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Septal lesion-induced hyper-reactivity anatomical and neurochemical aspects.

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SEPTAL LESION-INDUCED HYPER-REACTIVITY
ANATOMICAL AND NEUROCHEMICAL ASPECTS

A Thesis

by

Leanna J. Standish

Submitted to the Graduate School of the
University of Massachusetts in partial
fulfillment of the requirements for the degree of

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Approved as to style and content by



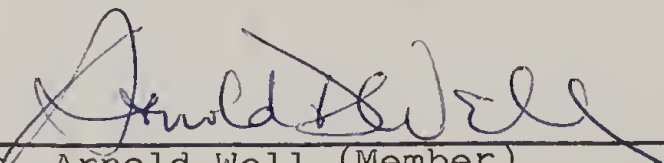
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PART I

Neurochemical Aspects of Septal Rage

The septum is one of the few areas of the brain where lesions result in readily observable changes in behavior. Septal lesions in rats and mice produce a dramatic hyper-reactivity to tactile stimuli, and these animals have been variously described as aggressive, hyper-emotional or ragic. For the sake of brevity, this hyper-reactivity will be referred to as septal rage. Septal lesions also lead to numerous profound behavioral changes such as over-responding on operant schedules of reinforcement (Lorens and Kondo, 1969), increased sensitivity to painful shock (Harvey and Yunger, 1973; Lints and Harvey, 1969), over-reactivity to appetitive stimuli (Beatty and Schwartzbaum, 1967), enhanced visual evoked potentials (Golden and Lubar, 1971; Lubar and Numan, 1973), and severe impairments of a number of species typical behaviors (Carlson and Thomas, 1968). The septum thus appears to be involved in the integration of wide varieties of behavior. Considerable evidence has accumulated suggesting that several of the effects of septal lesions are mediated via independent anatomical and neurochemical pathways. Normal operant responding seems to depend upon the integrity of the septal-fornix-hippocampal connections while septal hyper-reactivity or rage may be more dependent upon damage to septal-hypothalamic connections through the medial forebrain bundle (Beatty and Schwartzbaum, 1968; Ross, Grossman and Grossman, 1975).

Chlordiazepoxide has been found to suppress septal rage (Christmas and Maxwell, 1970; Sofia, 1969). It would be of interest if the neurochemical properties of CDP administration could be related to the anti-rage properties of this drug. Margules and Stein (1967, 1968), in animal experiments, demonstrated that benzodiazepines such as chlordiazepoxide and oxazepam have two opposing effects on behavior -- a response-decreasing or depressant action and a response increasing or disinhibitory action. They also showed that the disinhibitory effects of the benzodiazepines follow a different time course than the sedative action. With chronic administration, the sedative or depressant effects disappear in a few days as tolerance develops. The disinhibitory effects persist for as long as drug treatment is continued.

The behavioral effects of the benzodiazepines are paralleled by differential changes in the turnover of norepinephrine (NE) and 5-hydroxytryptamine (5-HT). Wise, Berger and Stein (1972) demonstrated that oxazepam blocks the turnover or utilization of NE and 5-HT in the midbrain-hindbrain region of rats. However, the decrease in NE turnover was transient and was no longer detectable after six daily injections. Serotonin turnover, however, was still substantially reduced for as long as the drug was given. Thus, the depressant action of the benzodiazepines seems to parallel the transitory blockade at NE turnover while the disinhibitory effects of the drug follow the time course of 5-HT turnover blockade. The present experiments were designed around these findings.

In the following experiment the effects of chronic administration of CDP in septal rage and on response rates during a variable interval schedule were measured. By observing the time course of CDP's action on these two response classes, it was possible to make inferences about the transmitter system responsible for the drug-induced changes in each of them. Also, the VI rate provided an independent measure of the non-specific depression of septal rage. Mice were chosen as experimental subjects because hyper-irritability produced by septal lesions does not dissipate with time or handling as it does in rats (Slotnick, McMullen and Fleischer, 1973). Fortunately, CDP's effects on brain amines in mice have been studied. Dominic (1973) has shown that, in mice, CDP doses as low as 20 mg/kg reduce significantly the turnover of 5-HT and its metabolite 5-HIAA.

It has been suggested that septal rage is mediated by serotonin (Dominquez and Longo, 1970) since p-chloro phenylalanine has been found to effectively reduce hyper-reactivity produced by septal lesions. If septal rage were related to changes in serotonergic neural pathways, as Dominquez and Longo suggest, repeated doses of CDP should depress the hyper-reactivity for as long as the drug is administered and little tolerance should be observed. If the depression of operant responding were at first depressed but then recovered, this would be strong evidence for an adrenergic mechanism being responsible for CDP's effects on response rates. If the

depression of VI rates recovered while the depression of septal rage did not, this would at least argue for different mechanisms being involved. More generally, if septal rage and septal operant over-responding are mediated via independent transmitter systems, then CDP's effects on both measures should be clearly dissociable.

Finally, it was of interest to compare the effects of CDP on VI rates between septal and normal mice. Normal control groups were run to give an idea of the effect of CDP on VI rates in mice would not in some unsuspected way be grossly different because they had septal lesions. Consequently, small groups of unoperated controls were treated identically to septal mice.

METHOD

Fifty-two male B6D2F₁/J hybrid mice procured from the Jackson Laboratory, Bar Harbor, Maine, served as subjects. They were 10-12 weeks of age at the time of surgery. Of these, 40 were given septal lesions, the rest served as unoperated controls. The mice to be lesioned were anesthetized with sodium pentobarbital (75 mg/kg i.p.) and placed in a Kopf stereotaxic apparatus equipped with a mouse headholder which eliminates the need for ear pins (Slotnick, 1972). Thermocoagulations of the septal nuclei were made by passing current from a Grass radio-frequency lesion maker through a size 00 (.3 mm diameter) stainless steel insect pin. Insulated except for the tip with enamel. Septal lesions were made

according to flat-skull coordinates (relative to bregma); anterior, 0.7 mm; ventral, -3.5 mm.; lateral, \pm 0.4 mm.).

During the experiment mice were housed in individual cages and maintained at 80% ad lib weight on wet lab chow mash. After completion of the experiment, the brain lesioned mice were anesthetized with sodium pentobarbital and perfused pericardially with 20 ml of .9% saline followed by 20 ml of 10% formalin in .9% saline. Frozen sections of 40 μ m were mounted and stained alternately with either cresylecht violet or hemotoxylin (Weil stain).

Apparatus

Reactivity scores were obtained from septal and normal mice by determining each animal's reaction to a 30 cc air puff directed at their dorsal surface. The mice were placed one at a time in a plastic cage (41 x 24 x 15 cm) which was covered by $\frac{1}{4}$ " metal mesh screening (7 mm mesh). The air puff was applied by placing the nozzle of a 50 cc syringe containing the air against the screen (15 cm from the animal) and quickly pressing the plunger.

Mice were trained on a VI-40 sec. schedule for food reinforcement in one of four identical operant chambers, each isolated in an insulated chest. The chambers were constructed of Masonite hardboard with a wire mesh floor and a perforated hardboard lid. Each chamber, 25.4 cm high, was trapezoidal in its horizontal cross-section; the parallel end walls were 5 cm and 15 cm wide. The length of the box (distance between

end walls) was 19 cm. Centered on the narrower end wall 2.8 cm above the wire mesh floor was a round aluminum tube (2.5 cm diameter) which protruded from the chamber. A photocell beam passed through holes in the sides of the tube. The operant response consisted of a head poke into the response tube, breaking the photocell beam. The source of the photobeam was a red light emitting diode. A light source at the end of the tube provided the only illumination. Reinforcements (20 mg. Noyes Co. rat pellets) could be delivered directly into the response tube via a plastic hose connected to a pellet dispenser. The presentation of stimuli and collection of data was controlled by an online computer.

Procedure

Following two weeks of post-surgical recovery time for the lesioned mice and 5 days of limited access to food, all mice were placed in operant chambers where reinforcements were delivered on a continuous reinforcement schedule. The operant level of head poking in mice was sufficiently high to render unnecessary the use of shaping techniques. This is especially true of mice with septal lesions. Over the period of 5 days the schedule of reinforcement was gradually changed from CRF to VI-10, 20 and, finally, VI-40 sec.

Once mice attained stable rates of responding they were tested for three days to obtain baseline measures of VI responding and reactivity to air puff. Prior to each 30-minute VI session all mice, lesioned and non-lesioned were

tested for their reactivity to the air puff. Since the behavioral effects of septal lesions in mice are slightly different from those seen in rats a different rating scale was used. Septal mice will typically respond violently to a puff of air directed anywhere on their body. Scores ranged from 0 to 4. An animal received 1 point for displaying each of the following behaviors:

- 1) Vocalizing and writhing while being picked up by the tail.
- 2) Escape running when the air stream was directed on the animal's back.
- 3) Jumping with all four feet off the floor of the testing apparatus in response to the air stream.
- 4) Jumping and hitting the wire mesh cover of the testing apparatus in response to the air puff stimulus.

Following three baseline days in which no drug was administered, animals were placed into groups receiving one of four drug treatments: saline, 20, 40, or 60 mg/kg CDP. Attempts were made to insure equal matching of baseline emotionality scores and VI response rates across the four groups. On day 4 drug treatment was begun. The daily procedure for the following 8 days was as follows:

- 1) Pre-drug reactivity scores were obtained.
- 2) Animals were weighed and injected i.p. with either saline or CDP.
- 3) Twenty minutes after injection a post-drug reactivity score was obtained.

- 4) Thirty minutes after drug administration mice were placed in their operant chambers where they responded on a VI-40 sec. schedule for a 30 minute daily session.

RESULTS

Histology

Mice with less than 50% damage bilaterally to both the medial and lateral septal nuclei were excluded from data analysis. Lesions typically were bounded ventrally by the decussation of the anterior commissure, dorsally by the genu of the corpus callosum and laterally by the lateral ventricles. In a few cases, the lesion extended through the lateral ventricle into the medial edge of the caudate nucleus, but this damage appeared uncorrelated with the behavioral measures observed in the experiment. Rarely did lesions encroach upon the diagonal band of Broca. However, lesions often included the columns of the fornix as their posterior boundary, whereas the fornix proper was rarely destroyed.

Examination of the histological material in correlation with baseline emotionality ratings led to the following general observation. Mice with high septal rage scores tended to sustain damage in the more anterior and dorsal parts of the septum. Animals with lesions extending as far anterior and dorsal as the hippocampal rudiment were invariably highly reactive to the air puff stimulus.

VI rates and septal rage.

First, five of the ten mice in the 60 mg/kg group died

between the second and fifth day of drug treatment. There were no fatalities in the 20 or 40 mg/kg group nor among the unoperated controls. Interestingly, four of the five mice who died were the animals with the highest baseline emotionality scores. Since these animals did not complete the experiment, their data, except in pre-drug comparisons, was not included in any of the figures of analyses. For this reason, the baseline emotionality scores of the 60 mg/kg group were considerably lower than that for the other groups. It appeared that animals with septal lesions were more susceptible to toxic effects of high doses of CDP.

The results are given as comparisons among scores of baseline activity and scores after early and late drug treatment. Also, some results are described which compare pre- and post-drug scores. Consequently all groups in these comparisons served as their own controls and matched-pairs t-tests and Wilcoxon matched-pairs signed-rank tests were used to establish the reliability of the findings.

For the septal mice VI rates (response/min) are plotted as a function of days of CDP treatment in Fig. 1. The VI rate for mice receiving 20 mg/kg was not reduced but rather this dose increased response rates. A comparison of the last two days of baseline with the last two days of 20mg/kg CDP treatment revealed a significant increase ($t = 2.82$, $df = 10$, $p < .02$).

The group receiving 40 mg/kg showed a marked depression of VI responding on the first two days of drug treatment.

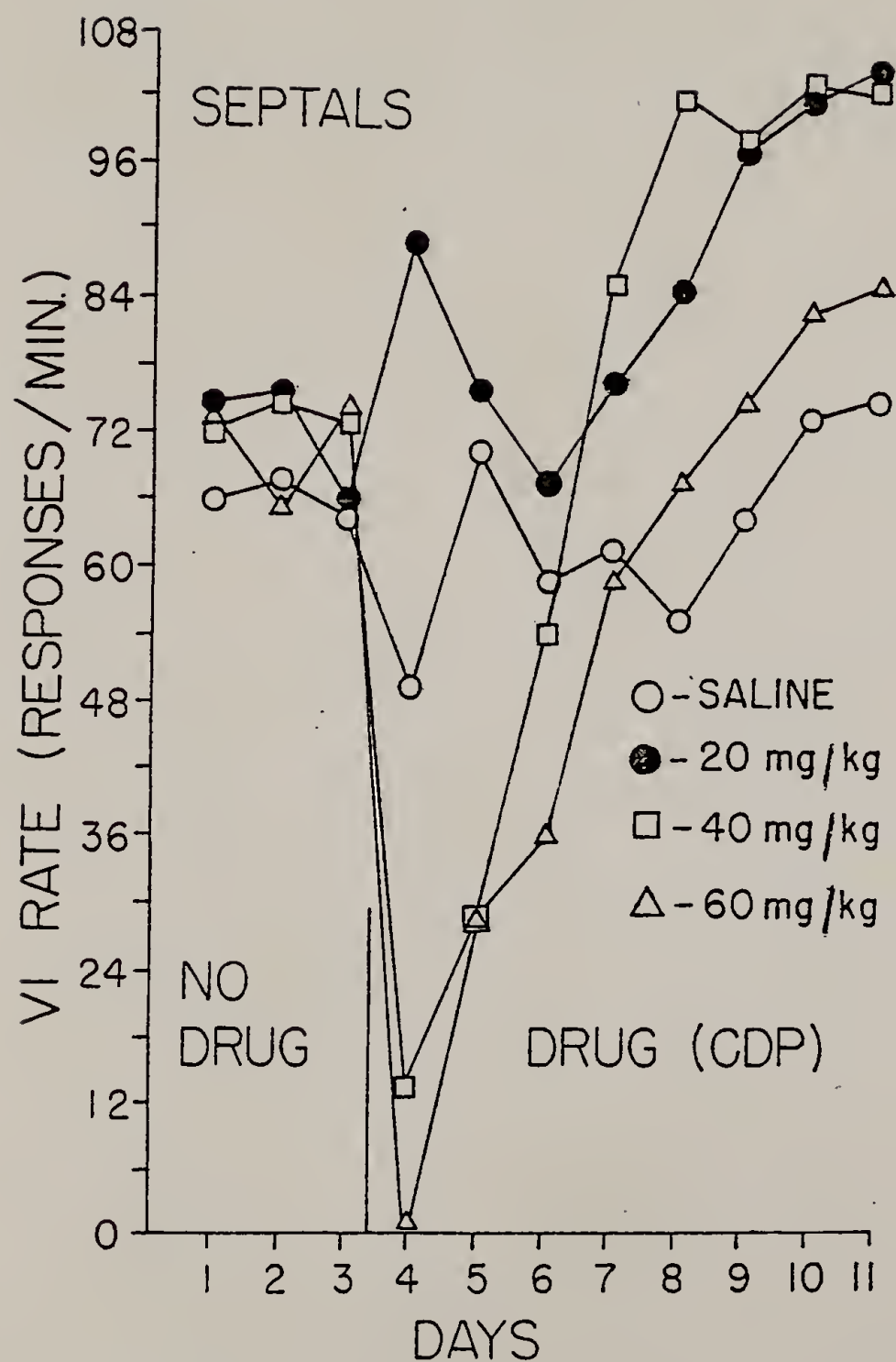


Figure 1. Rates of responding on a VI schedule in mice with septal lesions. No drug was administered on days 1, 2 and 3. The effects of saline, 20, 40 or 60 mg/kg CDP on rates of responding were observed beginning on day 4.

The difference between the first two days of drug administration and baseline was highly significant ($t = 6.80$, $df = 8$, $p < .001$). By the fourth day of drug tests response rates had not only returned to normal but surpassed baseline. On the last two days of 40 mg/kg administration response rates were 50% higher than on baseline days, a difference that was highly significant ($t = 9.32$, $df = 9$, $p < .001$).

The 60 mg/kg group showed a nearly complete suppression of VI responding on the first two days of CDP treatment. Response rates on the first two days of 60 mg/kg CDP administration were significantly lower than the last two days of baseline ($t = 8.25$, $df = 4$, $p < .005$). Response rates gradually returned to baseline levels within five days. However, the increase in response rates over baseline was not as striking as in the case of mice receiving 40 mg/kg, perhaps reflecting some residual depressant action of the drug at this high dose. Yet, a comparison of the last two baseline days with the last two days of 60 mg/kg treatment revealed a significant increase ($t = 3.22$, $df = 4$, $p < .05$).

Fig. 2 presents VI rates as a function of days for the unoperated controls. First, it is immediately apparent that baseline rates, where no drug was given, for these mice is half that of septal mice. Second, the drug test data show that 20 mg/kg produced a steady increase in VI responding, while 40 and 60 mg/kg first depressed responding before increasing response rates. No statistical comparisons were

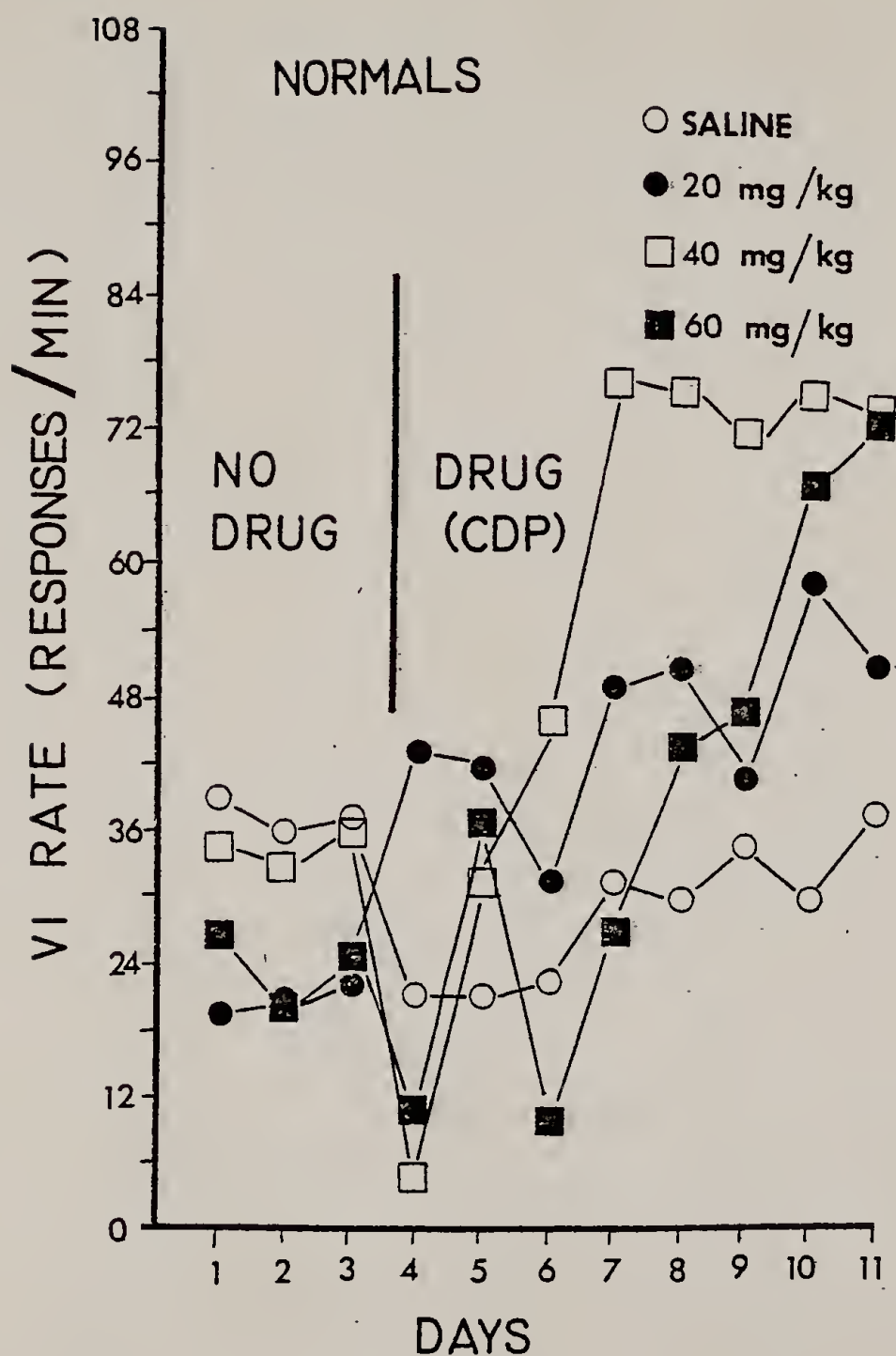


Figure 2. Rates of responding on a VI schedule in normal mice. No drug was administered on days 1, 2 and 3. The effects of saline, 20, 40 and 60 mg/kg CDP on rates of responding for food were observed beginning on day 4.

made because of the small samples in these groups (N = 3 each), but aside from overall response rates, these CDP effects were comparable to those of the septal mice.

Thus, CDP has a dose related depressant action on VI rates, but the depressant effect is only transient. Once animals developed tolerance to the depressant action of CDP, as shown in the case of 40 and 60 mg/kg groups, the response rates increased over baseline rates.

Fig. 3 presents pre- and post-drug reactivity ratings as a function of days of saline or CDP treatment for each dose group. A septal mouse expressing the full septal rage syndrome will typically receive a score of 4 by such a test, whereas normal mice display virtually no reactivity to being picked up by the tail or exposure to air puff and will typically receive a score of 0. Data from the saline group demonstrated that not only does septal rage persist with time in mice but actually increases. A Wilcoxin matched pairs signed-ranks test showed that there was no difference between pre- and post-saline measures. The dose of 20 mg/kg effectively reduced the hyper-reactivity of septal mice for the duration of drug test days. The overall difference between pre- and post-drug rages scores was highly significant ($p < .01$). CDP at 40 mg/kg suppressed rage severely on the first day of drug testing but on the second day there was some recovery. How-

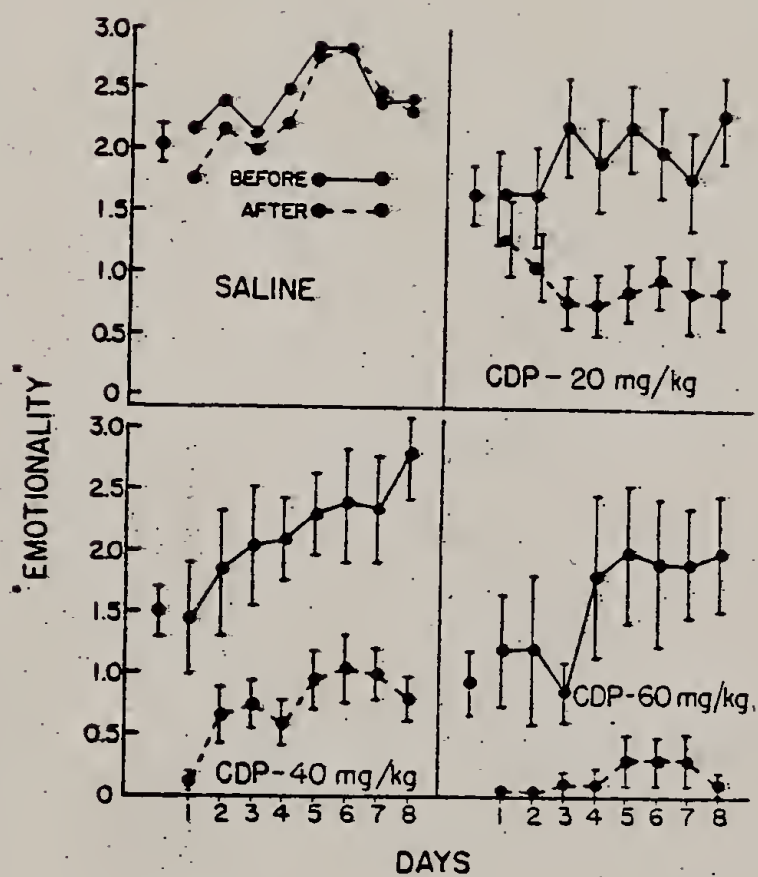


Figure 3. Pre- and post-drug reactivity ratings as a function of days of saline or CDP treatment for each dose group (indicated in each box). Post-drug reactivity scores are represented by dotted lines.

ever, post-drug scores for this group also remained low for the duration of the experiment. The overall suppression of hyper-reactivity for this group was also highly significant ($p < .01$) CDP at 60 mg/kg had an additional effect in that there was evidence on the third day of drug treatment that hyper-reactivity was suppressed in the pre-drug measure probably by residual amounts of CDP administered on the previous days. A statistical test for these effects was not feasible because of the small number of survivors. It can also be reported that when the unoperated controls were also tested for irritability with the air puff stimulus, their response was so minimal that any further reduction by CDP could not be detected.

To further examine the independence of septal rage and VI overresponding we computed a correlation coefficient between these phenomena. Comparing the baseline scores of septal rage and VI response rates, a Spearman Rank Order correlation corrected for ties yielded a correlation coefficient of only 0.15. Thus, high reactivity scores do not predict rates of responding on a variable internal schedule.

