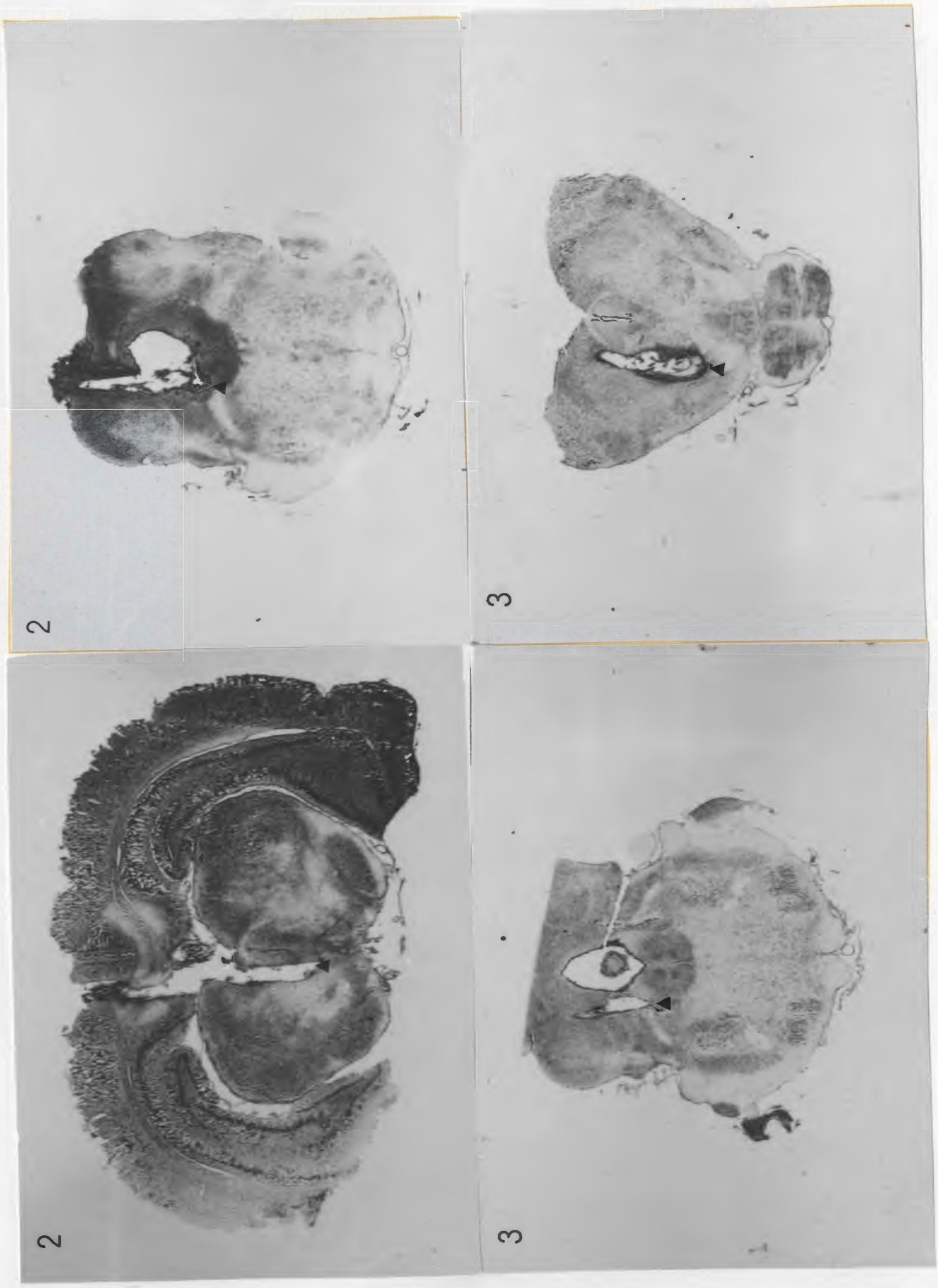



Plate 10. Location of dorsal NA bundle electrode and LC lesion in S DNB2 and dorsal NA bundle lesion and LC electrode in S DNB3.

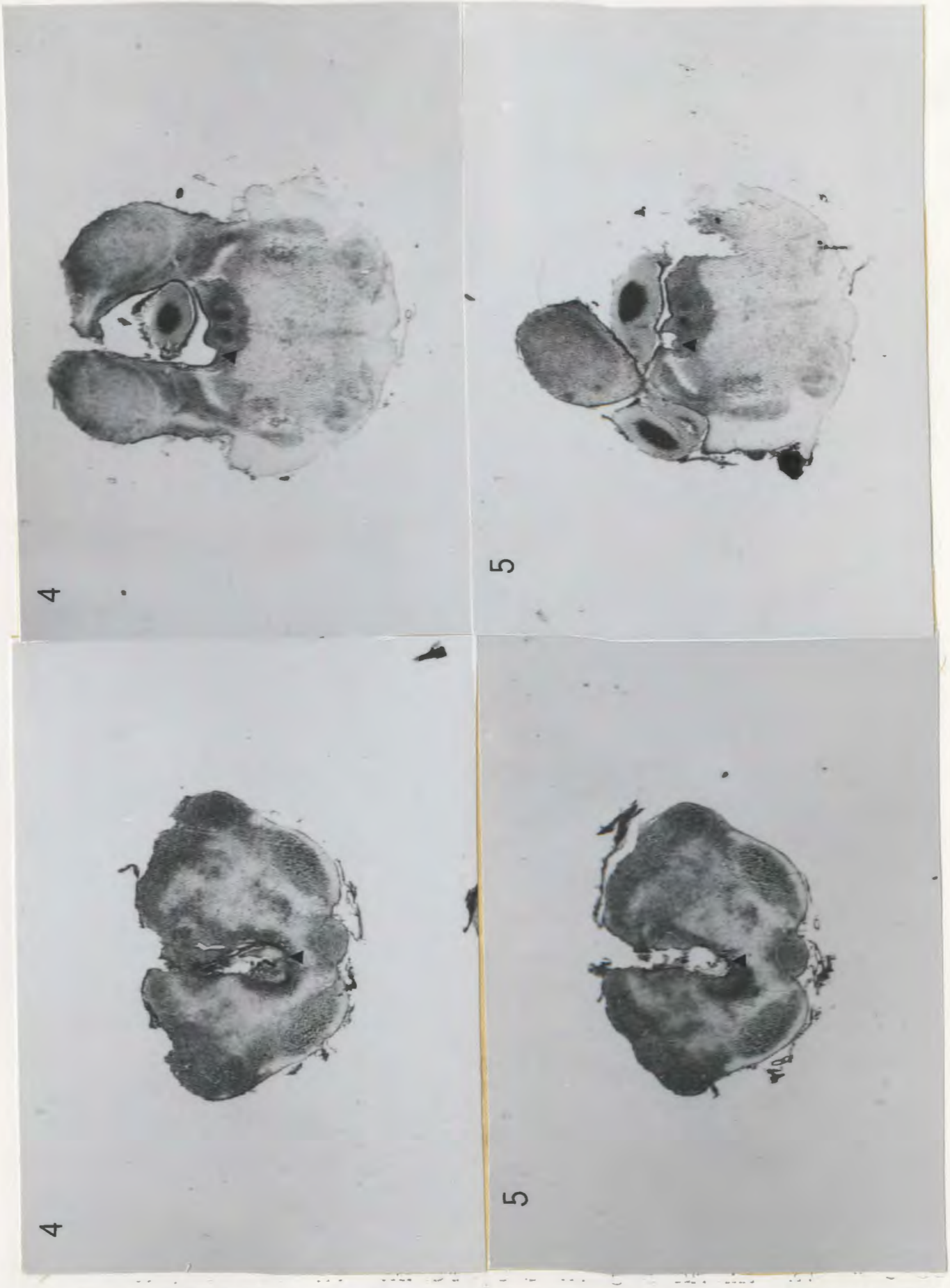



Plate 11. Location of dorsal NA bundle lesions and LC electrodes in S's DNB 4 & 5. The lesions clearly encompass the dorsaI NA bundle and have not damaged the dopamine containing A9 and A10 cell groups.

DNB5 had recovered to 68% of the pre-lesion level. These results were somewhat unexpected since neither 6-OHDA lesions anterior to A6 (Experiment 1) or electrolytic lesions in or adjacent to A6 (Experiment 2) had any effect on LH self-stimulation. The LH placements in DNB4 and DNB5 were more anterior and medial than other LH placements in the previous 3 experiments and were located approximately .3-.5 mm anterior to the illustration in Plate 9. It may be that these electrodes were activating the dorsal NA pathway to a greater extent than any of the LH electrodes in Experiments 1 and 2. Such a conclusion is conceivable since the dorsal NA pathway is well separated from the DA systems in the anterior hypothalamus (Ungerstedt, 1971a).