DISCUSSION

The results showed, first, that with repeated doses, CDP at 40 and 60 mg/kg depressed VI responding in a dose related fashion only transiently. Second, CDP's ability to reduce septal hyper-reactivity did not diminish with repeated drug doses. Third, there was a correlational independence

between septal hyper-reactivity and VI responding. This finding was not surprising since it is well known that deficits in DRL responding as well as high FI response rates in septal rats persist despite the dissipation of septal rage (Lubar and Numan, 1973). These data for septal mice strongly suggest not only separate neurochemical bases for these classes of behavior but separate neuroanatomical substrates as well. Wise, Berger and Stein (1972) have shown that tolerance develops to CDP's apparent ability to block NE turnover while the blockade of 5-HT turnover remains as long as drug administration is continued. The data from Experiment 2 suggested that CDP's suppression of septal rage parallels the time course of CDP's effects on 5-HT, and this was strong evidence to support the hypothesis that septal rage is mediated via serotonergic pathways. However, evidence for a 5-HT hypothesis is contradictory. First, septal lesions decrease whole brain levels of 5-HT (Heller and Moore, 1968). On the other hand, drugs which decrease whole brain levels of 5-HT such as parachlorophenylalanine (pCPA) (Dominquez and Longo, 1970) and CDP (Horowitz, Furgiuele, Brannick and Craver, 1963) reduce septal rage. If septal rage were mediated by depletion of telencephalic levels of 5-HT, it is unclear how further reduction of 5-HT utilization by CDP or pCPA can reduce septal rage. Experiment 2 was a further attempt to investigate the possible role of 5-HT in the septal rage syndrome.

Experiment 2

If septal rage were mediated by a change in 5-HT activity somewhere in the brain, then a serotonergic agonist should either attenuate or enhance this aspect of the septal syndrome. Further, if CDP attenuates septal rage by virtue of its ability to reduce the effectiveness of serotonergic systems, then a drug which potentiates the action of 5-HT should antagonize CDP's effect. Fuller and his colleagues (1974) have recently reported that Fluoxetine (Lilly-110140) selectively inhibits the re-uptake of 5-HT by synaptosomes and that the drug enhanced serotonergic transmission in vivo. Experiment 2 was designed to test fluoxetine's ability to alter septal rage and reverse the attenuating effect of CDP. Positive findings would give support to the hypothesis that 5-HT systems are implicated in septal rage.

METHOD

Eight male B6D2F₁/J mice were given septal lesions in a manner identical to that in Experiment 2. Approximately 30 days after surgery mice were tested for their reactivity to air puff using the same procedure as in Experiment 2. For two days emotionality tests were given immediately before and 30 minutes after injection of CDP (20 mg/kg i.p.). On the next two days mice were tested immediately before and 30 minutes after injection of fluoxetine. Immediately following the post-fluoxetine test mice were injected with CDP (20 mg/kg). Finally, a third reactivity test was given 30 minutes after the CDP injection. Three days of no testing

followed before testing with a higher dose of fluoxetine the next week. These procedures continued for five weeks. The dose of CDP was held constant at 20 mg/kg, but the dose of fluoxetine at 5 mg/kg the first week doubled each week. Thus, the effects of CDP and five doses of fluoxetine (5, 10, 20, 40 and 80 mg/kg) were observed.

RESULTS

Fig. 4 shows the effects of CEP, fluoxetine and their combined effects on septal hyper-reactivity. Each column expresses the average data of two days of tests; i.e., days 1 and 2, and days 3 and 4. The data were averaged in this way because there was no detectable differential effects within any two day period. For each week, the first column (open) expresses the average percent of the pre-drug rage score after CDP treatment on days 1 and 2. Thus, 30 minutes after 20 mg/kg CDP, emotionality scores were significantly reduced by about 50%. Wilcoxin matched-pairs signed-ranks tests were used in all comparisons. Tests comparing pre-CDP and post-CDP rage scores revealed significant differences each week, all below the .01 level of confidence (two-tailed).

The second column (black) shows the percent of pre-drug rage scores on days 3 and 4 after fluoxetine injection. During the first three weeks fluoxetine had no significant effect on septal rage. But on weeks 4 and 5, this drug at 40 mg/kg had a significant depressant effect on hyper-reactivity ($p < .01$).

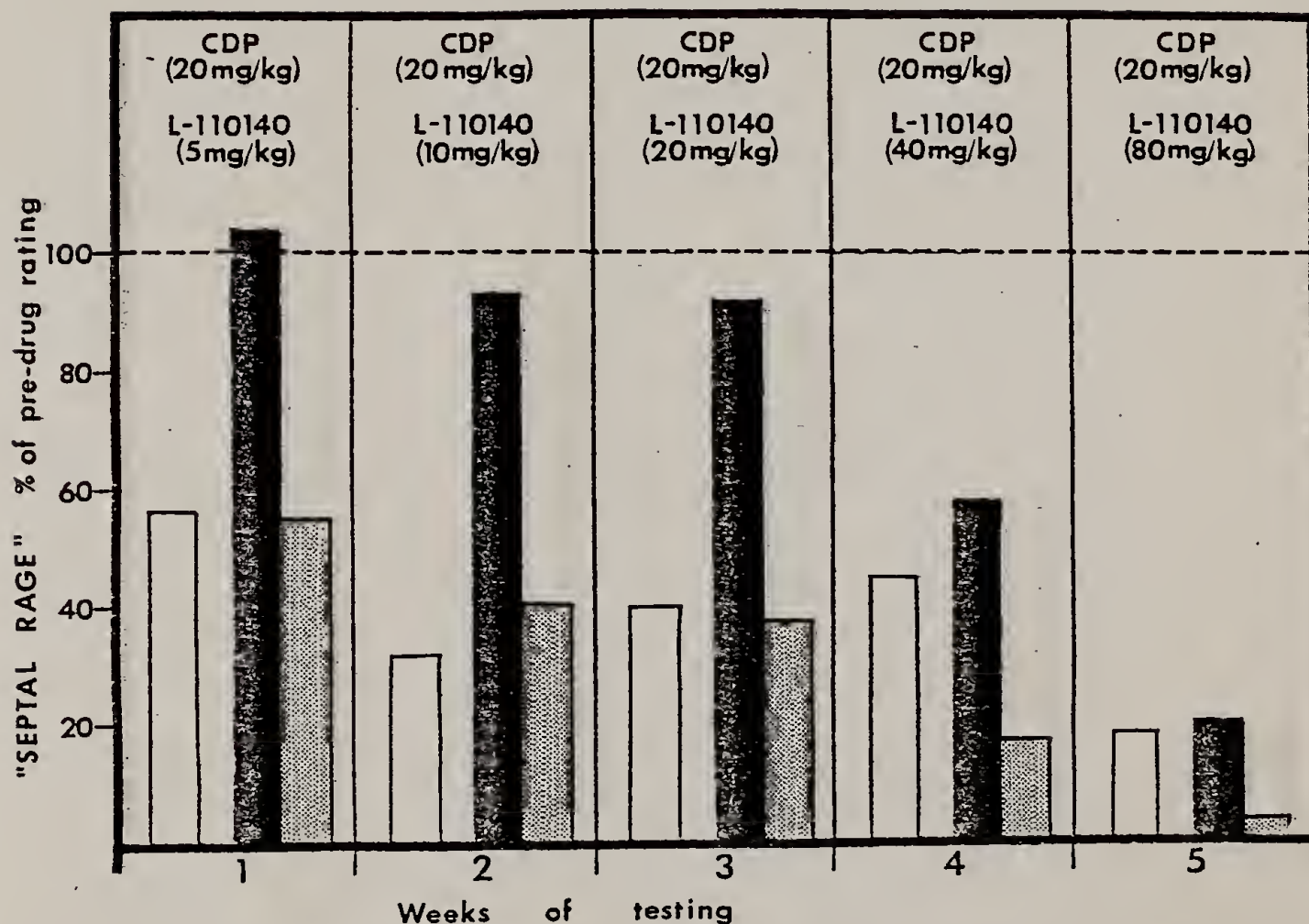


Figure 4. The effects of CDP (open column), fluoxetine (black) and their combined effects (stippled) on septal hyper-reactivity. The first column of each week expresses the average percent of the pre-drug reactivity scores after CDP treatment. The second column (black) shows percent of pre-drug reactivity scores after fluoxetine injection. The third column (stippled) presents the percent of pre-drug levels when reactivity scores were obtained after combined treatment of 20 mg/kg CDP and 5, 10, 20, 40 and 80 mg/kg fluoxetine.

The third column (stippled) presents the percent of pre-drug levels when rage was measured after combined drug treatment (1 hour after fluoxetine and 30 minutes after CDP). During weeks 1, 2 and 3, fluoxetine had no detectable effect on CDP's ability to suppress septal hyper-irritability in mice, as shown by the nearly identical percentage scores of the open and stippled columns. During weeks 4 and 5, however, the addition of CDP to fluoxetine further depressed septal rage ($p < .01$ and $< .05$ respectively). Thus, fluoxetine, rather than reversing the effects of CDP on septal rage had an additive effect at high doses and further reduced CDP-suppressed irritability. The ability of fluoxetine to suppress septal rage is further demonstrated by the lower septal rage scores seen after CDP treatment alone on week 5, the open column, compared to that of week 4. Apparently, residual effects of 40 mg/kg of fluoxetine from the previous week were able to interact with CDP in an additive fashion producing a considerably lower expression of septal hyper-reactivity after CDP during the last week of the experiment ($p < .01$).

DISCUSSION

Clearly, doses of fluoxetine that have been reported to block the re-uptake of 5-HT in rat brain did not antagonize CDP-induced reduction of septal rage. At the dose of 10 and 15 mg/kg fluoxetine not only effectively reduced

punished responding in rats in Geller/Seifter conflict tests but also antagonized CDP's disinhibitory action on punished responding (unpublished data from our laboratory). The action of 5-HT agonists in the suppression of punished responding and the release of punished responding by 5-HT antagonists has been shown repeatedly (Stein, Wise and Belluzzi, 1975). The fact that at high doses fluoxetine (40 and 80 mg/kg) depressed septal rage and CDP augmented the depression was most likely due to drug toxicity and general depression. During drug testing on week 4 and 5 animals appeared lethargic and sick. The data seem to suggest that septal rage is not mediated by a serotonergic mechanism and the effectiveness of CDP in suppressing this behavior is not readily explained.

PART II

Neuroanatomical Circuitry Involved in Septal
Hyper-reactivity

Since destruction of the septal forebrain area produces hyper-reactivity to tactile, visual and olfactory stimuli it seems clear that the septal nuclei are part of a larger system which mediates an organism's adaptive response to its environment. The fact that large septal lesions result in a multitude of behavioral changes argues that a unitary conceptualization of the septal area is an over-simplification. Experimentl, Part I demonstrated the low correlation between over-responding on operant schedules and irritability and these results suggested an anatomical and neurochemical independence of these two striking effects of septal lesions. The purpose of the following experiments was to determine the neural circuitry involved in the expression of septal lesion-induced hyper-reactivity.

The septal area lies in a special position relative to the rest of the limbic system and is in an excellent position anatomically to integrate the activity of a large number of brain structures. Raisman (1966) views the septal nuclei as a critical region integrating information between telencephalic limbic structures and the diencephalon. All parts of the septum receive massive input from a wide variety of limbic regions as well as from the hypothalamus. Its influence

over other brain regions is as widespread as its sources of incoming fibers. An analysis of the circuitry mediating the hyper-reactivity which results from destruction of the septum would greatly add to our understanding of how the brain integrates an organism's adaptive response to its environment.

The septal area lies below the most anterior portions of the corpus callosum, bounded anteriorly by the hippocampal rudiment and posteriorly by the postcommissural fornix. At the rostral end of the septum lies the medial septal nucleus and the nucleus of the diagonal band of Broca, while lateral to the medial septal nucleus is the anterior part of the lateral septal nucleus. Caudally, the medial septal nucleus becomes smaller and finally disappears as the lateral septal nuclei join together at the midline before disappearing at the level of the fornix. The medial septal nucleus is penetrated by fibers of the fornix which enter the nucleus at an oblique angle. The area between these two bundles of precommissural fornix is called the nucleus triangularis septi.

The cytoarchitecture of the septal area of the rat reveals homogeneous nuclear areas made up of medium sized neurons. Despite few differences in cytoarchitecture, the septal area has been divided into various subregions. In the anterior portion of the septal area, near the nucleus of the diagonal band, giant cells are found but only in the medial septal area. These cells project to the hippocampus

and undergo degeneration when the fornix is severed (Isaacson, 1974).

Raisman (1966) has subdivided the afferent and efferent connections of the septum into telencephalic and diencephalic projections, as follows:

TELENCEPHALIC AFFERENTS

- 1) All septal nuclei receive fibers from the hippocampus. These hippocampal projections are probably more complicated than anatomical studies have yet indicated. Freeman and Patel (1968) have shown that when the dorsal hippocampus is electrically stimulated the response recorded in the septum revealed six components. This suggests that there may be as many as six different routes by which fibers from the hippocampus can reach the septal area. Very little response can be elicited in the septum by stimulation of the ventral hippocampus. Powell et al (1968) reported that stimulation of the ventral hippocampus produced evoked responses in fibers of the diagonal band and in the nucleus accumbens septi, but not in the septal area proper.

There is some dispute over the fiber relations between the hippocampus and the septal area. Raisman, in the rat, found that lesions of the medial septal nucleus produced retrograde degeneration in both dorsal and ventral hippocampus. Siegel and Tassoni (1971) reported that medial septal lesions produced

widespread degeneration only in the dorsal hippocampus of the cat. Raisman also reports no hippocampal projection to the lateral septal nuclei (Raisman, 1966), whereas in the cat, Siegel and Tassoni report degeneration in ventral hippocampus after lateral septal damage. These are not simply species differences since Siegel and Edinger (1973) have found the same pattern of projections from the hippocampus to the septum as described by Siegel and Tassoni in rats, rabbits and gerbils.

- 2) Afferents to the diagonal band nucleus arise in pyriform cortex, basolateral amygdala and the olfactory tubercle. The diagonal band in turn projects fibers to the medial septum.

DIENCEPHALIC AFFERENTS

- 1) Diencephalic afferents to the septum are conveyed entirely by the medial forebrain bundle which recruits fibers from areas all along the base of the brain as far caudal as the dorsal and ventral tegmental nuclei.
- 2) Over and beyond the basic interconnections that the septum has with the medial forebrain bundle, the septal area is reciprocally interconnected with other hypothalamic regions; the supraoptic and periventricular nuclei. These hypothalamic connections are primarily with the medial septal nucleus.

TELENCEPHALIC EFFERENTS

- 1) The septo-hippocampal projection arises from the medial septal nuclei and the vertical limb of the nucleus of the diagonal band. The septum sends fibers to the ventral hippocampus via the fimbria. Dorsal fornix carries fibers from septum to dorsal hippocampus.
- 2) Fibers to the olfactory tubercle arise in the septum.
- 3) Medial septum projects to the basolateral amygdala via the diagonal band.

DIENCEPHALIC EFFERENTS

- 1) Fibers from all parts of the septum are distributed throughout the MFB to terminate diffusely in the lateral hypothalamic area, the preoptic area and terminate as far caudally as the tegmental nuclei.
- 2) The septofimbrial nucleus projects to the medial habenular nucleus via stria medullaris, which in turn sends terminating fibers to the interpeduncular nucleus via fasciculus retroflexus.

The hyper-reactivity produced by septal lesions may involve either septal afferents or efferents. For example, the characteristic behavior of an animal with a septal lesion may be the result of a release of inhibition on some other structure, thus suggesting septal efferents as responsible. If septal afferents are involved in hyper-reactivity the situation is then more complicated. Retrograde degeneration

in some other afferent structure, of which there are many, may be more critically involved. Another possibility that cannot be ruled out is the complex interactions among damaged and intact tissue within the septum itself.

In the past various attempts at localizing the critical area involved in lesion-induced hyper-irritability have led to contradictory and confusing conclusions. The usual method in these experiments is to produce septal lesions in a number of rats, observe the animals for several days and then determine the location of the smallest effective lesion. Thus, Thomas and Van Atta (1972) ascribe the hyper-responsivity following septal lesions to the percent damage to subseptal structures. Clody and Carlton (1969) presented evidence that medial septal lesions are not part of the "rage" circuitry and that damage to the lateral septal area is critical for the elicitation of hyper-reactivity. Turner (1970), on the other hand, holds damage to the bed nucleus of stria terminalis and the stria themselves as responsible for the hyper-reactivity induced by septal lesions. Harrison and Lyon (1957) asserted that there was no correlation between damaged structures and the emotional alterations observed. Schnurr's attempts (1972) to discover the septal area responsible for septal rage led her to the conclusion that lesions restricted to the anterior dorsal area of the septum resulted in the typical hyper-irritability. With few exception, posterior septal lesions have been reported to be ineffective in eliciting hyper-

reactivity.

These attempts to discover the septal areas involved in the elicited hyper-reactivity have focussed on the septal nuclei themselves and employing a single lesion technique have ignored the larger system involved in the hyper-irritability of which the septum may only be a small part. A more promising approach has been used by a few investigators. This approach studies the interrelationship of the septum with the rest of the brain through the use of a multiple lesion technique.

Lesions of structures outside the septal area influence the hyper-reactivity induced by septal lesions. Olton and Gage (1974) and Gage and Olton (1975) have studied the contribution of the fornix to the septal rage syndrome in rats and several authors have investigated the interaction between the septum and the amygdala (Joneson and Enloe, 1973; King and Meyer, 1958).

The literature concerned with the role of the amygdala in the hyper-reactivity induced by septal lesions is contradictory and confusing. The general conclusion of this literature however (for a review see Olton and Gage, 1974) is that the amygdala may be a structure directly involved in septal rage and that tissue damage in the septum leads to abnormal amygdala activity which may be directly responsible for the expression of hyper-irritability.

Olton and Gage (1974) and Gage and Olton (1975) have

concluded that it is the septal-hippocampal connections which are critically involved in hyper-reactivity as a result of septal destruction. In 1974 they showed that fornix lesions ten days prior to septal destruction eliminated the hyper-reactivity following septal lesions. In a second experiment they demonstrated that simultaneous septal and fornix lesions also prevent the appearance of hyper-irritability. In order to determine more specifically which components of the fornix system are critical for the appearance of hyper-reactivity following septal lesions, these authors in a 1975 paper made selective lesions placed in the medial fornix, lateral fornix, precommissural fornix, anterior hippocampus, posterior hippocampus and entorhinal area sixteen days prior to septal destruction. Both precommissural and postcommissural fornix lesions were effective in blocking the hyper-reactivity expected from the subsequent septal lesions. Results indicated that terminations of the fornix in the anterior hippocampus are critical for the appearance of hyper-reactivity while terminations in the posterior hippocampus are not. This evidence suggests that fornix-hippocampal-entorhinal connections are unimportant in the mediation of hyper-reactivity. The authors concluded that the following septo-hippocampal-hypothalamic circuits mediate the dramatic changes in behavior following septal lesions:

- 1) From the septum to the anterior hippocampus via the precommissural fornix.

- 2) From the anterior hippocampus to the hypothalamus via the postcommissural fornix.

These experiments are difficult to reconcile. Posterior septal lesions as well as fornix lesions have been generally reported to be ineffective in eliciting septal rage yet Olton and Gage have suggested that it is the posterior septal connections through the fornix that are responsible for the septal rage syndrome. Furthermore, medial septal lesions have proven not to be effective in eliciting septal rage. It is the medial septum to which the hippocampus projects. These data indicate that medial septum and its NPC connection are not involved in septal hyper-reactivity.

The study of the neural substrates of septal hyper-reactivity is complicated by the fact that, in rats, the irritability dissipates with time and handling. There is some indication that this "recovery of function" is mediated by cortical tissue. Yutsey, Meyer and Meyer (1967) have shown that the removal of as little as one half of either neocortex or limbic cortex when performed simultaneously with a septal lesion will prevent spontaneous attenuation of the hyper-reactivity. Mice, on the other hand, display the hyper-reactivity indefinitely and behavioral tests will show no diminution over weeks or months. The permanence of the effect of a septal lesion on the behavior of a mouse makes them ideal subjects for multiple lesion research on the neural circuitry mediating septal hyper-reactivity.

The purpose of the following experiments is threefold:

- 1) To confirm or repudiate Olton and Gage's claim of hippocampal mediation of hyper-irritability.
- 2) To clarify the involvement of the amygdala in septal hyper-reactivity using mice as experimental subjects.
- 3) To search out other brain structures whose destruction alters the syndrome.

In these experiments septal lesions are followed by three days of behavioral observation. On the third experimental day a second lesion, aimed at a major septal efferent and afferent structures, is produced bilaterally. Three days of behavioral observation is followed by perfusion and histological analysis. The effects of damage to the following structure on septal hyper-reactivity was studied: 1) fimbria-fornix 2) amygdala 3) stria terminalis 4) diagonal band of Broca 5) olfactory bulbs.

EXPERIMENT 1

This experiment investigated the involvement of septal-hippocampal connections in the mediation of septal lesion-induced hyper-reactivity. Olton and Gage (1974) demonstrated, in rats, that destruction of the fornix-fimbria prior to septal destruction prevented the appearance of the hyper-reactivity to somesthetic stimulation. These authors concluded that the relevant circuits involved fibers from the medial septal nuclei to the anterior hippocampus via the

precommissural fornix and from the anterior hippocampus to the hypothalamus via the postcommissural fornix. The purpose of the following experiment was to determine, in mice, the effectiveness of disruption of septal-hippocampal connections and hippocampal-hypothalamic connections by fimbria lesions on the hyper-reactivity produced by prior septal lesions.

Method

Six male mice weighing approximately 25 grams served as subjects. Five of these animals received a bilateral septal lesion three days prior to receiving a lesion destroying all of the fimbria. One animal received a fimbria lesion prior to septal destruction.

Surgical procedures were identical to those used in Experiment 1, Part I. Fimbria destruction required 5 cranial penetrations at the following coordinates relative to bregma: anterior, $-.5$ mm.; lateral $+1.1$, $+.5$, 0 $-.5$, -1.1 mm; ventral, -2.5 , -2.2 , -2.0 , -2.2 , -2.5 mm.

Reactivity ratings were determined at twenty-four hour intervals following each surgical procedure using the method outlined in Part I.

The effects of extraseptal structures on septal hyper-reactivity was determined using the following procedure: On Day 1, prior to receiving bilateral septal lesions, mice were tested for the reactivity to the air stream stimulus. Normal animals receiving a pre-operative score of over 1.0

were discarded from the experiment. At 24, 48 and 72 hours after septal destruction reactivity scores were obtained. During the third experimental day a fimbria (or septal) lesion was preceded by a second pre-operative rating. At 24, 48 and 72 hours after this second operation the animals were tested for their level of hyper-reactivity. Immediately following the last 72 hour, rating mice were perfused and brains removed according to the procedures outlined in PART I. Brains stood in 10% formalin solution for four days and a 30% sucrose-formalin solution for 24 hours before frozen sectioning. Alternate 40 μ m sections were mounted and stained with either cresyl violet or hematoxylin.

The size and location of each animal's lesions were drawn on serial diagrams of a mouse atlas. Diagrams of coronal sections caudal to the decussation of the anterior commissure were modified from Lehmann's mouse atlas (1974). Diagrams of brain sections rostral to the decussation of the anterior commissure were constructed by the author.

Results

Figures indicating each animal's reactivity scores over the six experimental days are shown in Figures 5 and 6. The order in which brain structures were destroyed is indicated by S/FIMB or FIMB/S. The abbreviation S/FIMB indicates that a septal lesion preceded a fimbria lesion and FIMB/S indicates that a fimbria lesion preceded septal destruction.