Dorsal NA bundle lesions in DNB3, 4, & 5 failed to significantly alter LC self-stimulation. Also, a lesion via an LC electrode in DNB2 that supported self-stimulation only at high current intensities (80-90μa) failed to affect dorsal NA bundle self-stimulation. These results suggested that the LC stimulating electrodes were not actually in A6 but perhaps in the PCS which is adjacent to LC and is known to support self-stimulation (German & Bowden, 1974).

## Experiment 5

One interesting behavioral characteristic of self-stimulation from NA placements is that the shaping process is prolonged, taking much longer to obtain than self-stimulation from mixed CA areas such as the LH or DA areas, e.g. A9 (Crow et al., 1972; Ritter & Stein, 1973; Micco, 1974). If the acquisition of self-stimulation behavior from pure NA electrode placements takes longer to obtain than from other areas, then the acquisition time to obtain self-stimulation could serve as a useful behavioral tool for mapping self-stimulation sites. In view of the potential importance of shaping duration, the time required to obtain self-stimulation from all electrode sites (LC, LH and dorsal NA bundle in the previous 4 experiments was carefully recorded.

### Subjects

Nine LC, 14 LH and 4 dorsal NA bundle self-stimulators from Experiments 1-4 were used in this experiment.

### Procedure

Each S was placed in the Skinner box with the stimulator set at a low current intensity (e.g. 4μa). As soon as the S oriented towards the lever he received "massed" priming stimulations by rapidly depressing the lever up and down from outside the testing chamber. At the same time the S was receiving the priming stimulations, the current intensity was being rapidly increased. Priming was term-

inated if the S oriented away from the lever of displayed escape reactions. When a S began bar-pressing without experimenter assistance he was allowed to lever press for the remainder of the 15 min. test session. If on the next day a S failed to begin bar-pressing of his own accord or after a few priming stimulations, he was deemed not to have acquired bar-pressing behavior and the shaping process was reinitiated.

Results

Table VI shows that the LH S's acquired bar-pressing after 1.5 sessions, the LC self-stimulators after 1.3 sessions while the dorsal NA bundle S's required 4.6 15 min. shaping sessions to acquire bar-pressing behavior. Also of interest was that the LC self-stimulators displayed stimulus-bound grooming and gnawing. By rapidly delivering 5 or 6 priming stimulations some S's would begin licking and chewing their forelimbs and pulling vigorously on the fingers of the forepaws. The LC S's that displayed stimulus-bound gnawing upon stimulation would immediately begin gnawing on the grid floor of the Skinner box or various other objects placed in the chamber such as food pellets, pens or cigarette butts. Upon termination of the stimulation, the object being gnawed was immediately released. Bar-pressing was also characterized by these gnawing and chewing behaviors. Some S's would sit quite placidly with the lever grasped in the teeth and rock it rapidly up and down. Other S's seemed to chew and lick at the lever, sometimes

with one paw placed lightly on the lever but no S pressed the lever solely with his paws.

Discussion

Previous reports of self-stimulation in and adjacent to LC have noted a slower acquisition of bar-pressing behavior than is the case for LH or SN self-stimulation (Crow et al., 1972; Ritter & Stein, 1973; Micco, 1974). It seems that the prolonged acquisition of self-stimulation behavior in the above studies may be due to the use of sub-optimal shaping procedures since the LC self-stimulators in the present series of experiments acquired bar-pressing as rapidly as LH self-stimulators. Dorsal NA bundle self-stimulators do acquire bar-pressing more slowly than LH S's, so it would appear that some NA self-stimulation is characterized by a prolonged shaping process. It should also be noted that all the LC S's who exhibited self-stimulation also displayed stimulus-bound grooming and gnawing. These stimulus-bound behaviors have been reported by Ball et al., (1974) from cerebellar structures, and by Micco (1974) from the PCS, LC and mesencephalic nucleus of the Trigeminal complex. Thus, in addition to the anatomical similarity of electrode placements in the present studies with those sampled by Crow et al. (1972); Ritter and Stein (1973) and Micco (1974) there also exists considerable behavioral evidence to suggest that the LC electrodes in the present studies were activating similar neural structures as those mentioned above.

Table 6

Acquisition of bar-pressing for LH, dorsal NA bundle and LC self-stimulation. The acquisition time is expressed as the mean number of 15 min. shaping sessions required to obtain self-stimulation

| Subject | Electrode Placement | Stimulus Intensity (for shaping) | Number of 15 min. shaping sessions to establish bar-pressing |
|---|---|---|---|
| DNB2 | Dorsal NA bundle | 24µa | 4 |
| DNB3 | Dorsal NA bundle | 20µa | 5 |
| DNB4 | Dorsal NA bundle | 20µa | 6 |
| DNB5 | Dorsal NA bundle | 30µa | 4 |
| | | | $\bar{X} = 4.6$ |
| DNB3 | LC | 30µa | 1 |
| DNB4 | LC | 22µa | 1 |
| DNB5 | LC | 14µa | 2 |
| FLM 14 | LC | 36µa | 1 |
| FLM 15 | LC | 40µa | 1 |
| FLM 17 | LC | 44µa | 2 |
| FLM 18 | LC | 60µa | 2 |
| FLM 23 | LC | 47µa | 1 |
| | | | $\bar{X} = 1.28$ |
| A6:1 | LH | 16µa | 1 |
| A6:2 | LH | 30µa | 2 |
| A6:3 | LH | 20µa | 2 |
| A6:4 | LH | 24µa | 3 |
| A6:5 | LH | 28µa | 1 |
| DNB2 | LH | 44µa | 2 |
| DNB3 | LH | 40µa | 1 |
| DNB4 | LH | 34µa | 1 |
| DNB5 | LH | 22µa | 1 |
| FLM 20 | LH | 20µa | 1 |
| FLM 21 | LH | 35µa | 2 |
| FLM 23 | LH | 24µa | 1 |
| FLM 24 | LH | 28µa | 1 |
| | | | $\bar{X} = 1.46$ |

Ritter and Stein (1973) do not describe their shaping procedure in sufficient detail to allow comparison with the shaping procedure used in the previous 4 experiments but Crow et al., (1972) start their animals at low current intensities and increase the intensity in steps of 25μa until the animals displayed behavioral signs of reinforcement or aversion. Micco (1974) shapes his animals by giving stimulation through a series of gradually increasing current levels. The shaping procedure used in the present studies differs from the above procedures in 2 important respects. Each S is given "massed" priming stimulation by rapidly manipulating the lever 5 or 6 times when the S's orient towards the bar, in addition the current intensity is rapidly increased during the massed priming. This procedure usually elicits stimulus-bound oral behaviors and it is then a simple matter to initiate bar-pressing by making the stimulation strictly contingent on these behaviors being directed towards the lever. There are several reasons to account for the success of this shaping procedure in obtaining rapid acquisition of LC self-stimulation. First of all, there is no apparent behavioral index indicating that a particular level of stimulation of LC is reinforcing. Positive stimulation in the LH-MFB area or A9-A10 areas typically yield excited exploratory behavior as indicated by forward locomotion and sniffing. No such behaviors occur with reinforcing LC stimulation. Thus, it is difficult to ascertain if a particular current intensity is above or

below threshold. By gradually increasing the current intensity, shaping is prolonged since at the very least the rapid increase in current intensity will determine if the stimulation is rewarding or at a certain level becomes aversive. Furthermore, Ball et al. (1974) and Micco (1974) report that the stimulus-bound oral behaviors are only elicited in the presence of a suitable goal object. The lever would seem to be such a goal object for these gnawing, chewing and licking behaviors. Secondly, LC stimulation seems to induce a relaxed state (Ritter & Stein, 1973). It would seem somewhat counter intuitive to deliver single or widely spaced priming stimulations for lever directed movement when massed stimulations by manual manipulation of the lever by the experimenter seem to be more effective for producing optimal arousal levels.

It would seem that LC self-stimulation is as easily obtained as LH self-stimulation when a sensitive shaping procedure is used. It may be that animals with NA electrodes must be highly aroused before they exhibit self-stimulation. Routtenberg (1974, personal communication) who does not shape his animals has noted that animals with LC electrodes will not initiate bar-pressing by themselves but when given an injection of d-amphetamine begin lever-pressing for LC stimulation. The finding that dorsal NA bundle self-stimulation is difficult to obtain in comparison to LC self-stimulation suggests that the complex stimulus-bound oral behaviors associated with LC self-stimulation

but not with dorsal bundle self-stimulation facilitates the acquisition of self-stimulation when the dependent measure is bar-pressing.