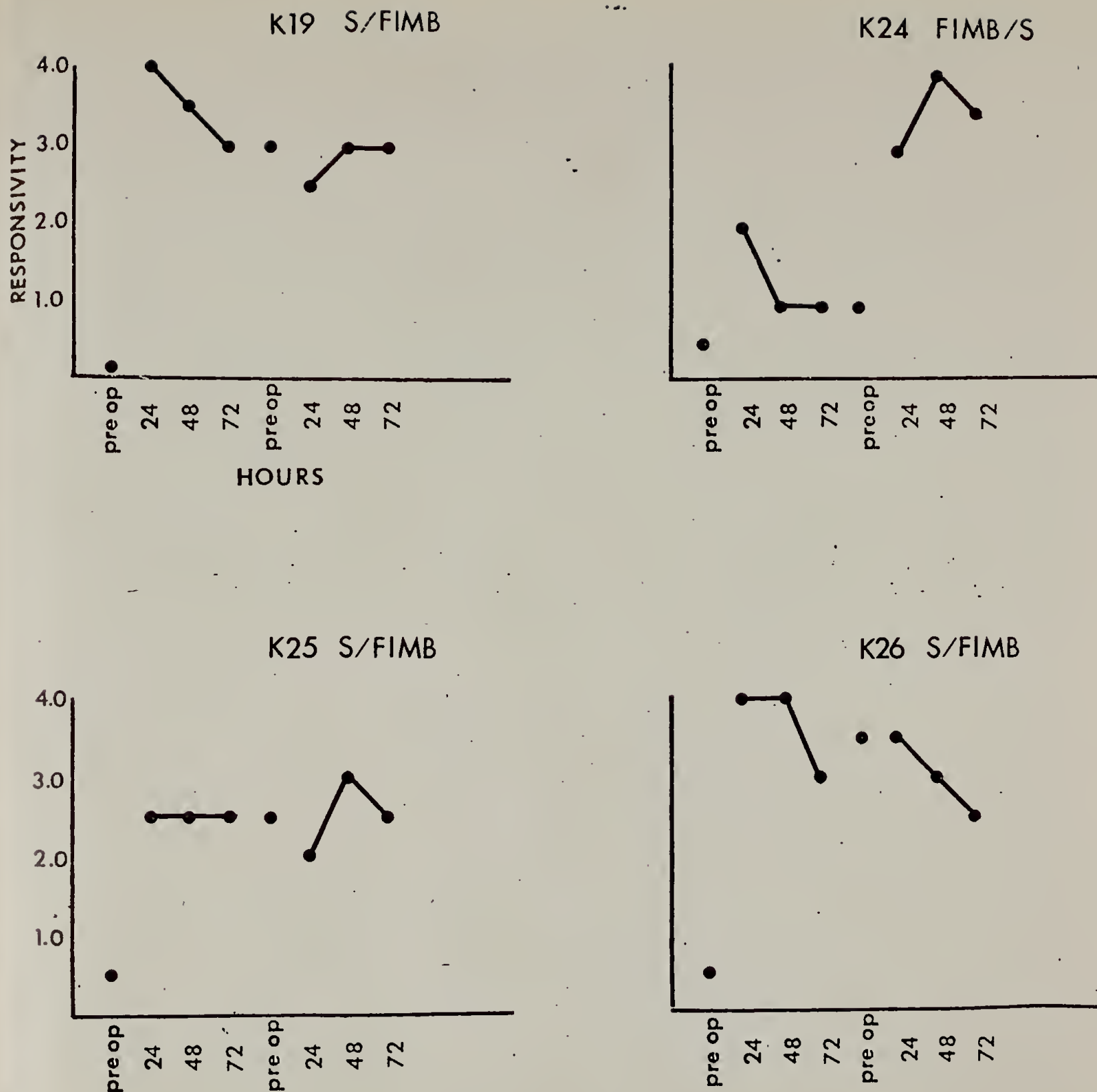


Figure 5. The effects of fimbria lesions on septal hyper-reactivity. Individual subjects' reactivity to air puff was measured over the six experimental days. The two pre-operative ratings are indicated as dots. Subjects K24, K25, K26 received fimbria lesions prior to septal area destruction. Subject K19 received a fimbria lesion before septal lesion. These figures indicate that fimbria destruction had little effect on the expression of septal rage.

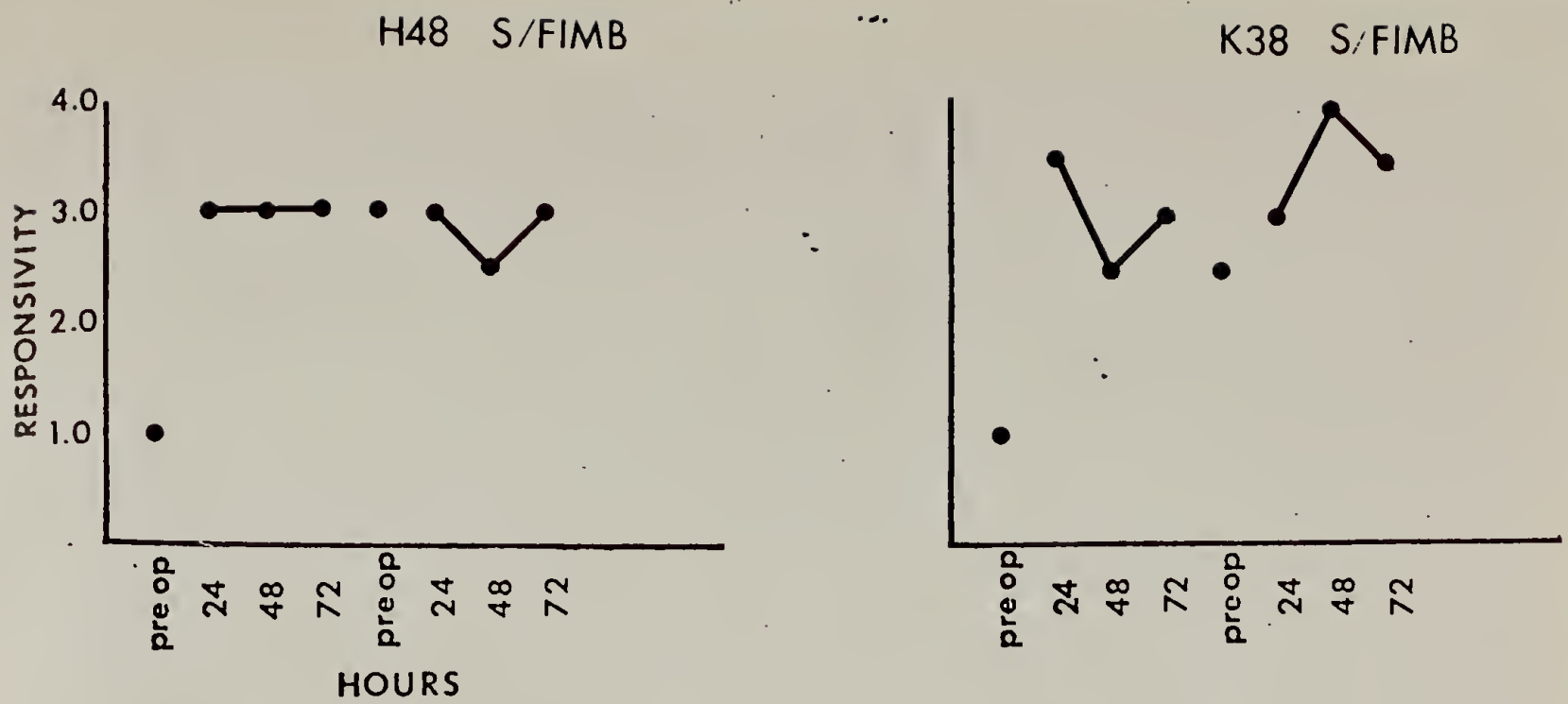


Figure 6. Subjects H48 and K38 received a fimbria lesion following a bilateral septal lesion. Two pre-operative reactivity ratings are indicated as dots. Fimbria destruction had little effect on the expression of septal hyper-reactivity.

Nonparametric statistics were used to evaluate the behavioral data since these tests do not make assumptions about the distribution of scores or the size of the intervals in the rating scale. A Sign Test (Siegel, 1956) compared the change in reactivity within each group after each surgical manipulation. The effectiveness of fimbria destruction in attenuating septal hyper-reactivity was evaluated in 5 S/FIMB animals; k19, k25, k26, H48 and K38. Median reactivity scores of the post-operative ratings after each lesion were compared for these 5 mice using the Sign Test data are presented in Table 1. This comparison yielded no significant difference between the median scores following septal lesions and those following fimbria destruction. Thus, results demonstrated that fimbria lesions were entirely ineffective in eliminating septal lesion-induced hyper-reactivity.

Because these results conflicted with the Olton and Gage finding that intact sept-hippocampal connections are necessary for the appearance of hyper-reactivity produced by septal lesions one animal, K24, received a fimbria lesion 3 days prior to septal destruction. The median reactivity score for this animal following fimbria destruction was 1.0. Although destruction of the fimbria was complete, as in all 6 mice in this experiment, this did not prevent the appearance of septal hyper-reactivity 4, 5 and 6 days later when the median reactivity score was 3.5.

TABLE 1

Subject no.	median reactivity score following septal lesion	median score following fimbria lesion	Sign
k19	3.5	3.0	+
K25	2.5	2.5	0
k26	4.0	3.0	+
H48	3.0	3.0	0
K38	3.0	3.5	-

N = 5 p = .5 (n.s.)

Lesion reconstruction for all 6 subjects are shown in Figures 7-12. In each case fimbria destruction was complete.

Discussion

Results from the 5 S/FIMB mice demonstrated that destruction of the fimbria following the expression of septal lesion-induced hyper-reactivity did not attenuate the syndrome in any measureable way. Furthermore, data from subject K24 indicated that a complete fimbria lesion does not prevent the appearance of hyper-reactivity 3 days later when septal surgery was performed. Thus, the conclusion to be drawn from this experiment was that septo-hippocampal connections through the fimbria-fornix play little role in the septal rage syndrome.

These results were in sharp contradiction to those of Olton and Gage (1974) and Gage and Olton (1975) who observed, in rats, that fimbria-fornix damage prevented the subsequent expression of septal lesion-induced hyper-reactivity 9 or 16 days later when the septal area was destroyed. Their conclusion that intact septo-hippocampal connections are critical for the expression of septal hyper-irritability was not substantiated by the present experiment in mice.

For the present, these contradictory results may be ascribed to species differences. There are other suggestions that mice and rats differ in the neural mechanisms responsible for the hyper-reactivity. In rats, the explosive reaction to tactile stimulation dissipates within two weeks, while in mice the hyper-reactivity induced by septal damage remains

K19 S/FIMB

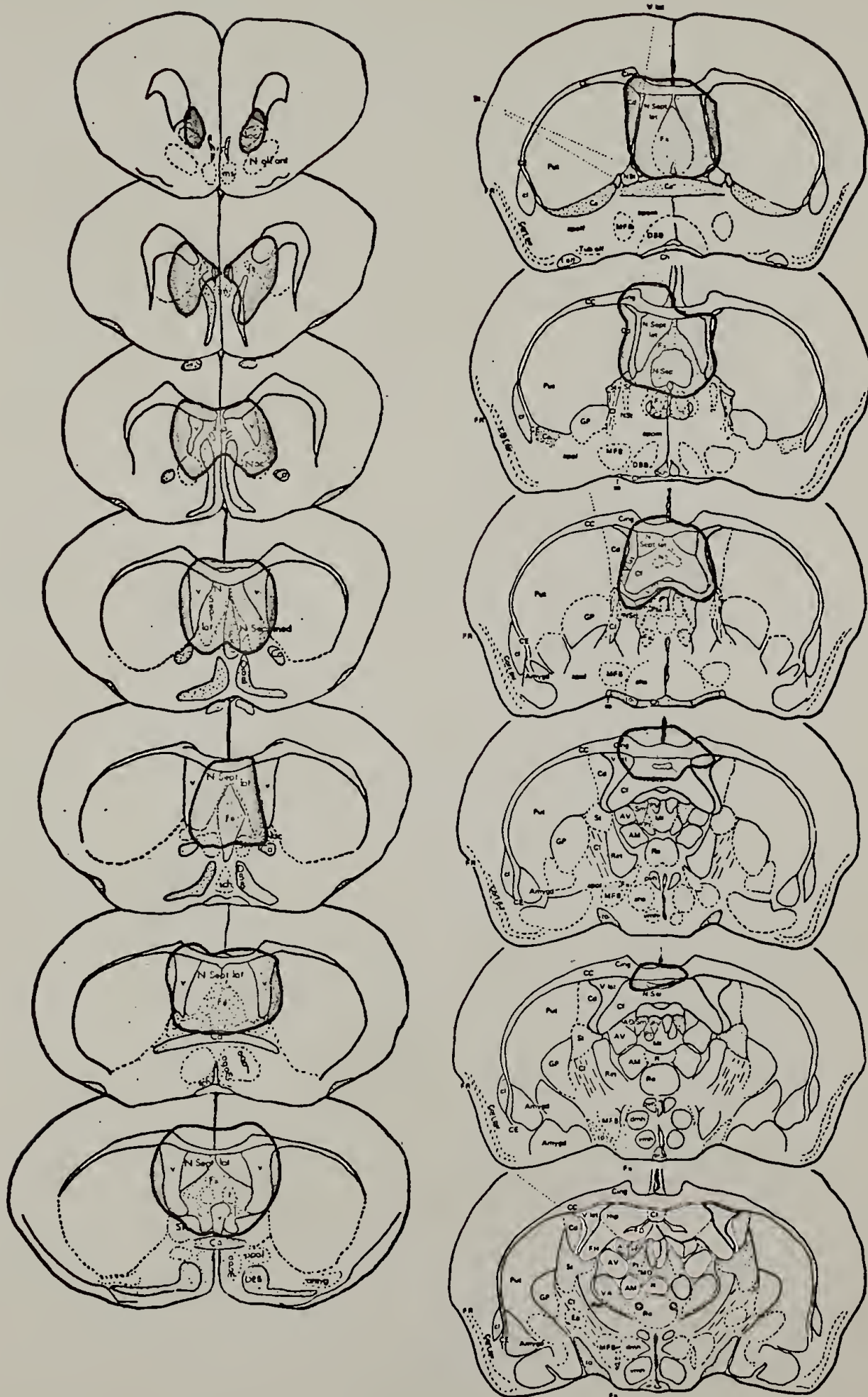


Figure 8

K24 S/FIMB

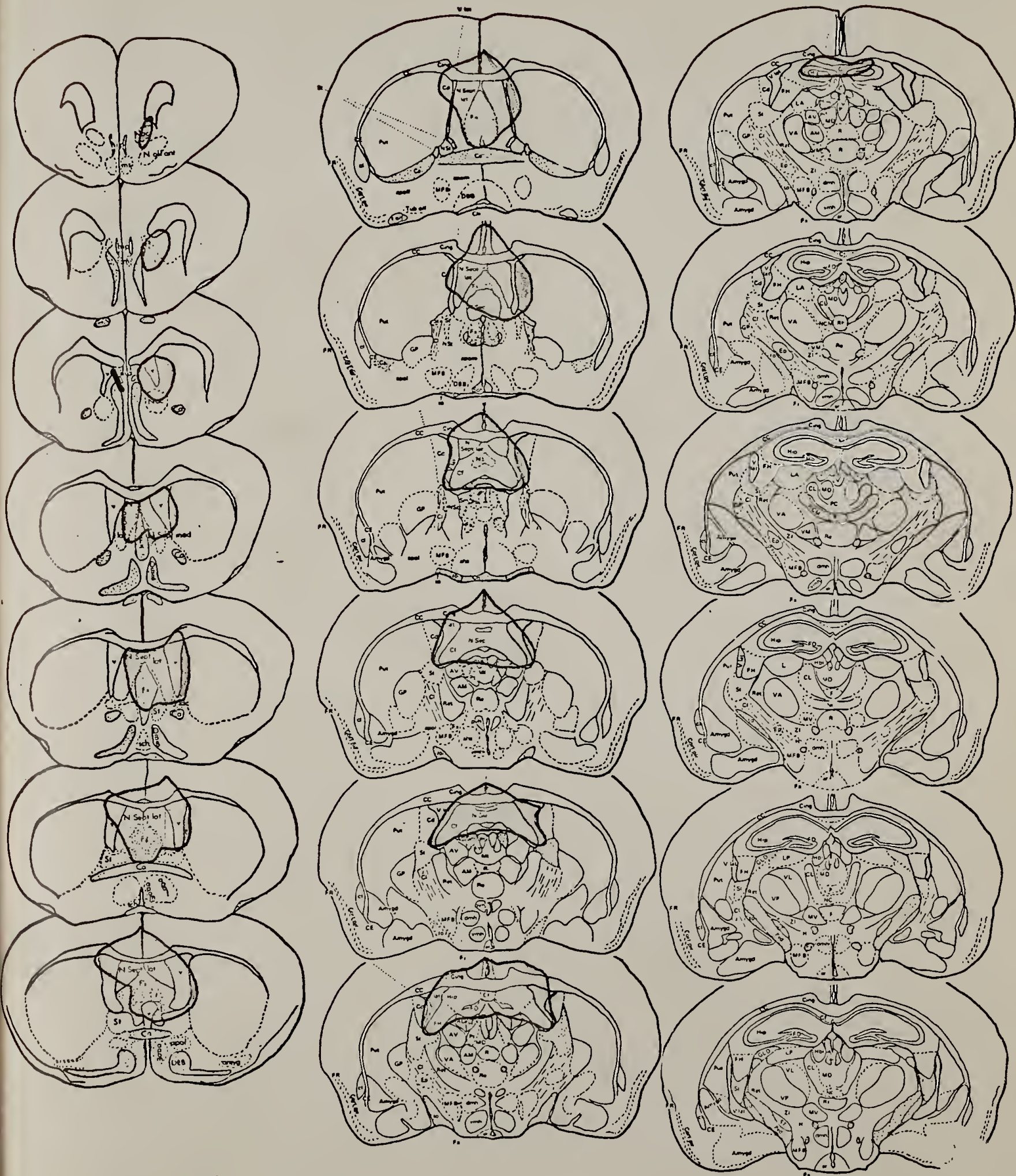
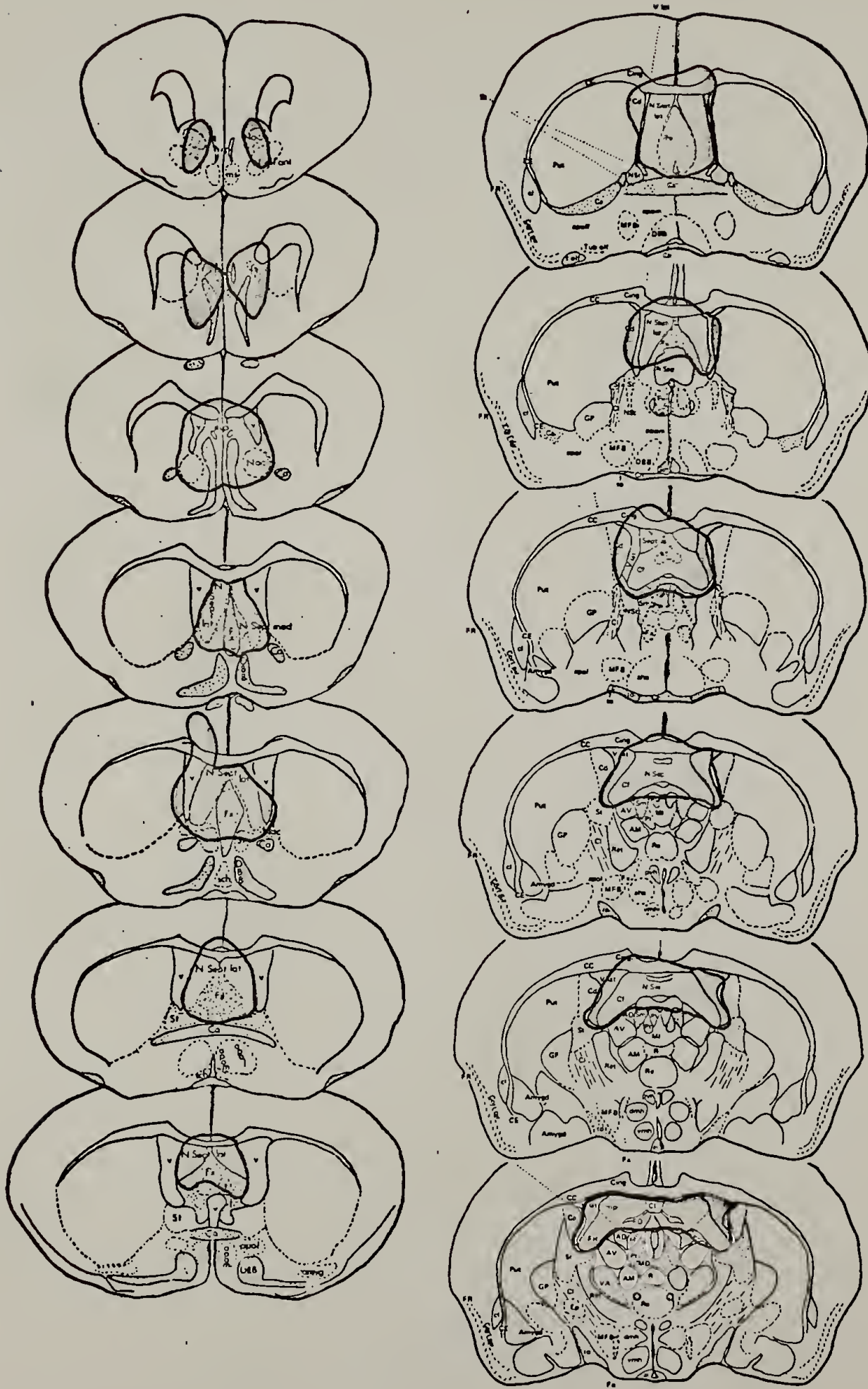


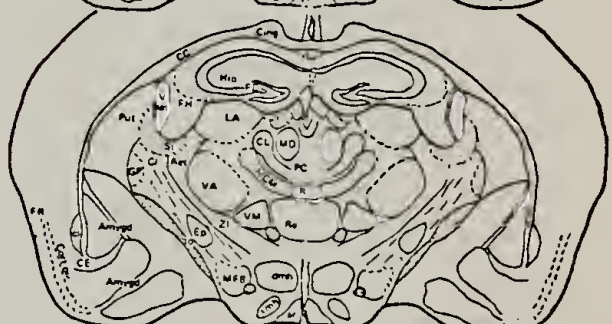
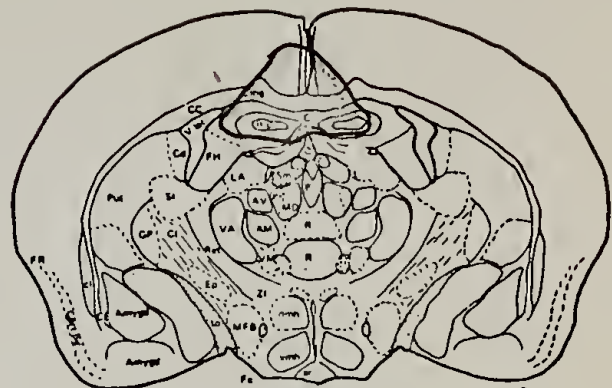
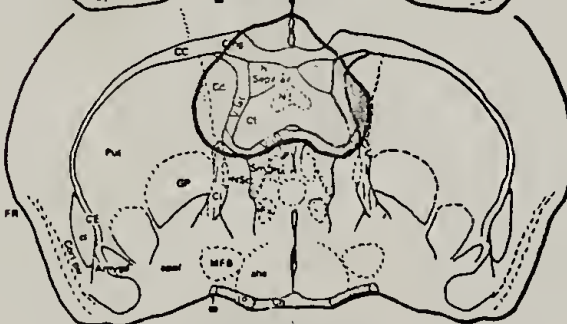
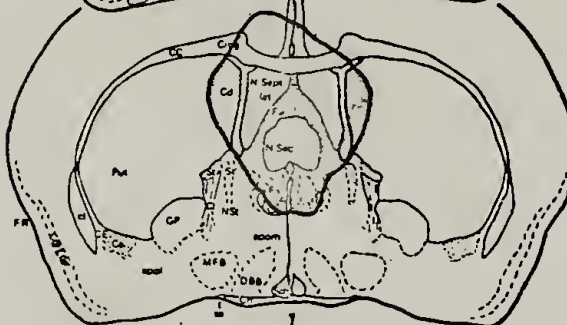
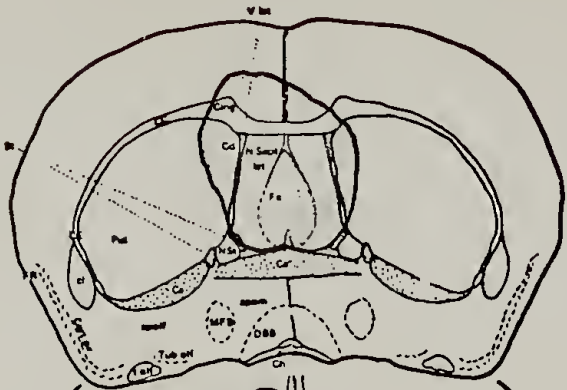
Figure 9