# CHAPTER IV

## DISCUSSION AND CONCLUSIONS

Since the discovery of self-stimulation by Olds & Milner (1954) there has been an increasing amount of evidence suggesting that the CA's play an important role in mediating this behavior. Self-stimulation rates are decreased by drugs such as 6-OHDA which deplete brain CA (Breese et al., 1971; Stein & Wise, 1971). Chlorpromazine, haloperidol, phentolamine and pimozide which block CA receptors also decrease self-stimulation rates (Stein, 1962; Lippa et al., 1973; Wise et al., 1973; Wise et al., 1974). Drugs such as amphetamine which augment the release of CA increase self-stimulation (Stein, 1964) as do monoamine oxidase inhibitors (Poschel, 1969). In a comprehensive review of the self-stimulation literature, German & Bowden (1974) have concluded that self-stimulation may be obtained with perhaps one or two possible exceptions only within the boundaries of CA systems as mapped by Ungerstedt (1971a) and the most effective lesions for disrupting self-stimulation are those affecting CA systems. More specifically, self-stimulation can be elicited by activation of any one of 3 CA systems: (1) the nigrostriatal DA system (Huang & Routtenberg, 1971; Crow, 1972a; Crow & Arbuthnott, 1972); (2) the mesolimbic DA system (Crow, 1972a) and (3) the dorsal NA system (Crow et al., 1972; Ritter & Stein,

1973). The ventral NA system does not seem to support self-stimulation (Clavier & Routtenberg, 1974; Crow, 1974, personal communication).

A series of 6 experiments were undertaken in order to ascertain the role of the nigrostriatal DA system and the dorsal NA system in LH self-stimulation. The results of these experiments strongly suggest that LH self-stimulation is mediated by several CA systems as indicated by severe deficits in this behavior after damage to the nigrostriatal and mesolimbic DA systems and the dorsal NA system. These studies also suggest that NE and DA or DA alone are the crucial neurotransmitters for LH self-stimulation. Support for this argument is based on findings that lesions of the dorsal NA system, the only NA system known to support self-stimulation failed to have any substantial effect on LH self-stimulation suggesting that current theories purporting to demonstrate a NA basis for self-stimulation (Stein & Wise, 1969; Wise & Stein, 1970; Stein & Wise, 1971; Wise et al. 1973) must be reconsidered.

The substantial reductions in LH self-stimulation after 4.0µl injections of 6-OHDA in the SN would appear to result from extensive damage to all 4 major CA systems. Less severe damage to these systems by 2.0µl injections of 6-OHDA in SN fail to significantly alter LH self-stimulation (Phillips, 1973). The difference between 2.0µl and 4.0µl injections of 6-OHDA is that the 2.0µl injections in SN would spread to the ventral NA system and would possibly

affect the mesolimbic DA system while 4.0µl injections would affect these systems as well as the dorsal NA system (Agid et al., 1973; Sotelo et al., 1973). A second critical factor in producing deficits in self-stimulation is the extent of the CA depletion. Reductions of NE to 29% of control levels after 200µg of 6-OHDA injected intraventricularily result in only temporary decreases in LH self-stimulation (Stein & Wise, 1971). When NE is selectively depleted to 10% of control levels, LH self-stimulation recovers to pre-injection levels in approximately 7 days (Lippa et al., 1973). Phillips (1974, personal communication) has shown that 250µg of 6-OHDA injected intraventricularily reduces whole brain NE and DA to 30.9% and 53.4% of control values respectively and results in only a temporary reduction in LH self-stimulation returning to control levels 5-6 days after the injection. Intraventricular injections of 6-OHDA (250µg) in combination with the monoamine oxidase inhibitor pargyline reduced whole brain DA and NE to 10% of control levels and resulted in a long lasting suppression of LH self-stimulation until the termination of testing 34 days after the injections.

Since there is a tissue concentration of 6-OHDA for producing an optimal amount of degeneration of CA neurons (Sotelo et al., 1973) it could be argued that the 4.0µl injections of 6-OHDA in the present study by their sheer volume would diffuse to other CA systems in the proximity of the nigrostriatal DA system in a sufficient

concentration to produce marked degeneration whereas 2.0µl of 6-OHDA (Phillips, 1973) would probably also reach these adjacent CA systems but in a concentration that would produce less marked degeneration. It should not be too surprising that extensive depletion of brain CA and damage to several CA systems is required to produce deficits in LH self-stimulation. Traditionally, self-stimulation has been most reliably obtained from the LH-MFB area. Electrodes here yield the highest rates of self-stimulation and the LH-MFB was thought to be the center for self-stimulation (Olds, 1969). It would seem that the reason for the LH-MFB area being such an optimal site for self-stimulation is that all the CA pathways which support self-stimulation converge through this area (Ungerstedt, 1971a). Thus, destruction of a single CA pathway may not be sufficient to disrupt LH self-stimulation.

There is a large body of evidence which has been interpreted as suggesting that NE is the neurotransmitter mediating self-stimulation (Stein, 1964; Stein & Wise, 1969; Wise & Stein, 1970; Stein & Wise, 1971; Wise et al. 1973). However, these pharmacological studies are subject to the criticism that most of the drugs affecting self-stimulation were altering other putative neuro-transmitters such as DA in addition to NE. For example, the observation that amphetamine, which facilitates self-stimulation (Stein, 1964), releases NE in vivo as does electrical stimulation of the LH-MFB area (Stein & Wise, 1969) has been interpreted

as support for the hypothesis of the NA mediation of reward (Stein & Wise, 1969; Wise & Stein, 1970). However, amphetamine is known to release DA and serotonin as well as NE (Fuxe & Ungerstedt, 1970). Electrical stimulation of an area subserved by several monoamine systems will release all of these monoamines (Sheard & Zolovick, 1971). If Stein & Wise (1969) had analyzed their perfusates for several monoamines, it is likely that other monoamines such as DA and serotonin would have been detected. Inhibition of NE biosynthesis by administration of disulfiram has been reported to produce deficits in self-stimulation (Wise & Stein, 1969). This study has been criticized by Roll (1970) who found that disulfiram produces sleep and drowsiness and if animals treated with disulfiram are kept aroused by handling, etc., show no deficits in self-stimulation. Stein & Wise (1971) reported decreases in LH self-stimulation after intraventricular injections of 6-OHDA which reduced brain NE to 20% of controls and increased brain DA to 168% of controls. The increase in brain DA after 6-OHDA is at odds with every other study employing intraventricular injections of 6-OHDA, which report substantial reductions of brain DA (Breese et al., 1971; Iverson & Uretsky, 1970; Uretsky & Iverson, 1970; Phillips, 1974). Thus, the evidence that NE solely mediates self-stimulation is questionable yet NE must play some part in the mediation of self-stimulation since self-stimulation can be obtained from the dorsal NA system

(Crow, 1972; Crow et al., 1972; Crow & Arburhnott, 1972; Ritter & Stein, 1973).

Considering the controversy surrounding the role of NE in self-stimulation, 5 experiments were conducted to test the involvement of the dorsal NA system in LH self-stimulation. These experiments generally suggest that LH self-stimulation is not affected by destruction of the dorsal NA system and therefore NE cannot be the sole mediator of self-stimulation.

In the first experiment 4.0µl of 6-OHDA was injected just anterior to A6, the origin of the dorsal NA system (Ungerstedt, 1971a). These injections produced no appreciable alterations in LH self-stimulation. However, 6-OHDA may not be the most appropriate lesioning tool for NA neurons since the cell bodies do not degenerate (Ungerstedt, 1971a; 1973) and 6-OHDA does not seem to produce as severe neuronal reactions as electrolytic lesions of this system (Chu & Bloom, 1974). The second experiment confirmed the results of Experiment 1 by demonstrating that both 1.0µl injections of 6-OHDA in the dorsal NA bundle at the level of the FLM and electrolytic lesions of LC via electrodes which supported self-stimulation failed to appreciably affect LH self-stimulation. Several S's showed a slight transitory increase in LH self-stimulation after the LC lesions. Histological examination of the lesion sites did not seem markedly different in the S's who increased LH responding and the S's who did not increase their response

rates. Since this increase in LH self-stimulation was not a consistent finding, it would seem that these increases were merely fluctuations in response rate unrelated to the LC lesion. However, Routtenberg (1974, personal communication) has noted similar increases in BC responding after LC lesions but again a finding not easily replicated. It may be that there is an inhibitory system in the region of LC that when lesioned more extensively would result in somewhat more consistent and permanent increases in LH self-stimulation.