K25 S/FIMB



K26

S/FIMB



H48 S/FIMB

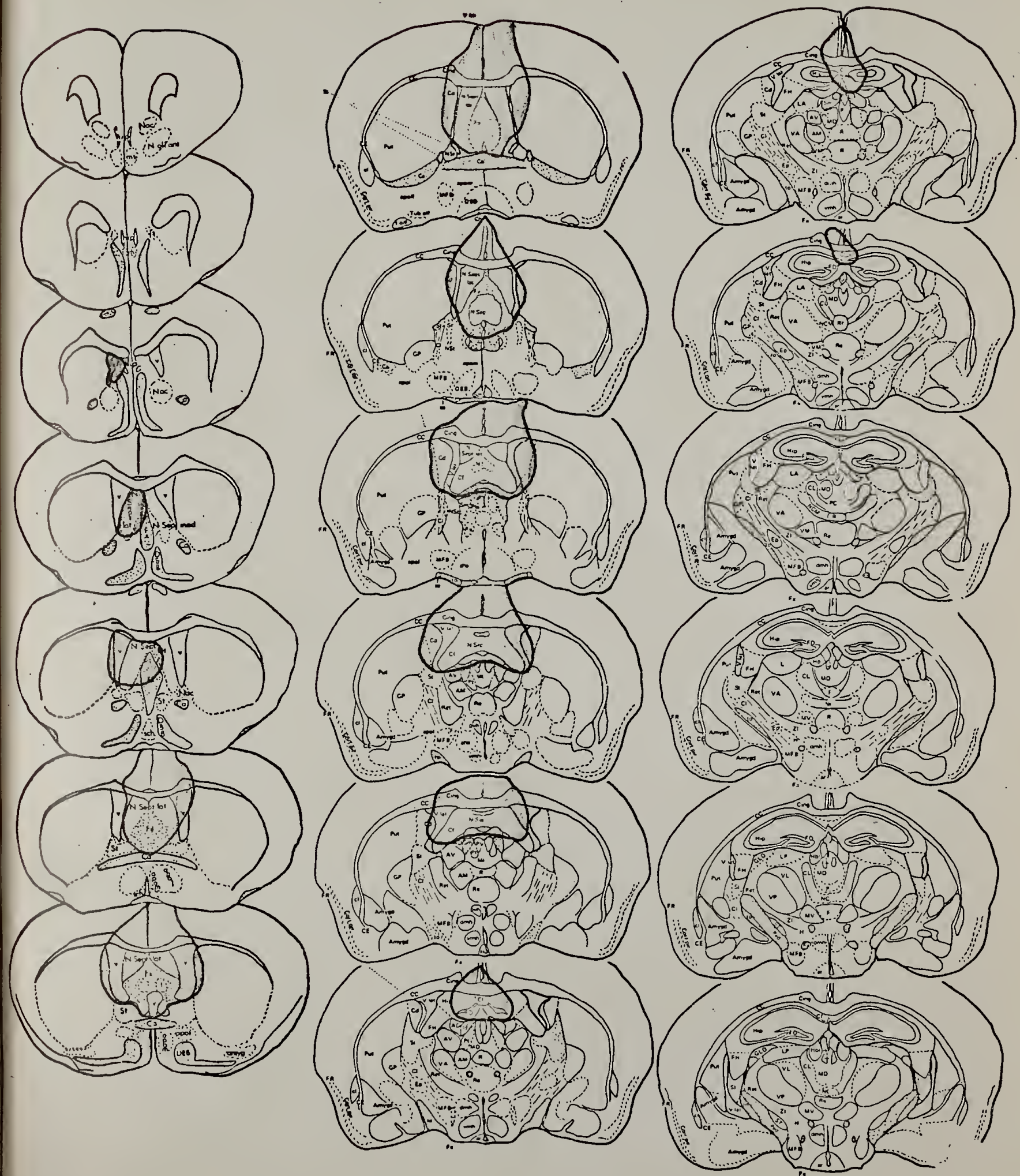
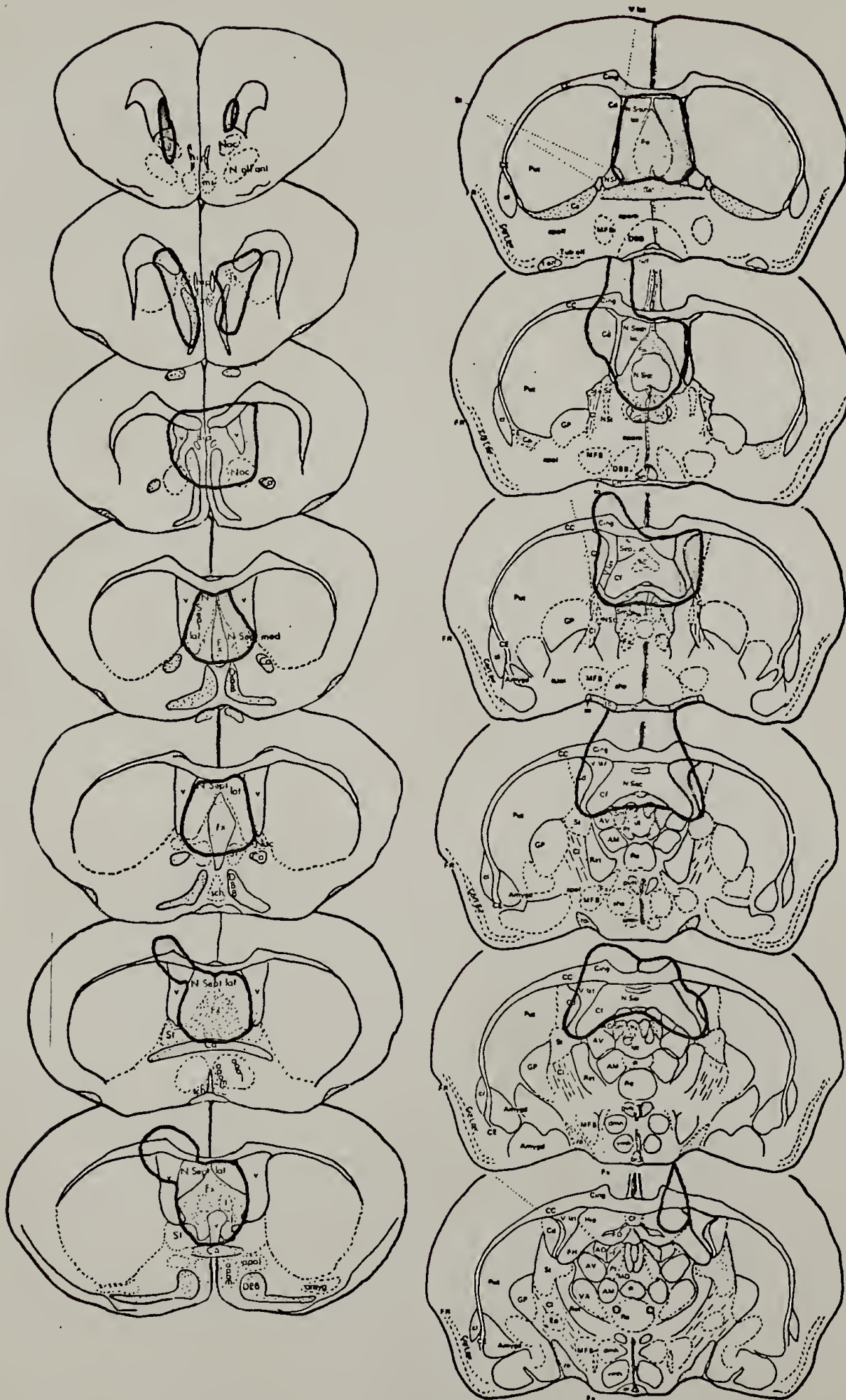


Figure 12

K38 S/FIMB



for as long as the animals are tested. Since, in rats, cortical lesions can prevent the disappearance of hyper-reactivity it is possible that cortical control over limbic structures is more elaborated in rats and cortical-hippocampal interrelationships play a more important role in the expression of septal hyper-reactivity.

EXPERIMENT 2

It has been reported (King and Meyer, 1958) that amygdala destruction in rats attenuates septal lesion-induced hyper-reactivity. Thus, the amygdala may be a structure directly involved in the mediation of a septal rodent's abnormal response to its environment. Given the apparent species differences between mice and rats shown in Experiment 1 it was of interest to determine whether amygdaloid lesions are effective in attenuating septal irritability in mice.

Method

Eight male mice of the same age, weight and breed as used in previous experiments served as subjects. Surgical, behavioral and histological procedures were identical to those used in Experiment 1. Briefly, the procedure was as follows: mice were rated for their reactivity to the air stream stimulus prior to septal surgery. Animals receiving a pre-operative score of over 1.0 were discarded. Mice then received a septal lesion bilaterally (coordinates

relative to bregma; anterior +1.7mm, lateral \pm .4 mm., ventral -3.5 mm). At 24, 48 and 72 hours following septal lesions each animal's reactivity was rated. On the third day bilateral lesions were aimed at the entire amygdaloid complex (coordinates relative to bregma; anterior -.5, -1.6, lateral \pm 3.3, \pm 3.3, ventral -5.2, -4.8). Thus, complete destruction of the amygdaloid complex required two cranial penetrations bilaterally. Just prior to this second operation a pre-operative rating was obtained. At 24, 48 and 72 hours after amygdala destruction animals received tactile reactivity ratings.

Following the last 72 hour test, mice were perfused and brains removed according to previous procedures. Lesion location evaluation methods were identical to those of Experiment 1.

Results

Behavioral

Figures 13 and 14 present reactivity ratings over the six experimental days for each subject. S/A indicates that a septal lesion preceded the amygdala lesion by three days. In each animal's case, bilateral amygdaloid destruction reduced or eliminated the hyper-reactivity produced by the previous septal lesion.

A Sign Test evaluated the difference between median post-operative scores following septal lesions and median post-operative scores after amygdaloid lesions. The ability

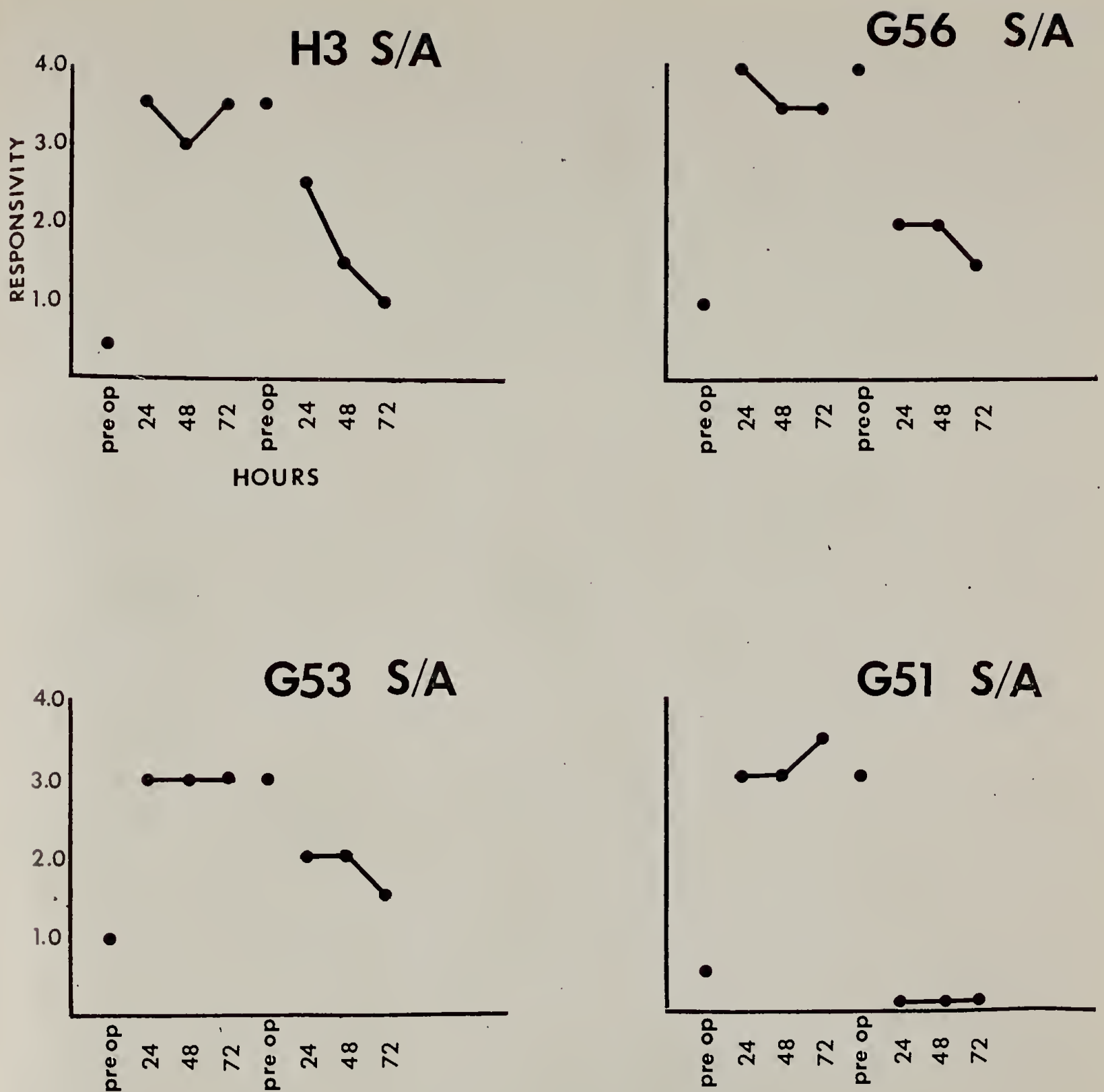


Figure 13. The effects of bilateral amygdala lesions on septal lesion-induced hyper-reactivity. The abbreviation S/A indicates that septal lesions preceded amygdala lesions. Pre-operative ratings preceded each operation and are shown as dots. In each subject, H3, G53, and G51, amygdala destruction significantly reduced the hyper-reactivity produced by septal lesions.

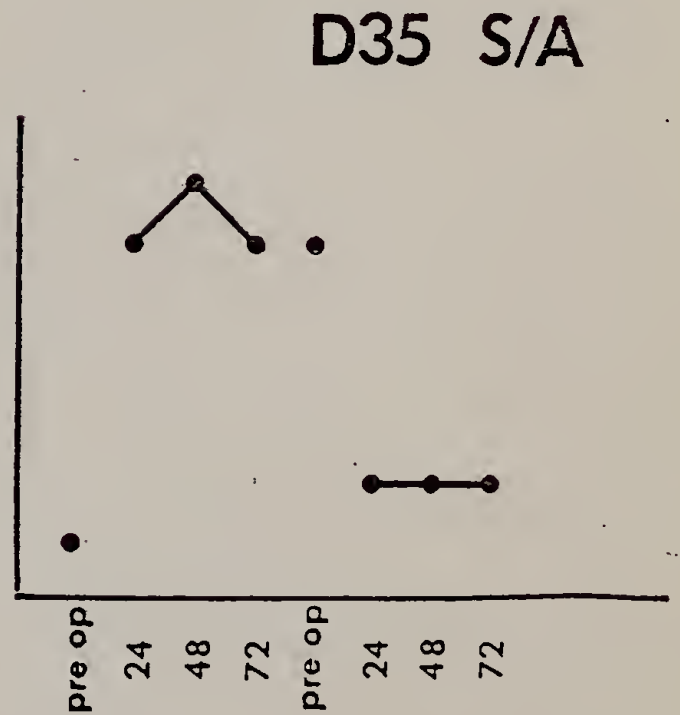
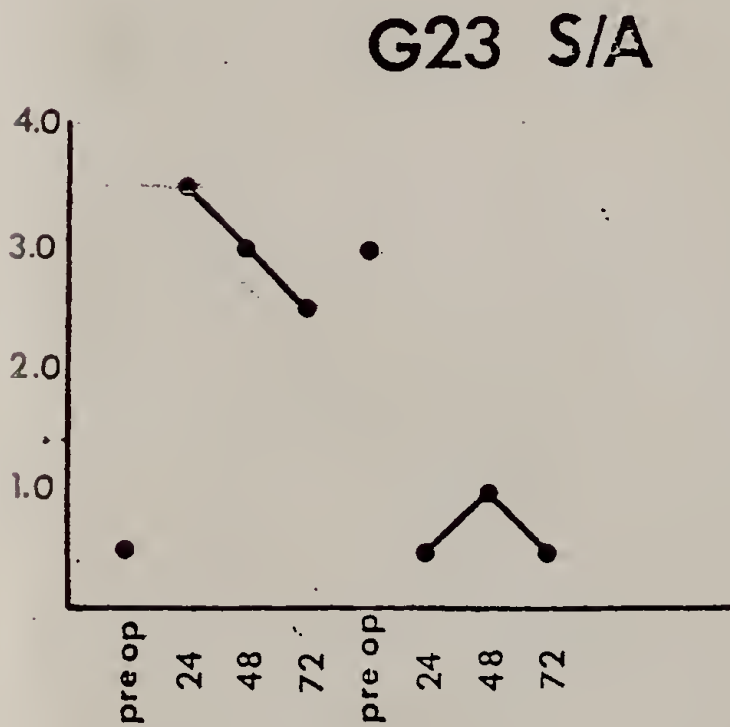
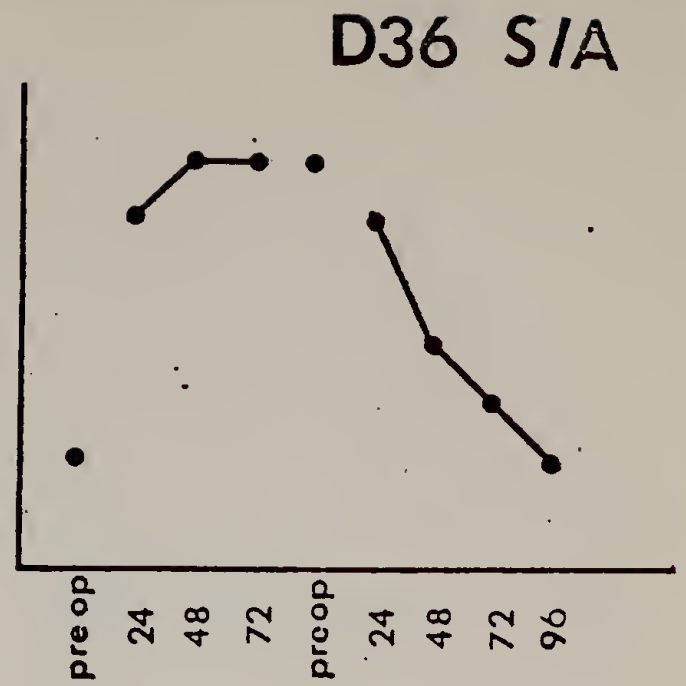
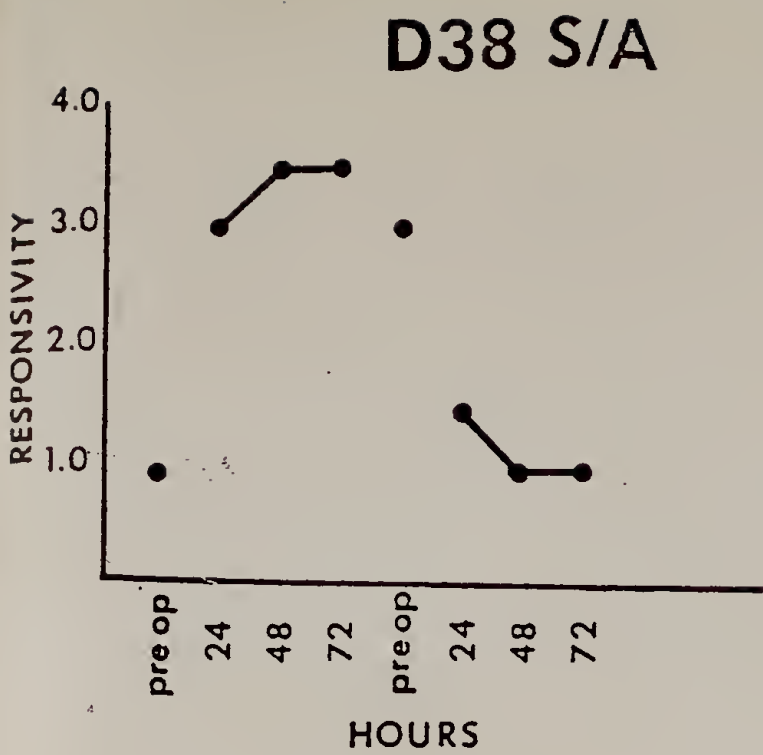


Figure 14. Subjects D38, D36, G23 and D35 received amygdala lesions 3 days after septal lesioning. In each case, destruction of the amygdala reduced septal lesion-induced hyper-reactivity.

of amygdala lesions to reduce septal lesion-induced hyper-reactivity was significant at the .004 level. Table 2 shows that, in every animal, amygdala lesions reduced irritability to normal or near-normal levels. It is important to point out that the effectiveness of amygdala destruction in this regard was not due to motor or feeding impairments or a general malaise in the mice. Animal's weights were recorded each day and no significant drops in weight were observed in any of the eight subjects. Weight loss, because of the high metabolic rate in mice, is an excellent measure of malaise or aphagia.

Histological

In most instances, amygdaloid destruction involved unrelated structures such as the claustrum, external capsule and pyriform cortex. Generally, lesions which were effective in reducing septal hyper-reactivity tended to involve the basolateral division of the amygdala. Lesion reconstructions are shown for each subject in Figures 15-22. Reduction of hyper-reactivity was not dependent on destruction of all of the amygdala bilaterally or even all of the basolateral division. Lesion reconstructions for subjects H3, G23 and D38 exemplify this point. The lesions of G23 suggest that pyriform cortex may be intimately involved in the reduction of hyper-irritability. Relatively little damage was observed to the left amygdaloid complex itself. Rather, damage involved pyriform cortex lateral to the basolateral amygdala

TABLE 2

Subject n.	median reactivity following septal lesions	median reactivity following amygdala lesions	Signs
H3	3.5	1.5	+
G56	3.5	2.0	+
G53	3.0	2.0	+
G51	3.0	0	+
D38	3.5	1.0	+
D36	3.0	1.75	+
G23	3.0	.5	+
D35	3.0	1.0	+