To further assess the function of the dorsal NA system in LH self-stimulation procaine, d-amphetamine and glutamic acid were injected in LC. On the basis of the lesion results from Experiments 1 and 2, it was predicted that procaine and d-amphetamine injected in LC would have no effect on LH self-stimulation while glutamate, a neural excitant, might be expected to increase LH self-stimulation. All 3 drugs suppressed LH self-stimulation which suggested that the 1.0µl injections of these drugs in LC were affecting systems not affected by the electrolytic lesions of LC in Experiment 2 or the 6-OHDA injections anterior to A6 in Experiment 1. The LC cannulae in the 3 S's used in Experiment 3 were all in close proximity to the IVth ventricle, thus unlike electrolytic lesions, the injected solutions may have diffused into the ventricle as well as seeping up the cannula shaft in the cerebellum. If the procaine, d-amphetamine and glutamate injections diffused

into the ventricular system it may be that the suppression of LH self-stimulation was due to pharmacological actions of these drugs quite distal to LC perhaps affecting the DA systems. Another possibility is that the suppression of LH responding was due to a completely non-specific interference with systems unrelated to self-stimulation. For example, the d-amphetamine treated S's exhibited motor impairment after the injections, crouching low to the floor of the testing chamber and moving slowly about displaying extension of the fore and hind limbs. The S's injected with glutamate showed marked signs of agitation and excitation, moving quickly back and forth with their ears laid flat on their heads. Perhaps the dosage of glutamate (4.4 mg/ml) was too high, resulting in excessive behavioral stimulation with a subsequent disruption in self-stimulation behavior. Support for this argument is provided by Wise and Stein (1970) who have observed substantial reductions in LH-MFB self-stimulation after high doses of d-amphetamine, presumably as the result of excessive behavioral stimulation. The suppression of LH self-stimulation after injections of procaine in LC is very difficult to reconcile since these S's showed no obvious signs of motor impairment or sedation. There are now a growing number of reports illustrating opposite effects on self-stimulation with electrolytic versus procaine lesions. Valenstein and Campbell (1966) report no effect of lesions of the afferent fibre projections to the anterior olfactory area (AO) on AO self-stimulation

while Nakajima and Iwasaki (1973) report strong suppression of AO self-stimulation after procaine injections in the LH. Stiglick (1974) reports no effect on LH self-stimulation after extensive electrolytic lesions of BC, whereas Nakajima and Corbett (1972, unpublished observations) found strong suppression of LH self-stimulation following injections of procaine in BC. The discrepant results between procaine and electrolytic lesions on self-stimulation suggests that procaine is exerting some subtle effect on self-stimulation behavior that electrolytic lesions do not or perhaps there are peculiarities of the testing paradigm with temporary lesions that are not common to the electrolytic lesioning paradigm. The recovery of self-stimulation following electrolytic or 6-OHDA lesions of system thought to mediate self-stimulation is well documented (Valenstein, 1966; Stein & Wise, 1972; German & Bowden, 1974). Receptor supersensitivity and/or axonal sprouting have been suggested as possible mechanisms to account for recovery of self-stimulation behavior (e.g. Stein & Wise, 1971; 1972); however, neither of these mechanisms seems to occur rapidly enough following a lesion to account for the recovery of self-stimulation behavior (Antelman et al., 1972; Katzman et al. 1971). The fourth experiment in this series illustrates this phenomenon of recovery of function. Dorsal NA bundle lesions disrupted LH and LC self-stimulation in a few S's when tested 24 hr. after the electrolytic lesion. In some cases the suppression was nearly total

after 24-hr. but recovered substantially or completely over a period of 2-5 days. These results raise some important questions concerning the evaluation of a lesion's effect on self-stimulation behavior; how soon after a lesion should testing for self-stimulation be resumed? If deficits in self-stimulation are observed initially but recover over time, are the deficits to be interpreted as indicating interference with reward mechanisms per se or simply non-specific interference with systems unrelated to self-stimulation? Perhaps the suppression of LH self-stimulation after injection of procaine in LC is somewhat analogous to deficits in self-stimulation observed soon (e.g. 24 hr.) after electrolytic lesions. Although LH self-stimulation was not affected 24 hr. after electrolytic LC lesions (Experiment 2) it may be that deficits would have occurred had the S's been tested several hours after the lesion. It is possible that the effects of temporary lesions, that is, lesions introduced while an animal is self-stimulating should not be compared with the effects of electrolytic lesions on self-stimulation 24 hr. - 1 or more weeks after the lesion.