N = 8, $p < .004$

Figure 15

H3 S/A

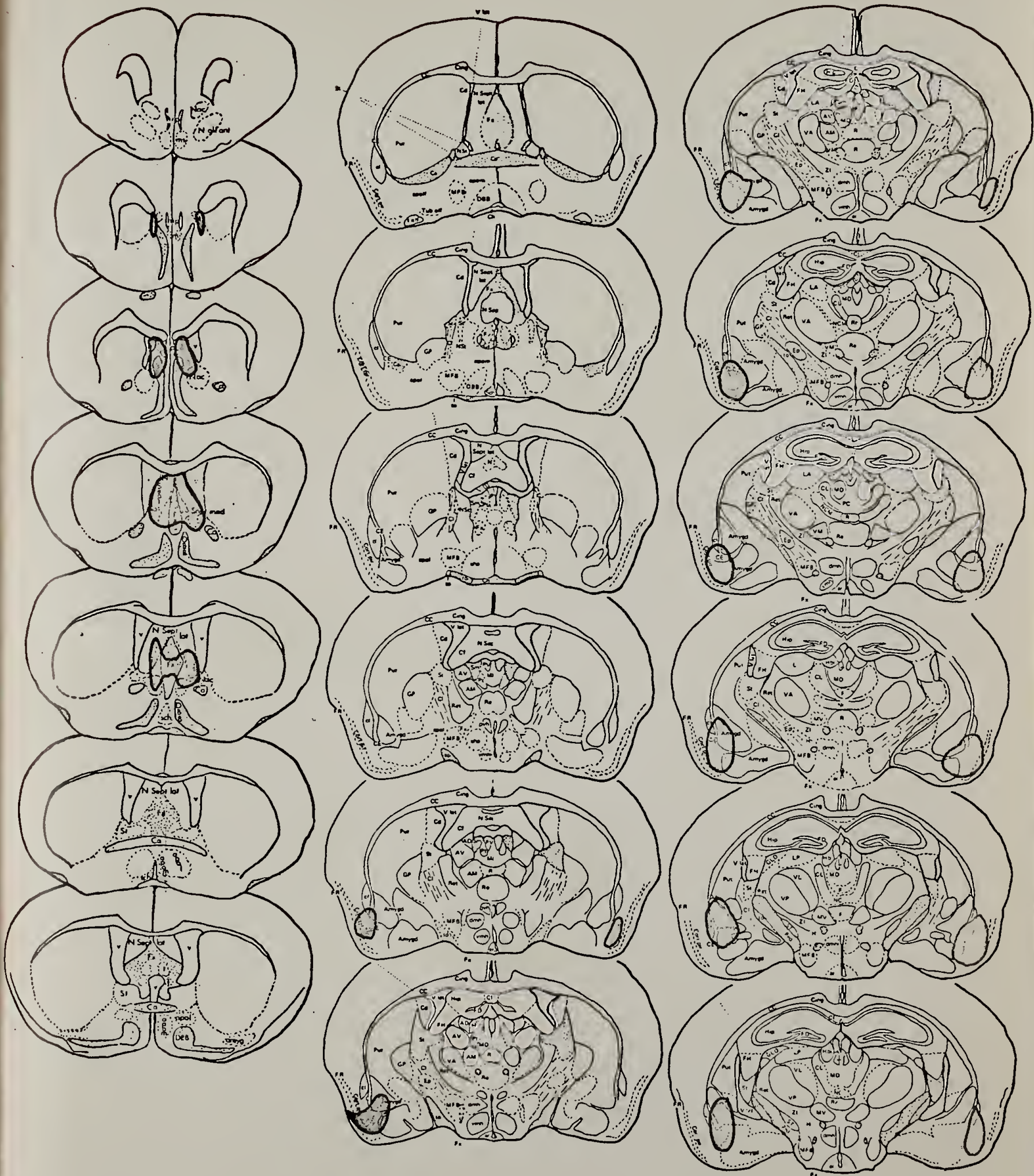


Figure 16

G56 S/A

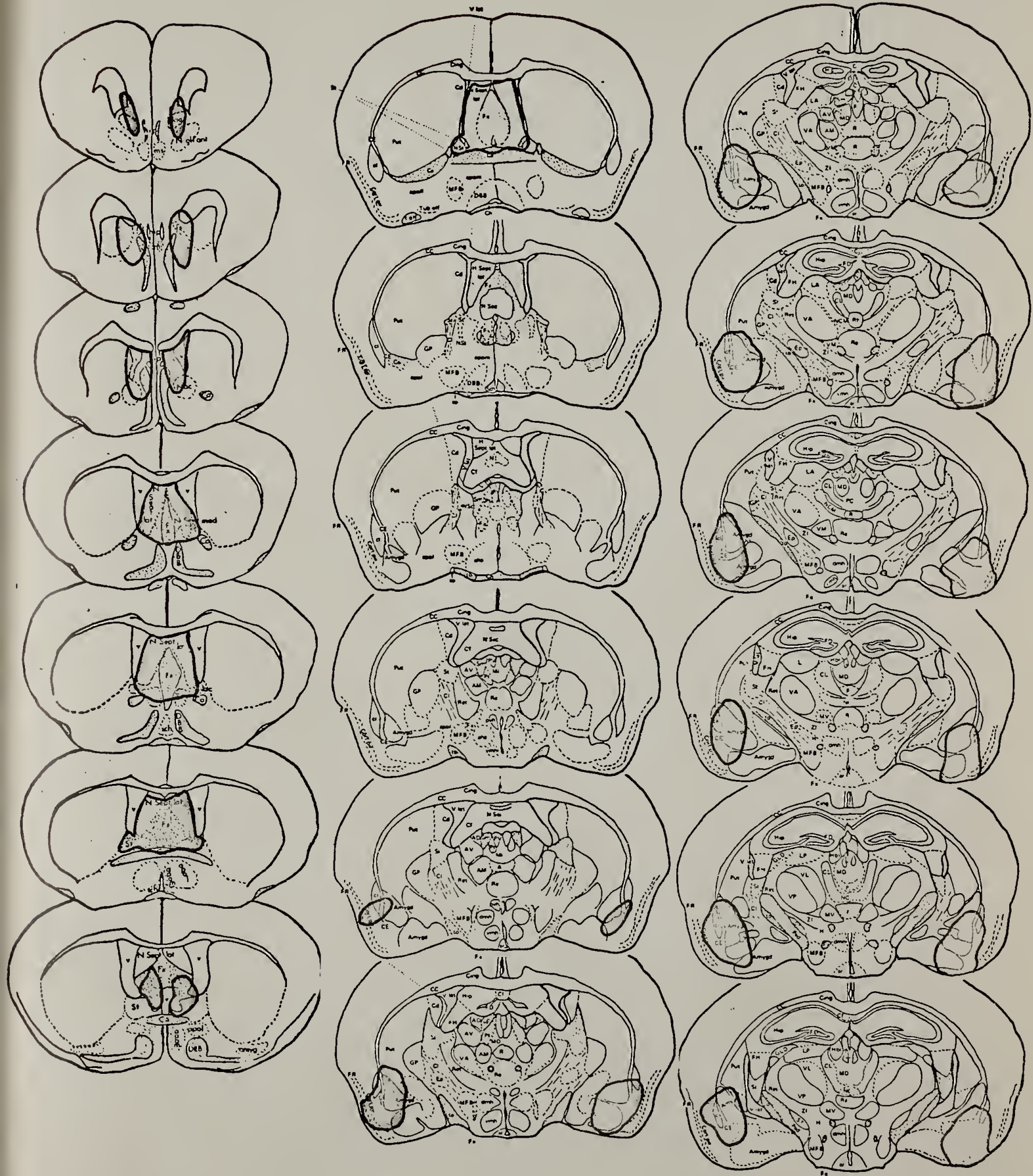
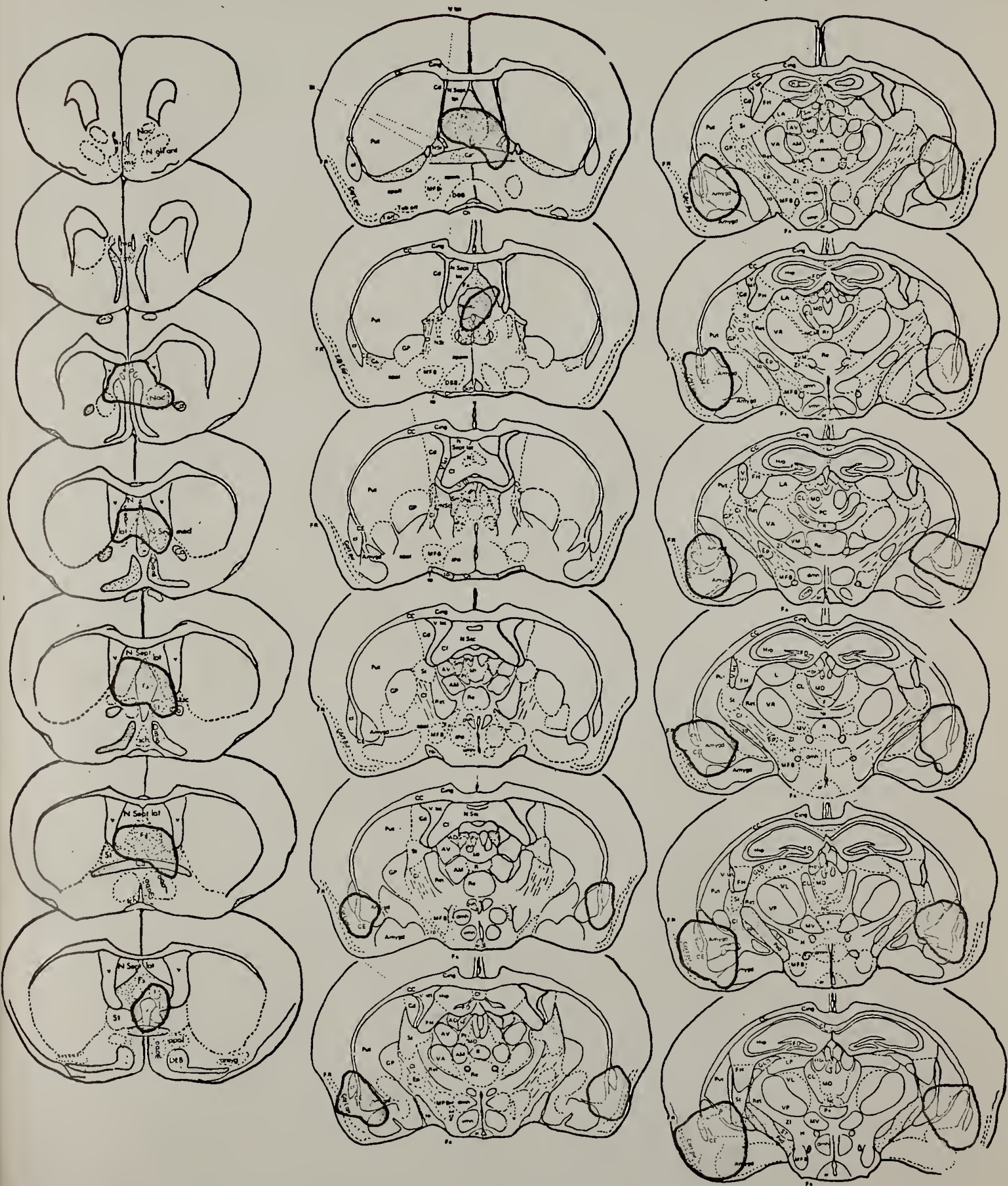
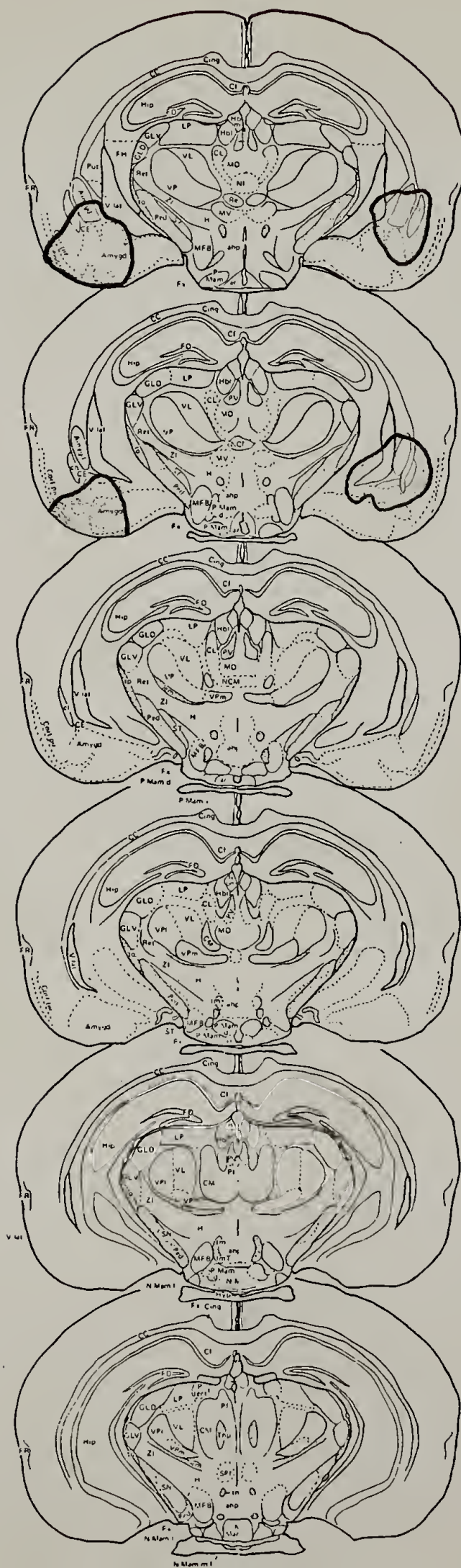


Figure 17

G53 S/A



G53 S/A



G51 S/A

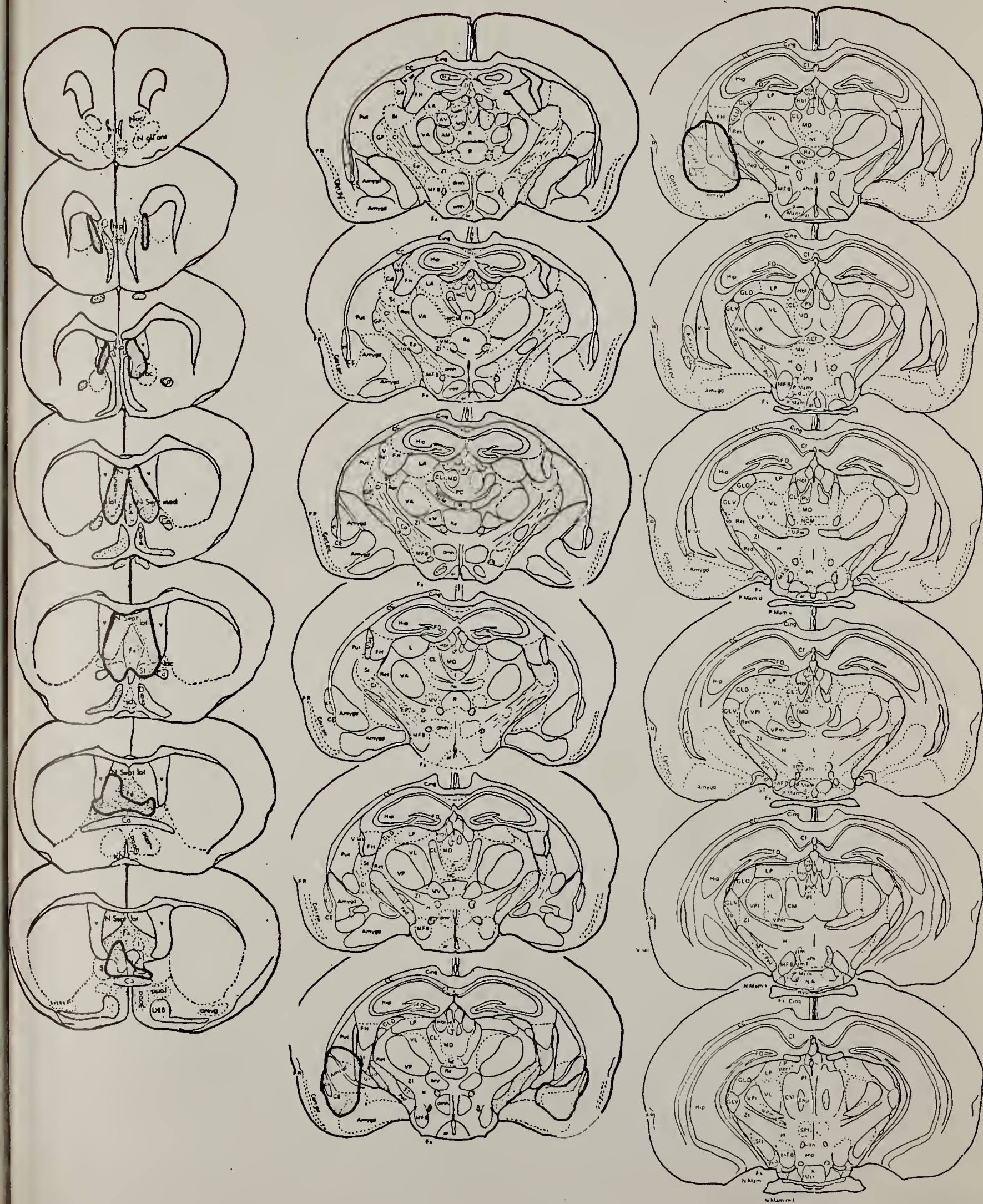
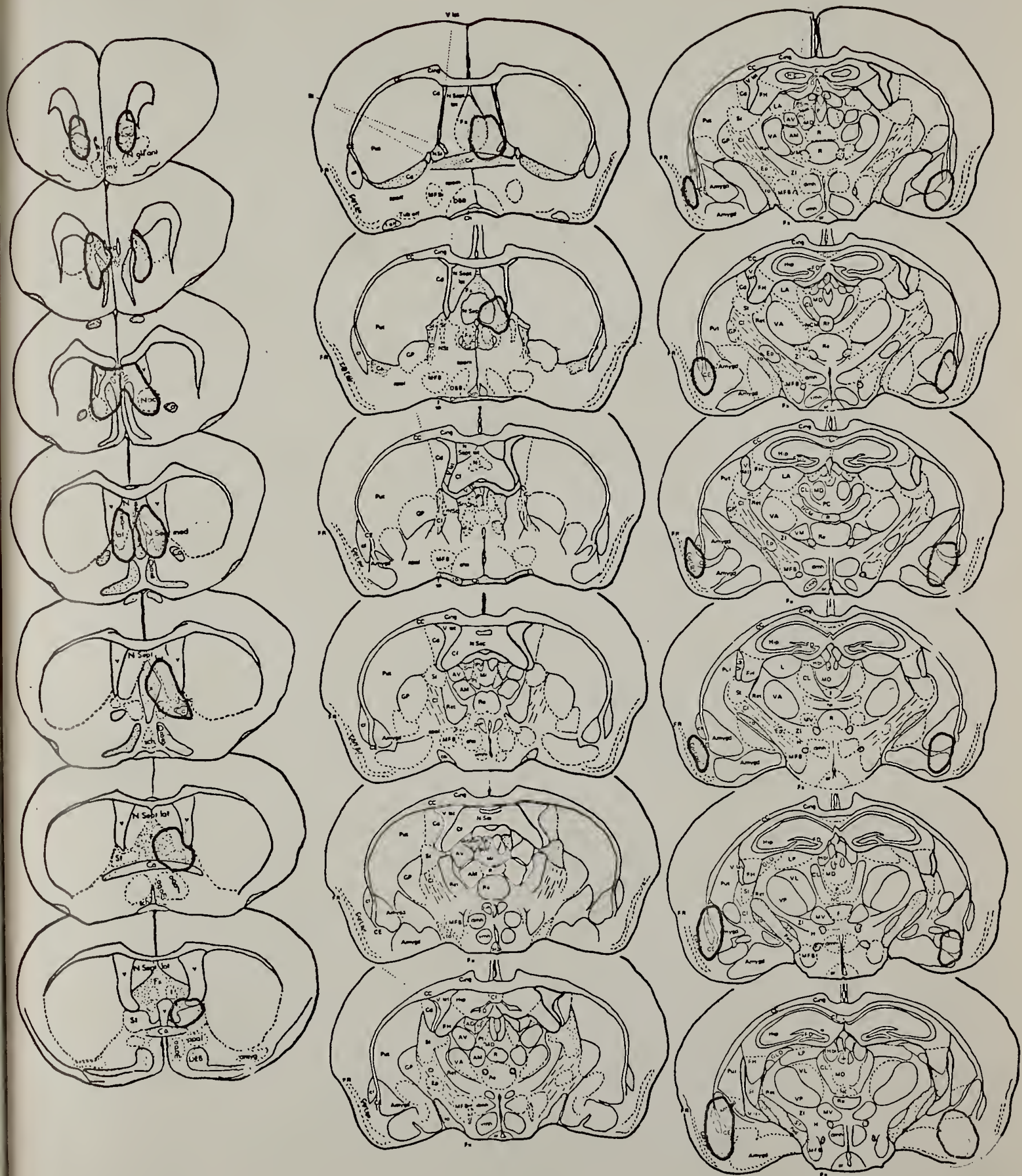


Figure 19
D38 S/A



D38 S/A

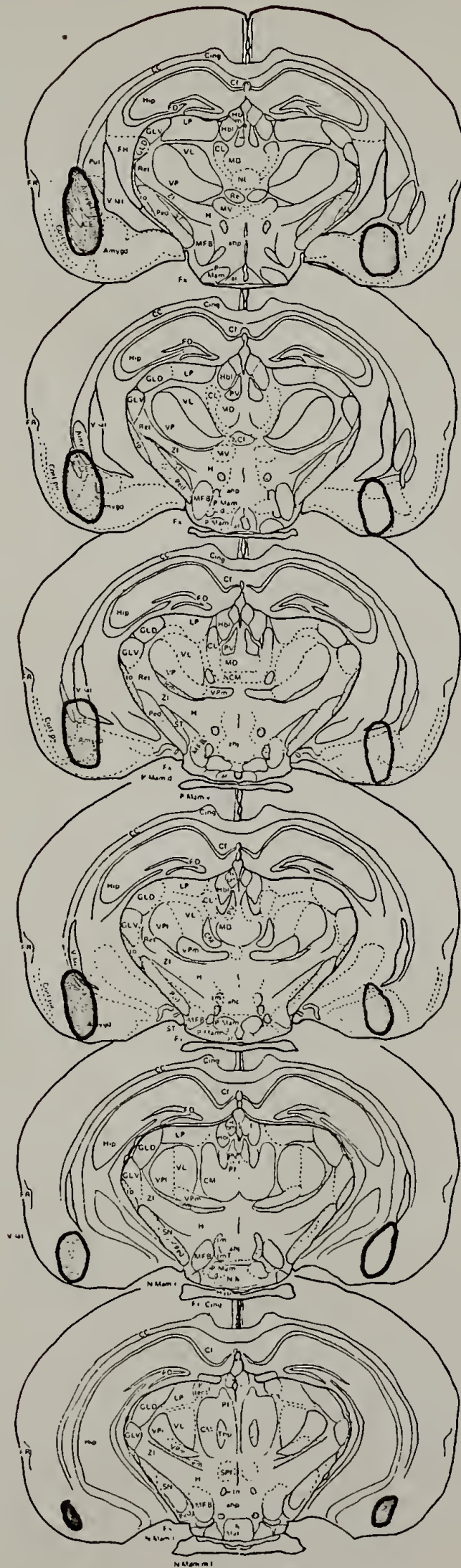


Figure 20
D36 S/A

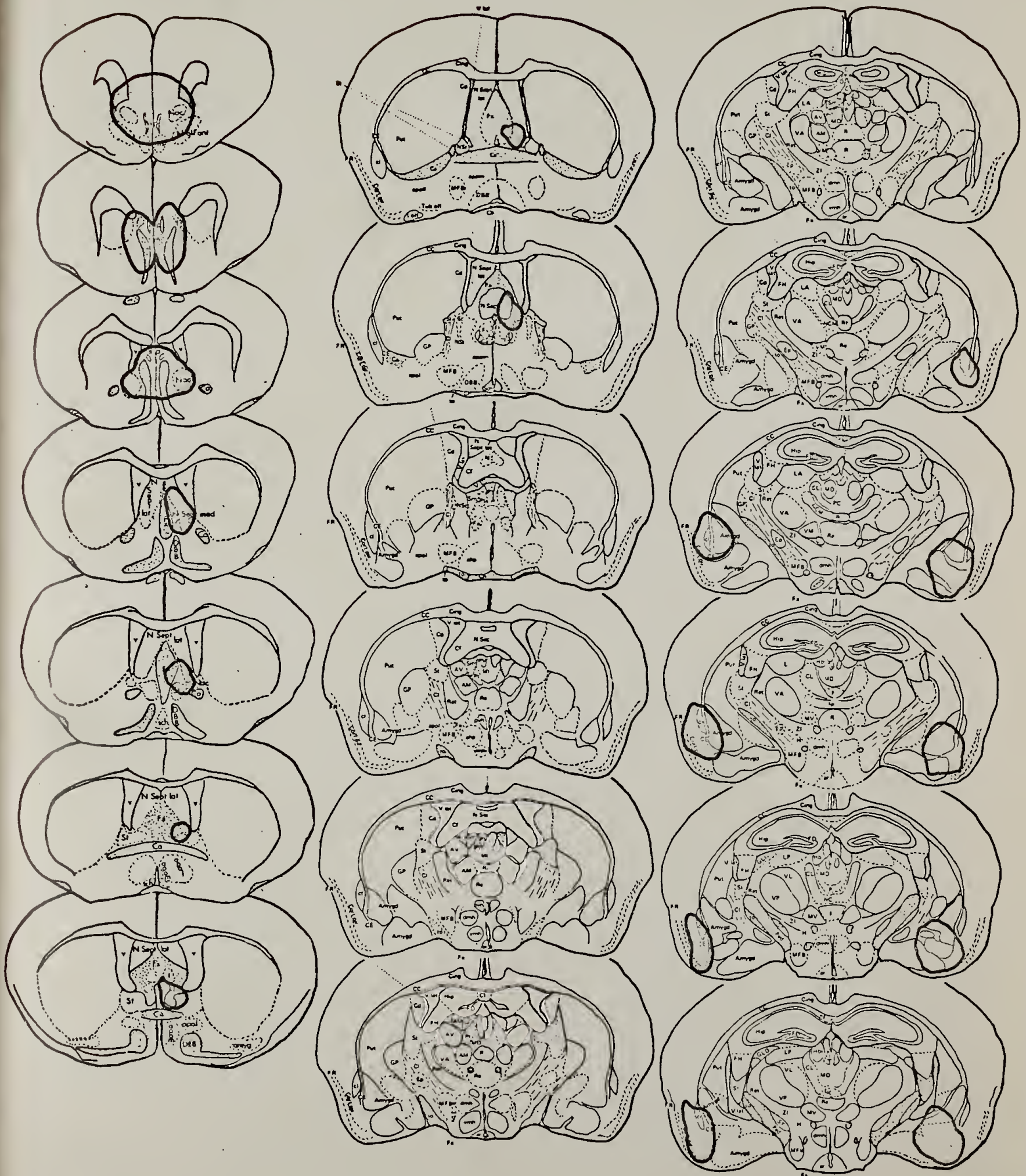


Figure 20 cont'

D36 S/A

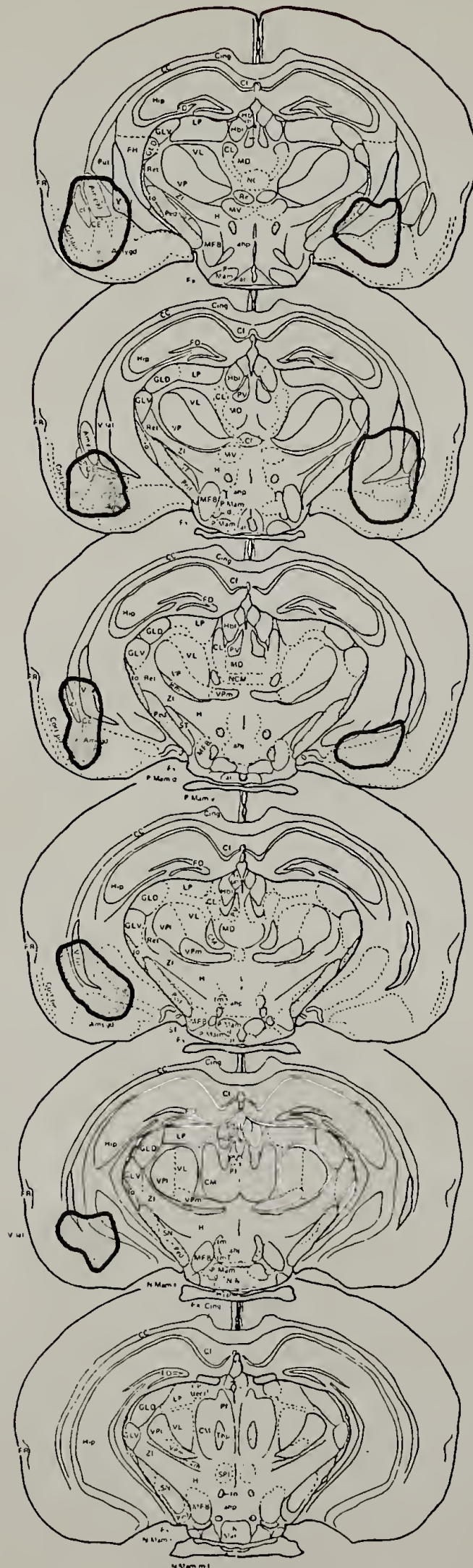


Figure 21

G23 S/A

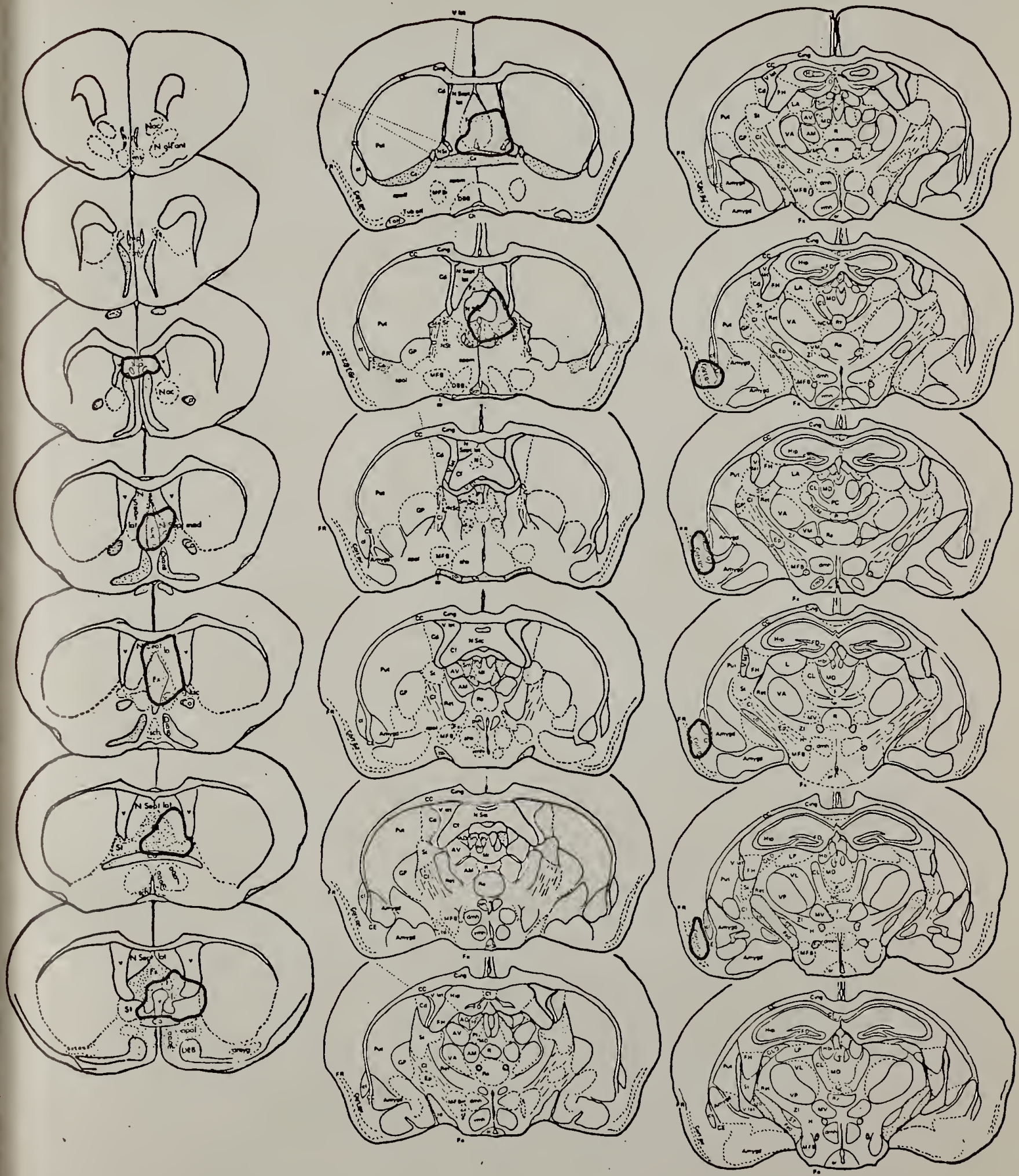


Figure 21 cont'
G23 S/A



D35 S/A

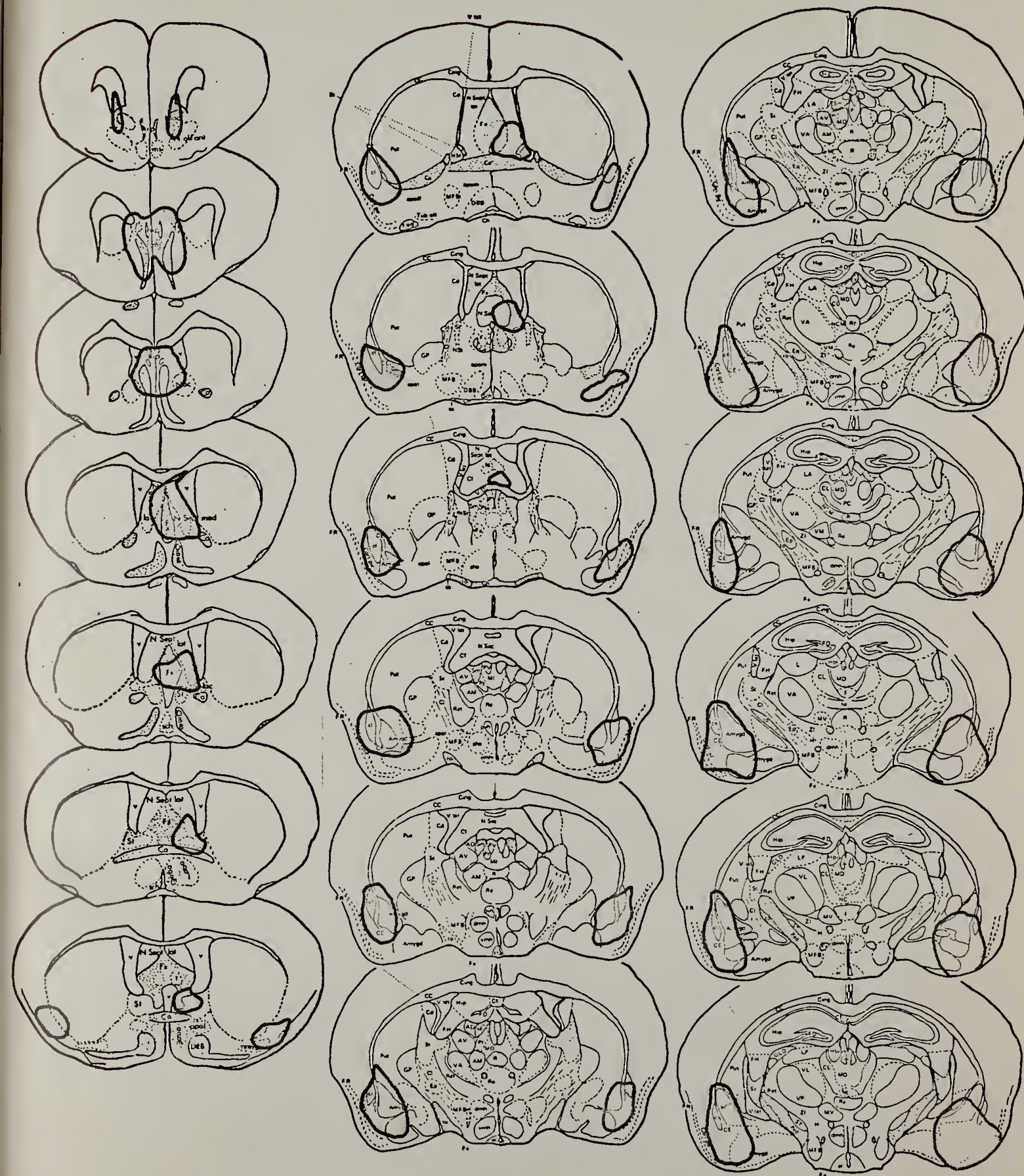


Figure 22 cont'

D35 S/A



and left relatively undamaged the lateral amygdala.

The results of this experiment demonstrated that destruction to the basolateral division of the amygdaloid complex or the adjacent pyriform cortex was highly effective in attenuating septal-lesion induced hyper-reactivity. This situation in mice is paralleled by the effectiveness of amygdala lesions in rats with regard to septal rage.

Discussion

Since amygdala lesions were extremely effective in reducing septal hyper-reactivity a brief review of the neuroanatomy of the amygdaloid complex is in order.

The two major subdivisions of the amygdaloid complex are the corticomedial division and the basolateral division. The corticomedial division is generally thought to be comprised of the cortical, medial and central nuclei as well as the nucleus of the olfactory tract. The basolateral division is made up of the lateral, basal and accessory basal nuclei. The corticomedial complex is found more medial and is closely related to fibers from rostral olfactory structures carried by the lateral olfactory tract. The basolateral nuclei, on the other hand, are thought to have strong interconnections with neocortex (Isaacson, 1974).

Two main efferent systems arise from the amygdaloid complex; stria terminalis and the ventral amygdalofugal pathways. Stria terminalis originates predominantly from the corticomedial division of the amygdala, although the

basolateral nuclei also contribute some fibers. The ventral amygdalofugal pathways to the hypothalamus are believed to arise from both nuclear divisions (Isaacson, 1974), but many fibers in this pathway arise from periamygdaloid cortex rather than from the amygdala itself. This appears to be true of many species, including the rat.

According to DeOlmos (1972) there are three components to the stria terminalis; dorsal, ventral and commissural. The dorsal component sends fibers to the bed nucleus of the stria terminalis, the basal part of the lateral septal nucleus, the posterior-medial part of the nucleus accumbens septi, the olfactory tubercle, the anterior olfactory nucleus, the granular layer of the accessory bulb, the medial preoptic area, the area surrounding the ventral medial nucleus of the hypothalamus and an area just anterior to the mammillary bodies. The ventral component distributes its fibers to the bed nucleus of stria terminalis, the junction between the preoptic area and the hypothalamus, the central core of the ventromedial nucleus of hypothalamus and the premammillary area. The commissural component connects the amygdalae of the two hemispheres through the anterior commissure.

According to Isaacson the distribution of fibers originating in the amygdala and reaching forebrain sites through the ventral amygdalofugal pathway have been difficult to establish owing to the difficulty in producing small restricted amygdalar lesions. DeOlmos (1972) reports, however, that

short fibers of the ventral system project from to the claustrum and anterior olfactory nucleus. Fibers also interconnect the several nuclear groups and others extend toward the pyriform and entorhinal cortex. Fibers from the basolateral amygdala and olfactory tubercle project through the diagonal band into the ventral septum. Medial septum also projects fibers to the basolateral amygdala via the diagonal band. Fibers originating in the olfactory bulb project via the lateral olfactory tract to terminate in the corticomедial nuclear group. The basolateral amygdaloid nuclei receive an indirect olfactory input via relays in the prepyriform cortex (Carpenter, 1972). Thus, nearly all parts of the amygdaloid complex receive either direct or indirect olfactory fibers.

The fact that lesions which were effective in reducing septal hyper-reactivity involved the basolateral division of the amygdaloid complex suggested that the hyper-reactivity may involve neocortical connections. This was hypothesized since the corticomедial amygdaloid nuclei receive heavy input from olfactory structures while the basolateral nuclei receive fibers from the medial septum via the diagonal band. Isaacson (1974) states that the basolateral nuclei has fiber connections with neocortex. Unfortunately he does not specify which areas of cortex are involved.

Because of the effectiveness of amygdaloid destruction in reducing septal rage it would be of great interest to

ascertain whether the critical circuits involved in the expression of hyper-reactivity are direct fiber connections between the septum and the amygdala. Experiments 3 and 4 investigated the importance of the two main efferent pathways from the amygdala - the ventral-amygdalofugal pathway via the diagonal band and stria terminalis.

EXPERIMENT 3

Experiment 2 clearly demonstrated that hyper-reactivity induced by septal lesions was reduced by subsequent bilateral amygdaloid destruction. Furthermore, histological evaluations suggested that the basolateral division of the amygdaloid complex may be more intimately involved in the expression of hyper-reactivity than the corticomедial area. The efficacy of amygdala lesions in suppressing septal irritability argued that this brain area may be directly responsible for the expression of a septal rodents abnormal reaction to tactile stimulation. Two possibilities exist 1) destruction of the relevant septal area may release from inhibition an area of the amygdaloid complex which plays a direct role in the rage reaction or 2) amygdala efferents to the remaining intact portions of the septum may be more important in mediating the hyper-reactivity. The first question to be answered is: are the relevant septo-amygdala circuits direct fiber connections? This question may be readily answered by attempting to replicate the amygdaloid lesion's effects on

irritability with destruction of the two major fiber systems which interconnect septum and amygdala; stria terminalis and the diagonal band.

The stria terminalis arises predominantly from the corticomedial division of the amygdala and projects to the bed nucleus of stria terminalis, lateral septal area, nucleus accumbens septi, olfactory tubercle, anterior olfactory nucleus, medial preoptic area and the hypothalamus. There is some indication that fibers between the septum and amygdala course in both directions through the stria.

The purpose of the following experiment was to test the ability of stria terminalis lesions to suppress septal lesion-induced hyper-reactivity as amygdaloid lesions do. Stria terminalis lesions were performed bilaterally three days after septal destruction and the lesion's effects on the expression of septal hyper-irritability were observed.

Method

The surgical, behavioral and histological procedures followed in this experiment were identical to those used in the previous two experiments. Briefly, the procedure was as follows: eleven male mice who scored below 1.0 on the first pre-operative reactivity test received septal lesions bilaterally. At 24, 48 and 72 hours each subject's response to the air stream stimulus was determined. During the third experimental day these animals received bilateral stria terminalis lesions (coordinated relative to bregma; posterior

-.8 mm., lateral \pm 2.0 mm., ventral -3.0 mm). At 24, 48 and 72 hours following stria terminalis lesions reactivity tests were administered. Following the 72 hour test mice were perfused, brains removed and histological material evaluated.

Results

Behavioral

Figures 23, 24 and 25 present reactivity scores for each animal over the six experimental days. S/ST indicates that septal lesions preceded stria terminalis lesions by 3 days. Examination of these figures revealed an inconsistent effect of ST lesions on septal lesion-induced hyper-reactivity. In some cases, bilateral destruction of these fiber bundles interconnecting septum and amygdala had no effect on septal hyper-reactivity. In others, this second operation reduced septal hyper-reactivity and in one case, K52, ST lesions increased reactivity. A Sign Test comparing median reactivity scores after septal destruction with those following ST lesions over all 11 subjects revealed a non-significant effect of ST lesions to attenuate septal hyper-reactivity. Table 3 presents individual median reactivity scores after each of the surgical manipulations and the sign assigned to each subject.

Histological

Figures 26-36 indicate the location and extent of lesions produced in each animal. Stria terminalis lesions

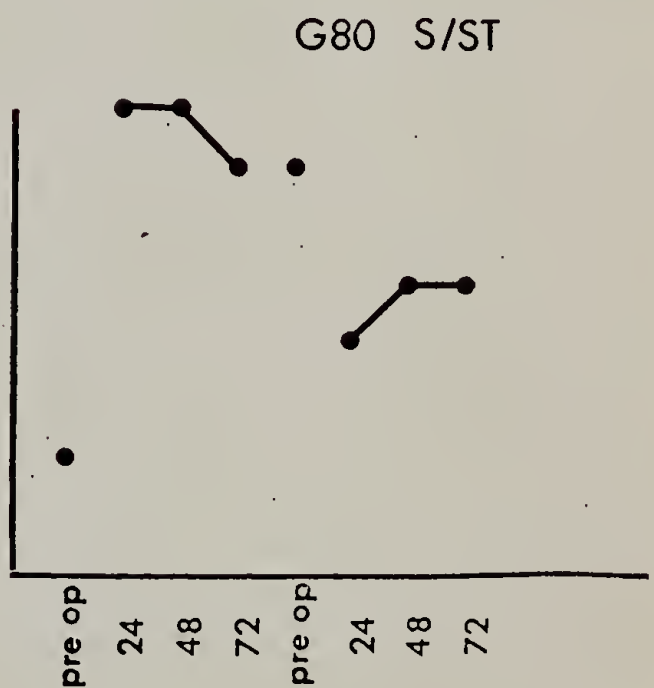
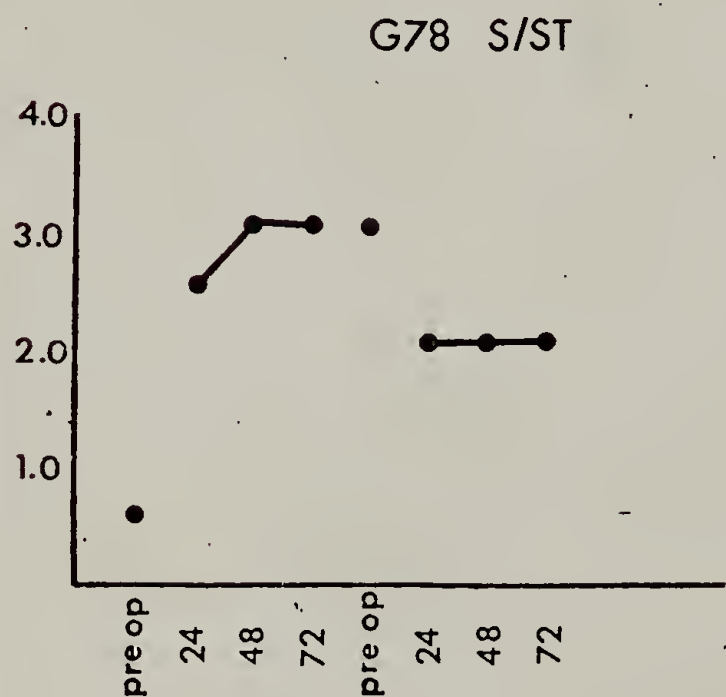
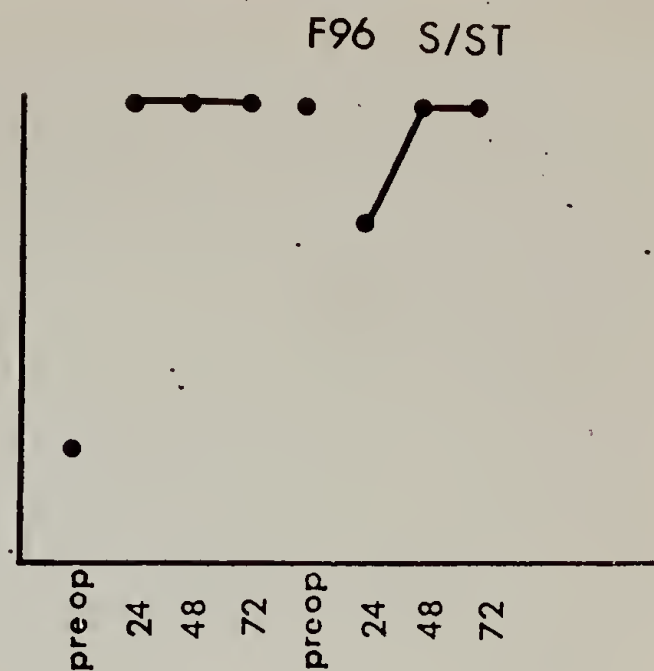
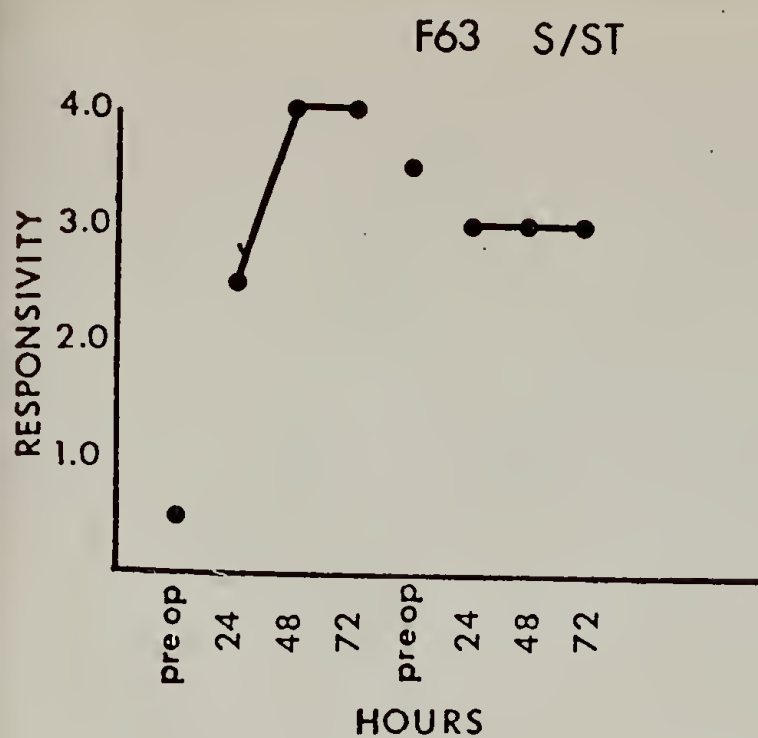
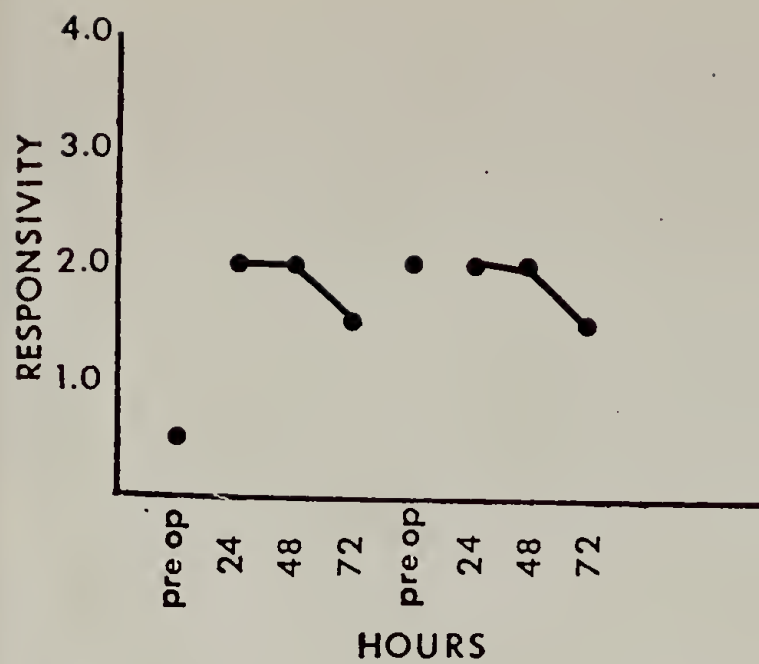
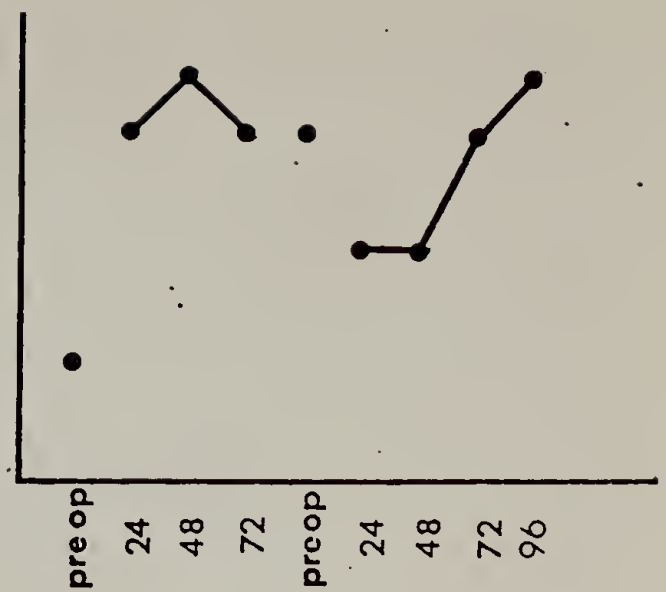


Figure 23. Subjects F63, G78, F96 and G80 received bilateral lesions of stria terminalis 3 days following septal destruction. Pre-operative reactivity ratings were administered before each operation and are indicated as dots.