The finding in Experiment 4 that LH self-stimulation was disrupted in 2 S's (DNB4 & DNB5) following dorsal NA bundle lesions via electrodes that supported self-stimulation was somewhat surprising in view of the fact that LC lesions (Experiment 2) failed to produce any decrease in LH self-stimulation. On close examination of the location of the

LH electrodes in these S's (DNB4 & DNB5) if was found that the electrodes were located at the anterior level of the VMH, a region where the dorsal and ventral NA pathways are somewhat separated from the DA pathways, whereas caudal to this region the NA and DA pathways intermingle to a greater extent (Ungerstedt, 1971a; see page 37, Fig. M & N). Since the stimulation levels of the LH electrodes were relatively low (25μa and 10μa for DNB4 and DNB5, respectively), it is possible that the NA pathways rather than the DA pathways were primarily being activated. Support for this argument has been reported by Stiglick (1974) who found substantial decrease in LH self-stimulation in 2 S's who had anterior LH electrodes after lesions of the dorsal NA pathway at the level of the BC. These electrodes seemed to be in a similar area to the LH electrodes of DNB4 and DNB5 and would appear to have been activating the dorsal NA pathway. It is not too surprising then that the dorsal NA bundle lesions disrupted LH self-stimulation since the LH electrodes appear to have been preferentially stimulating this pathway.

Of considerable interest was the finding that dorsal NA bundle lesions had little or no effect on LC self-stimulation. There are 2 main fibre projections from LC: (1) One ascends through the midbrain and joins the MFB, and (2) The cerebellar projection which passes through the PCS (Ungerstedt, 1971a; Hoffer et al., 1973). Self-stimulation is readily obtained from both of these pro-

jections (Routtenberg & Malsbury, 1969; Huang & Routtenberg, 1971; Micco, 1974; Ball et al., 1974; Crow et al., 1972). It is known that NA cell bodies do not degenerate after lesions of their ascending axons (Ungerstedt, 1971a; 1973). Thus, lesions of the dorsal NA bundle would result in retrograde degeneration back to A6 but would not affect LC cells or the cerebellar projection from LC. It seems that the LC electrodes in DNB3, 4 & 5 were in the cerebellar projection of A6 rather than A6 proper. Since A6 and its cerebellar projection would remain intact after a dorsal NA bundle lesion no effect on self-stimulation from the cerebellar projection would be expected. Similarly, a lesion of the cerebellar projection of A6 but not A6 itself should not affect dorsal NA bundle self-stimulation. Such was the case with DNB2 who sustained a lesion of the cerebellar projection of A6 that did not seem to encroach upon A6 and did not affect dorsal NA bundle self-stimulation. Together, these results suggest that due to the unique property of NA cell bodies not to exhibit degeneration after transection of their efferent axons (Ungerstedt, 1971a) there exist 2 functionally independent self-stimulation systems arising from LC, the dorsal NA bundle and the A6 cerebellar projection. An experiment to test this hypothesis would be to lesion the dorsal NA bundle at the level of the FLM and observe subsequent effects on BC and cerebellar projection self-stimulation. On the basis of ideas expressed above, it would be predicted that BC

self-stimulation would be disrupted by a dorsal NA bundle lesion while cerebellar self-stimulation would be unaffected.

Summary and Conclusions

Extensive damage to both DA and NA systems at the level of the A9-A10 cell groups resulted in a strong suppression of LH self-stimulation. 6-OHDA lesions of the dorsal NA bundle, electrolytic lesions of the cerebellar projection of A6 alone and lesions which encompassed both the cerebellar projection and A6 proper failed to affect LH self-stimulation. Dorsal NA bundle lesions disrupted LH self-stimulation when the LH electrodes seemed to be primarily activating fibers of the dorsal NA bundle. Injections of procaine, d-amphetamine and glutamate in LC suppressed LH self-stimulation but these results were rendered questionable due to possible non-specific effects as well as the theoretical validity of the temporary lesion method for investigating the neural substrates of self-stimulation.

Lateral hypothalamic self-stimulation seems to depend upon the neural integrity of the DA systems alone or in combination with the dorsal NA system but not the dorsal NA system by itself. Theories proposing an exclusive NA basis for self-stimulation (e.g. Stein & Wise, 1971) must be reconsidered.

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