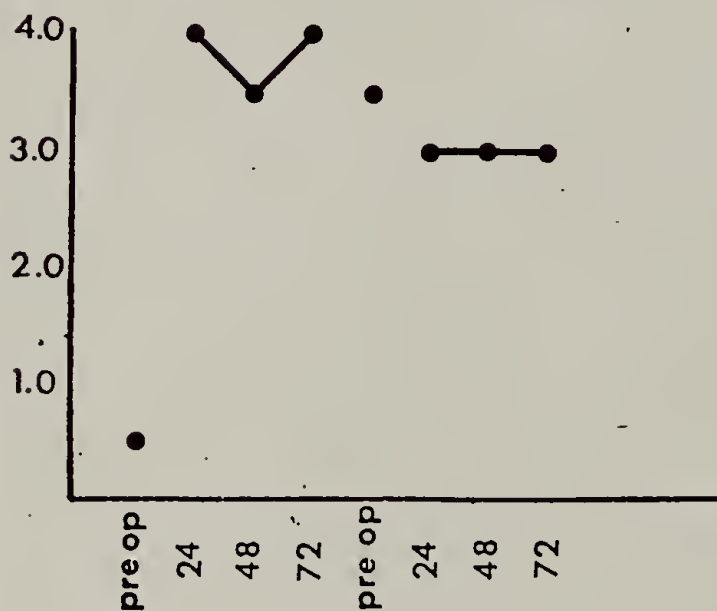
G83 S/ST



J80 S/ST



K18 S/ST



K39 S/ST

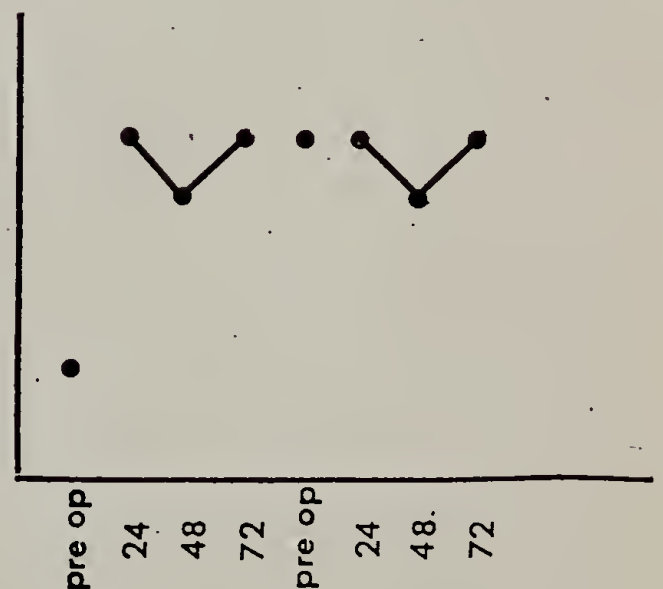


Figure 24. Subjects G83, J80, K18 and K39 received lesions to stria terminalis 3 days following septal lesioning. Pre-operative reactivity ratings were administered before each operation and are indicated as dots.

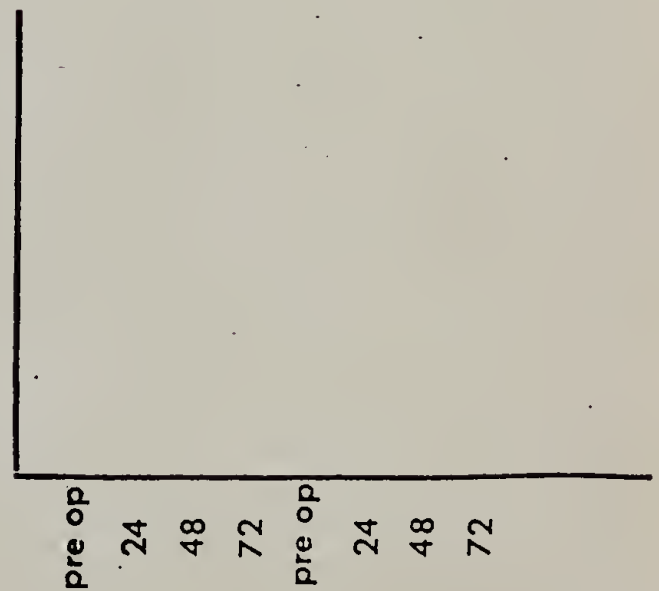
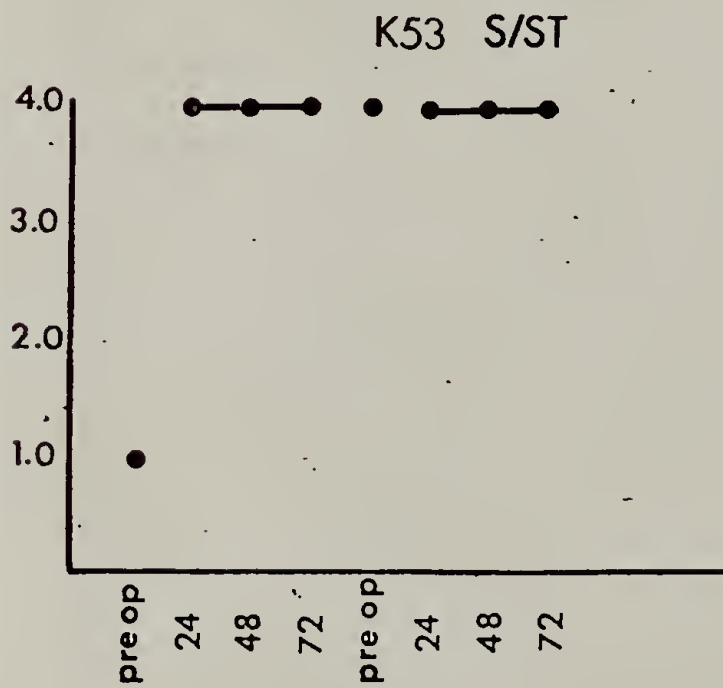
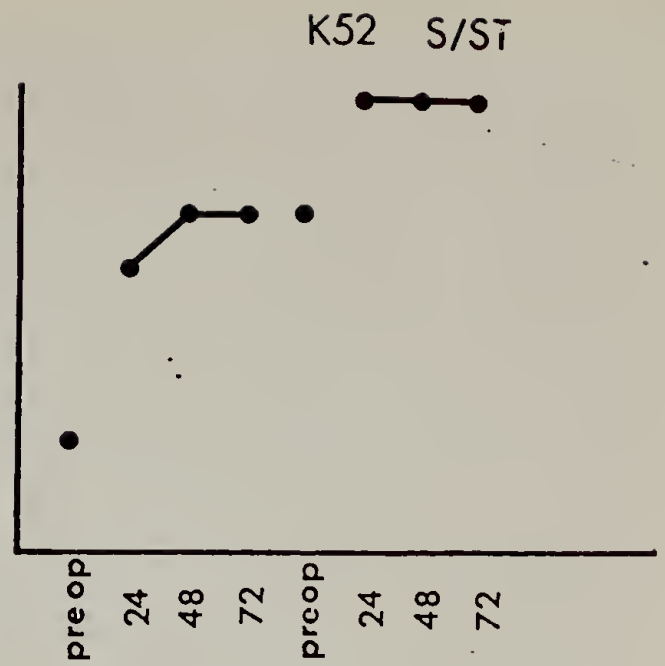
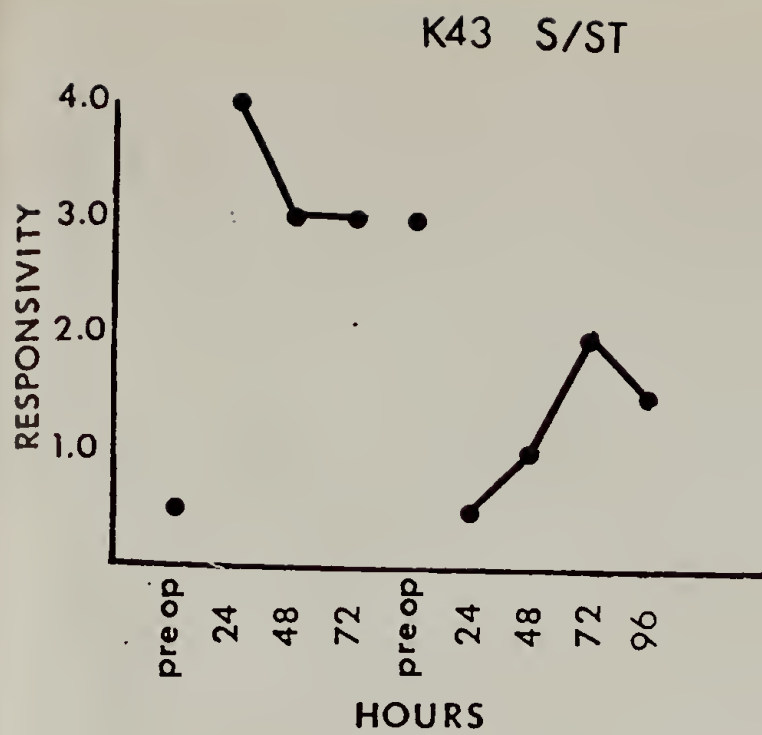


Figure 25. Subjects K43, K52, K53 received bilateral stria terminalis lesions 3 days after septal destruction. Reactivity to an air puff directed at each animal's back was determined at 24 hour intervals following each operation. Pre-operative scores are indicated as dots.

TABLE 3

Subject no.	median reactivity score after septal lesions	median reactivity score after ST lesions	Sign
f63	4.0	3.0	+
F96	4.0	4.0	0
G78	3.0	2.0	+
G80	4.0	2.5	+
G83	2.0	2.0	0
J80	3.0	2.5	0
k18	4.0	3.0	+
K39	3.0	3.0	0
K43	3.0	1.25	+
K52	3.0	4.0	-
K53	4.0	4.0	0

N = 11 p = ~~4~~274 n.s.

F63

\$/ST



A detailed schematic diagram of a coronal section of a rat brain. The diagram shows various anatomical regions and structures, each labeled with an abbreviation. Key labels include: CC (Caudate Cerebellum), CP (Caudate Putamen), SI (Superior Infratentorial), CL (Caudate Lateral), CI (Caudate Inferior), PC (Pretectal Cortex), SC (Substantia Nigra), SNr (Substantia nigra pars reticulata), STN (Striatum), SN (Substantia nigra), SNr (Substantia nigra pars reticulata), SNc (Substantia nigra pars compacta), SNd (Substantia nigra pars dorsalis), SNv (Substantia nigra pars ventralis), SNl (Substantia nigra pars lateralis), SNm (Substantia nigra pars medialis), SNp (Substantia nigra pars posterior), SNA (Substantia nigra pars anterior), SNB (Substantia nigra pars basalis), SNF (Substantia nigra pars fascicularis), SNH (Substantia nigra pars horizontalis), SNI (Substantia nigra pars intermedia), SNJ (Substantia nigra pars junctional), SNK (Substantia nigra pars kaudate), SNL (Substantia nigra pars lateral), SNM (Substantia nigra pars medial), SNP (Substantia nigra pars posterior), SNO (Substantia nigra pars oralis), SNP (Substantia nigra pars posterior), SNO (Substantia nigra pars oralis). The diagram uses solid lines for major boundaries and dashed lines for internal subdivisions.

F96 S/ST

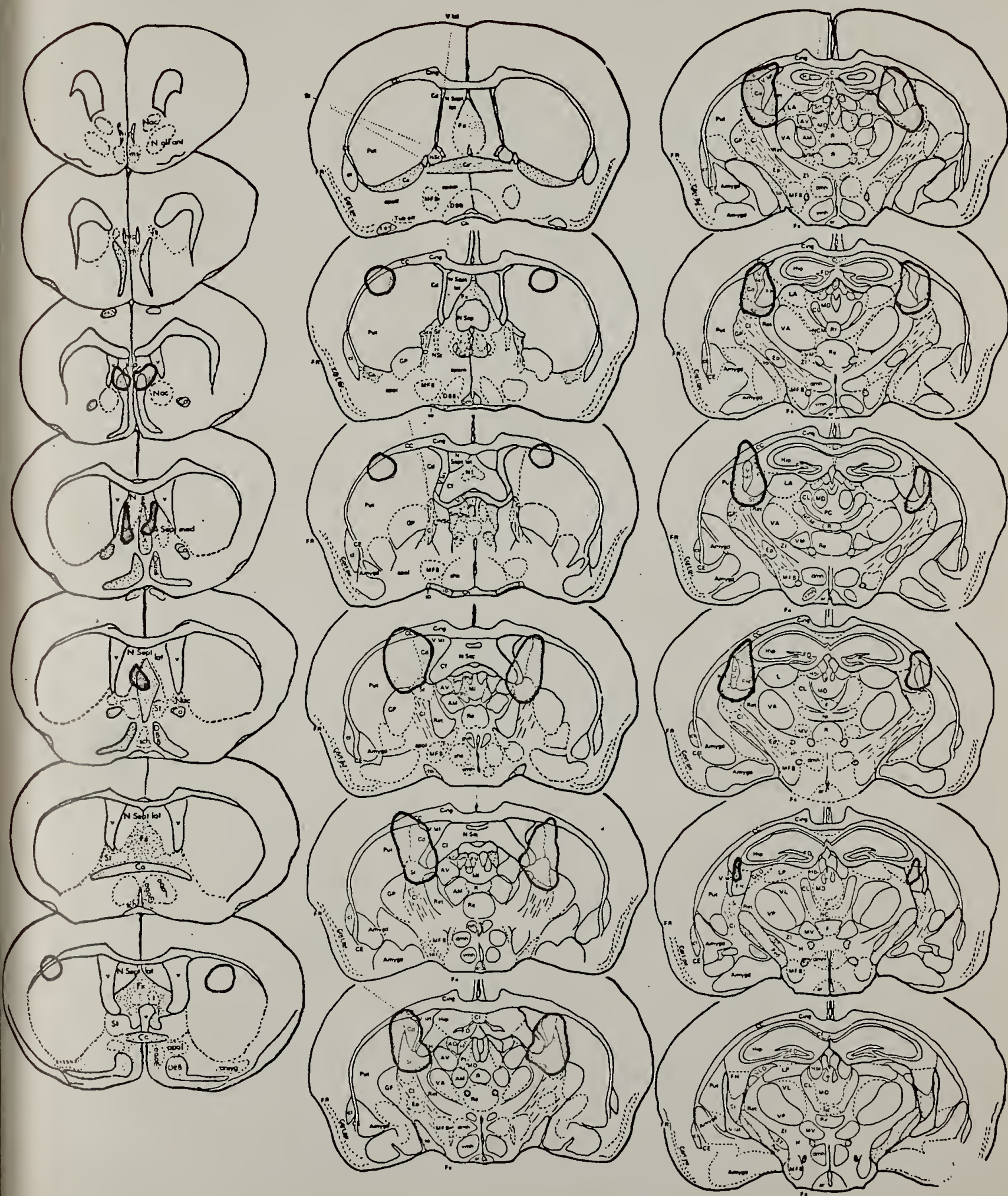


Figure 28

G78 S/ST

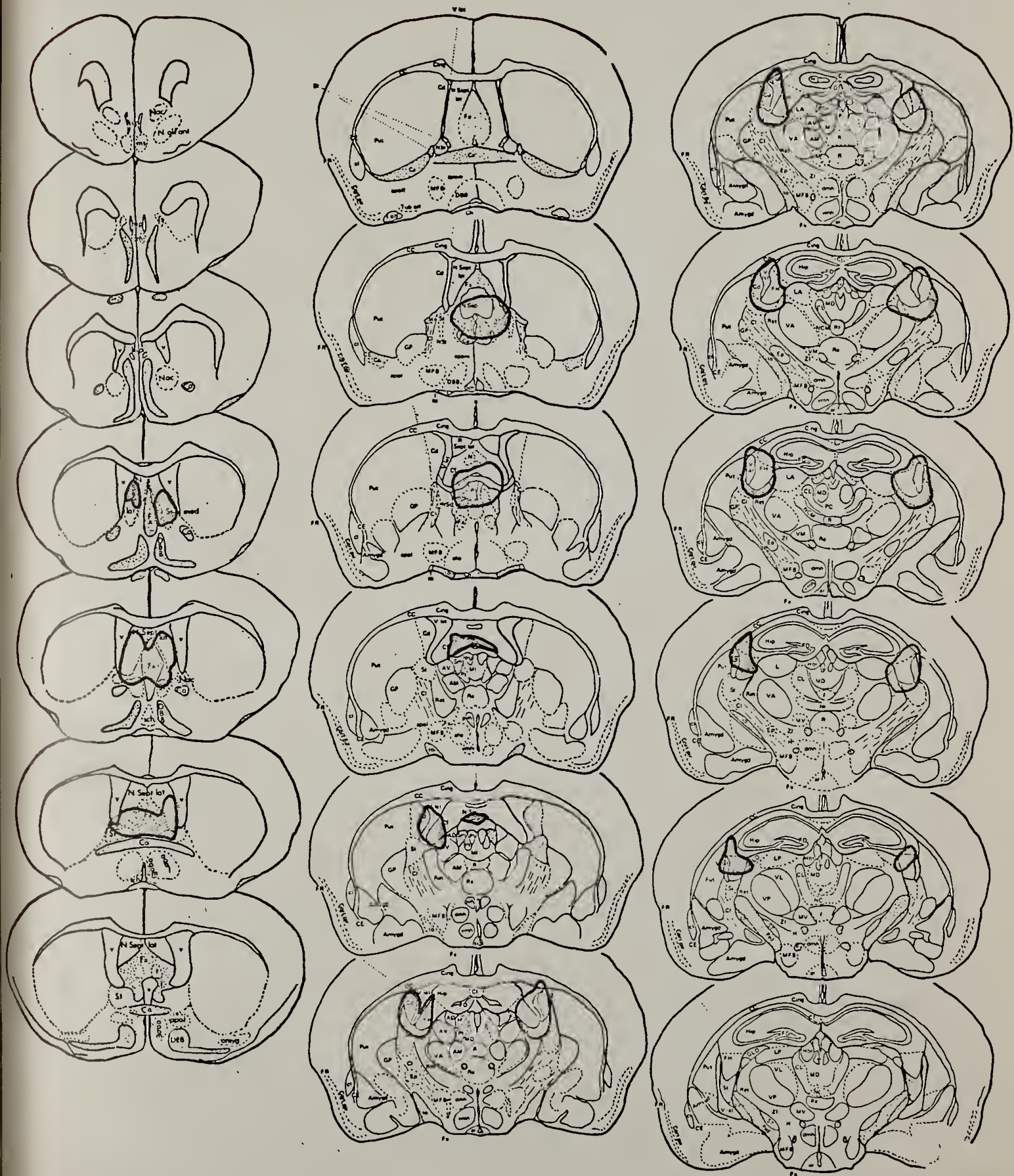


Figure 29
G80 S/ST

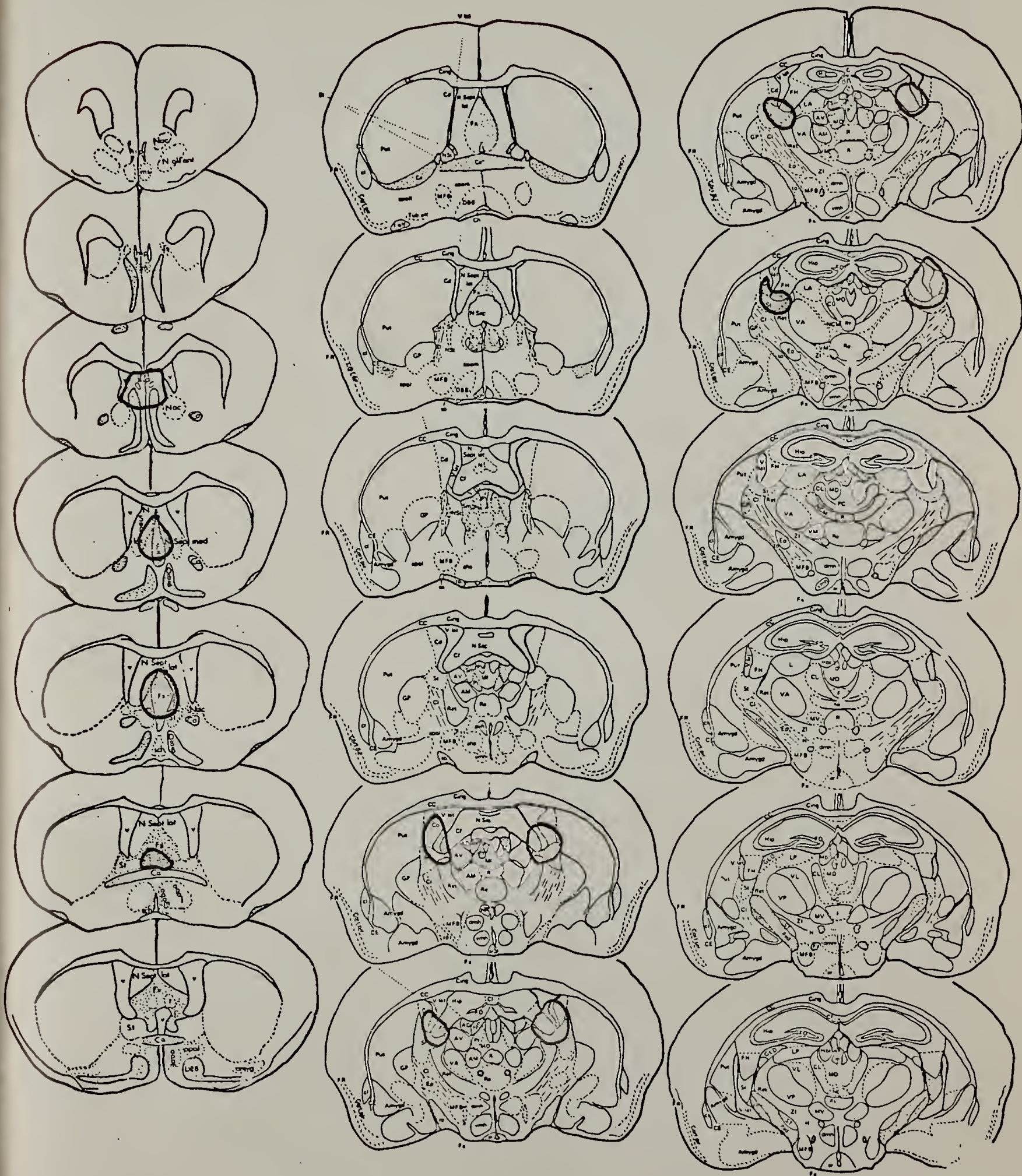
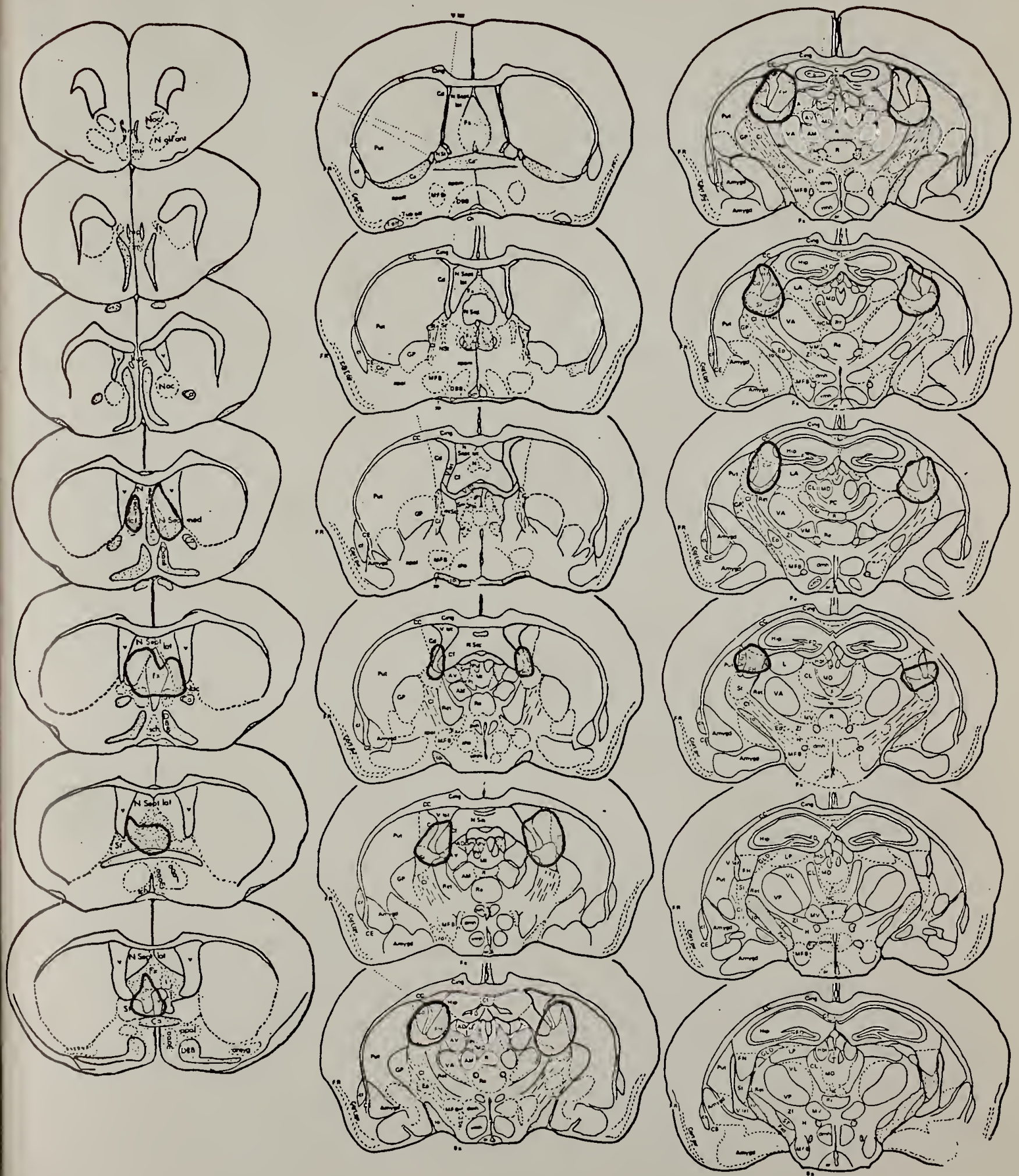


Figure 30

G83 S/ST



J80 S/ST

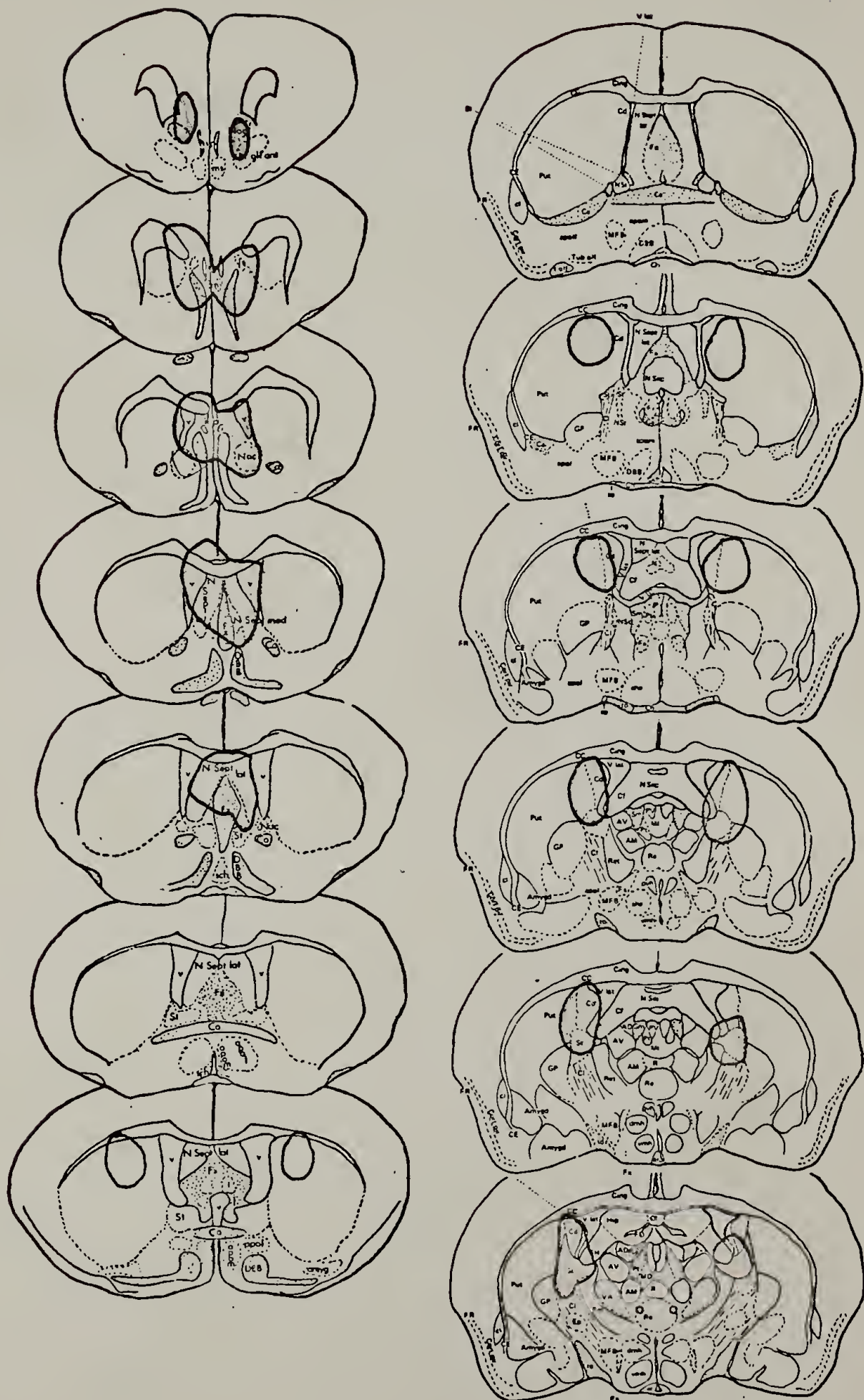
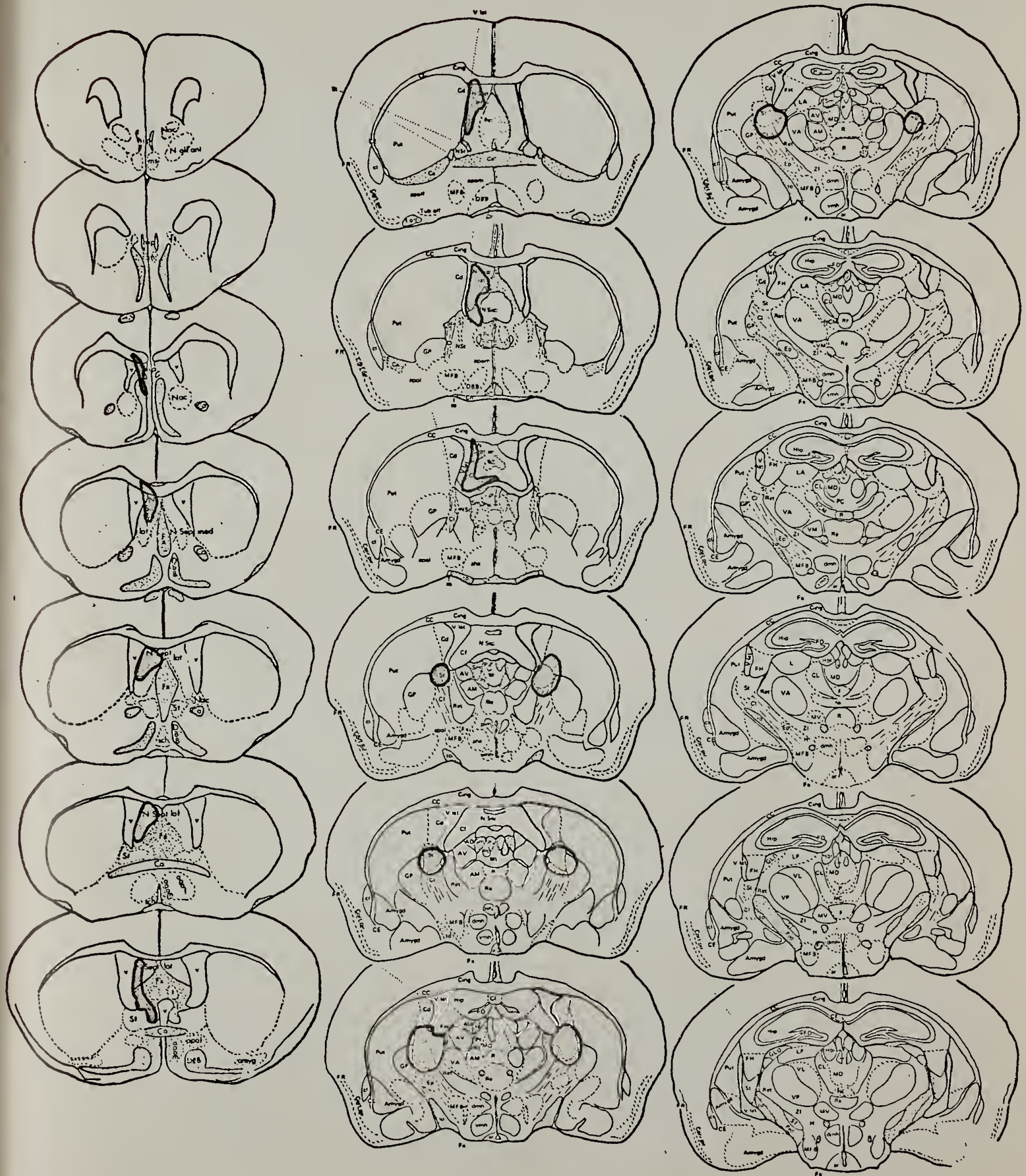
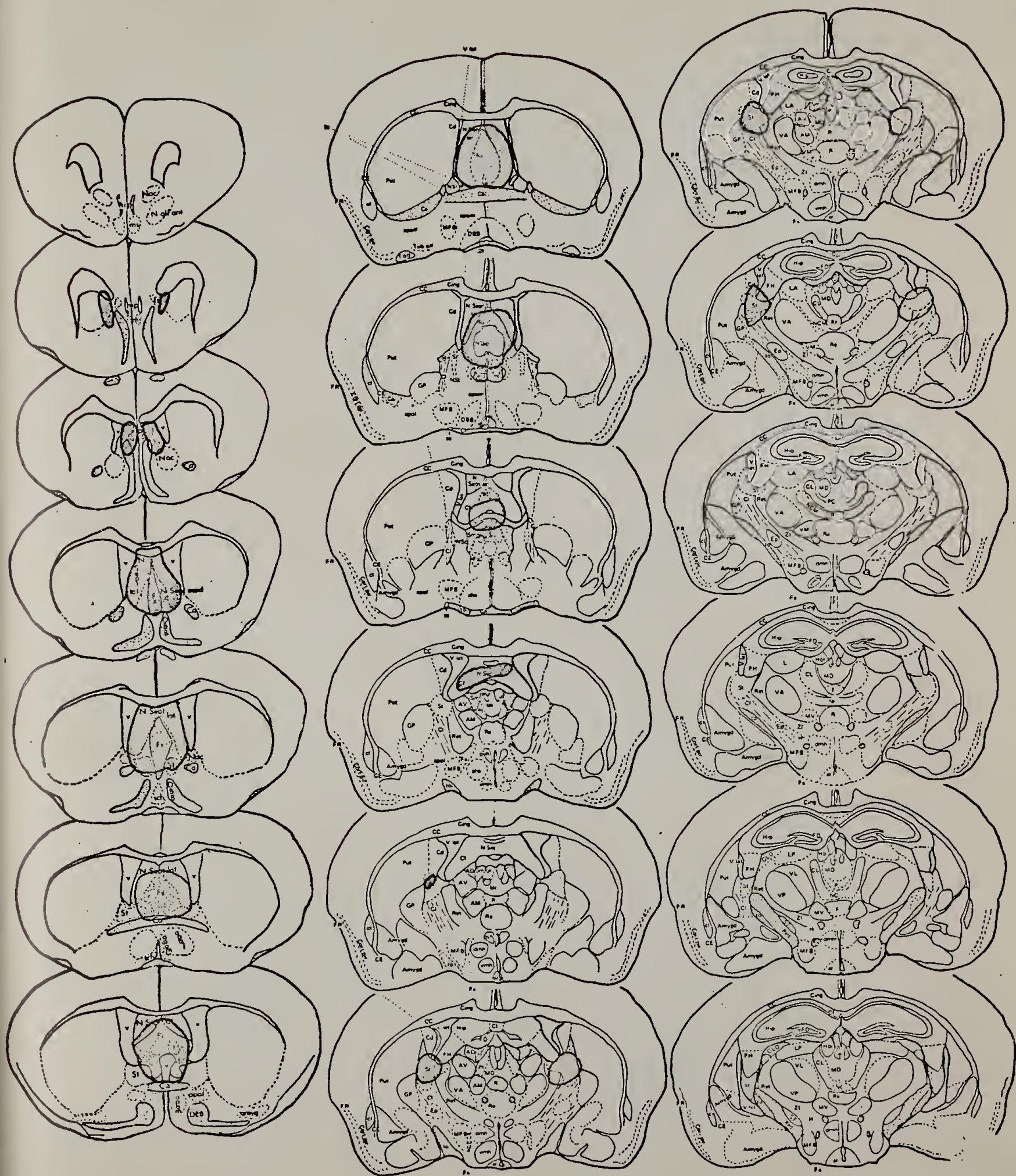


Figure 32

K18 S/ST



K39 S/ST

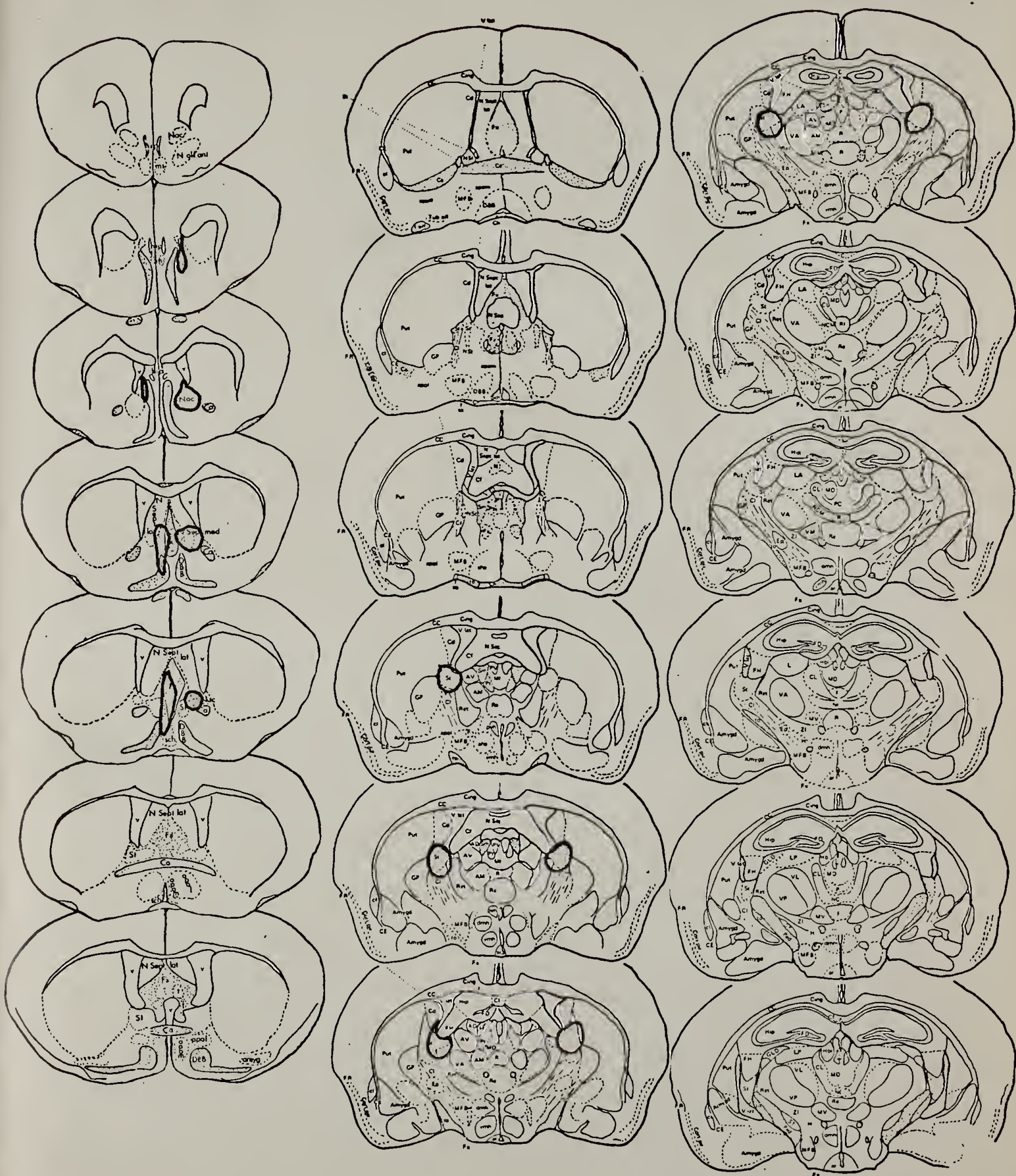


K43 S/ST

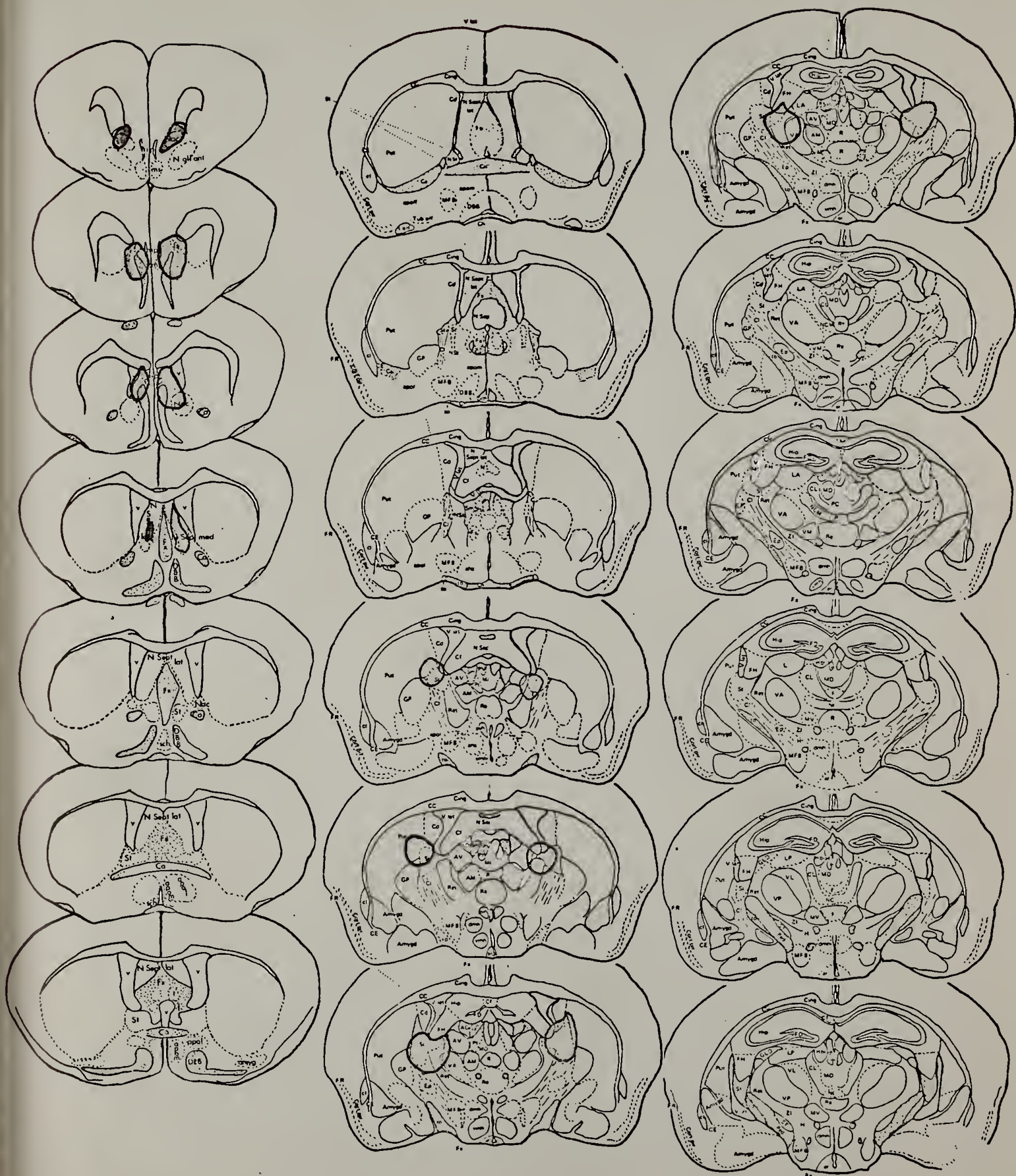


Figure 35

K52 S/ST



K53 S/ST



generally invaded fimbria tissue medially and caudate-putamen laterally. This unintended tissue destruction, however, appeared uncorrelated with behavioral measures. In all cases, stria terminalis was bilaterally severed at some point along its course. Although results from this experiment revealed inconsistent effects of ST lesions on septal hyper-reactivity it was possible to conclude that, in general, ST lesions were not nearly as effective in reducing irritability as an amygdala lesion. Examination of histological material revealed nothing obviously different about the size or location of lesions in subjects whose reactivity was reduced by ST lesions versus lesions which had no effect or an enhancing effect of hyper-reactivity. A composite drawing of lesions in mice whose reactivity was unchanged by these lesions is presented in Figure 37. Likewise, a composite diagram of ST lesions which were somewhat effective in reducing septal hyper-reactivity is shown in Figure 38. Lesions in Figure 38 which had some efficacy in reducing septal hyper-reactivity are generally larger than those in Figure 37 showing lesions which were ineffective in changing the reactivity of septal mice.

Discussion

Stria terminalis lesions were found to have a small and inconsistent effect on septal hyper-reactivity. Composite lesion drawings indicated that lesions which had some attenuating

effect on irritability tended to be slightly larger than lesions in animal's whose reactivity was unchanged by these lesions. This suggested that perhaps smaller lesions did not destroy all ST fibers interconnecting relevant brain areas, and that even small reduction in hyper-reactivity was dependent on destruction of all fibers of this bundle.

While the present experiment produced somewhat inconclusive results concerning the role of stria terminalis in septal lesion-induced hyper-reactivity there is no doubt but that the dramatic reduction in reactivity by even the largest ST lesion did not mimic the magnitude of the effect of amygdaloid lesions on septal hyper-reactivity. Thus it was possible to conclude that if direct septo-amygdaloid connections are involved in septal rage they do not travel along the stria terminalis.

EXPERIMENT 4

The septum is interconnected with the amygdaloid complex via two routes 1) stria terminalis 2) diagonal band as part of the diffuse ventral amygdalofugal pathway. Fibers from the basolateral amygdala and olfactory tubercle project through the diagonal band into the ventral septum. Medial septum also projects fibers to the basolateral amygdala via the diagonal band.

Since amygdala lesions were effective in normalizing a septal mouse's reactivity to tactile stimulation and stria

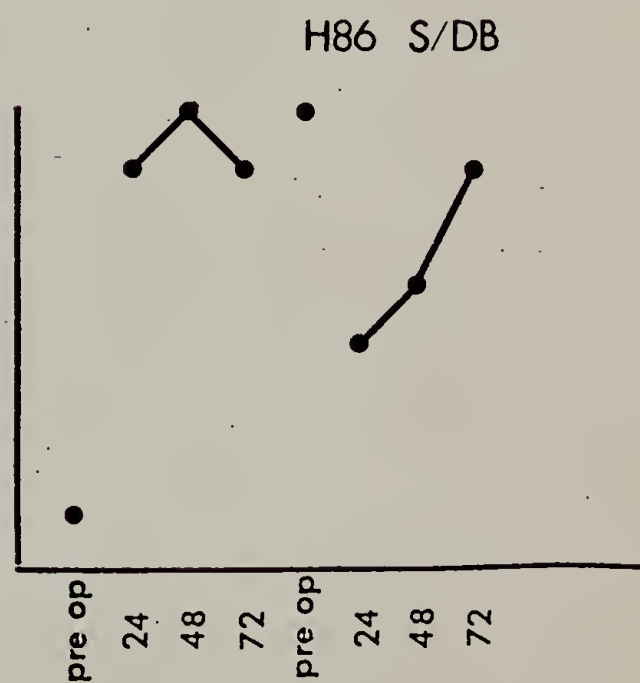
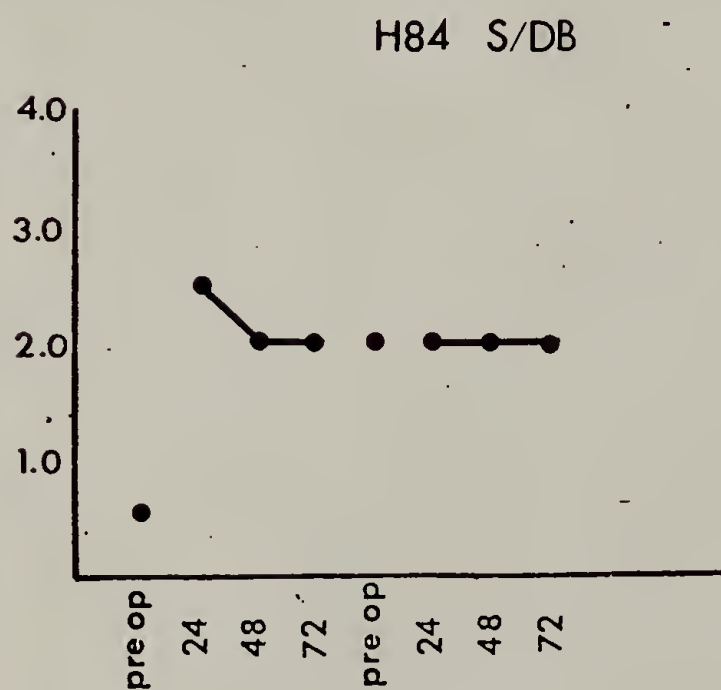
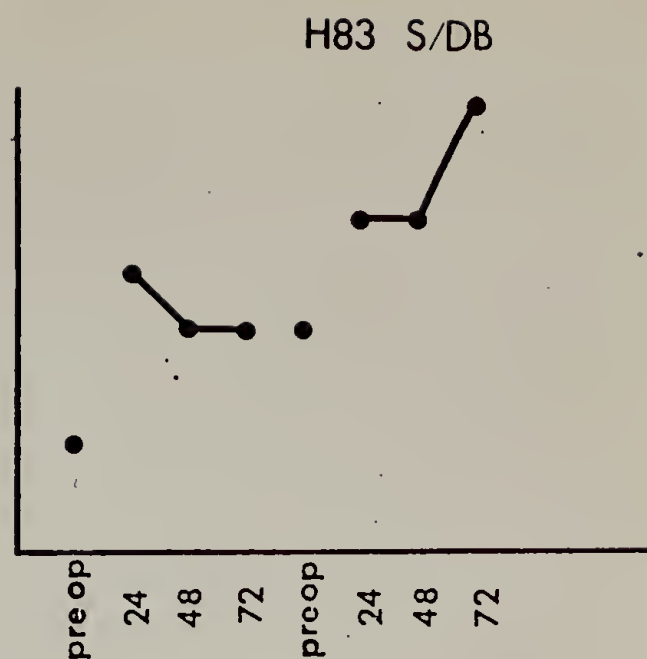
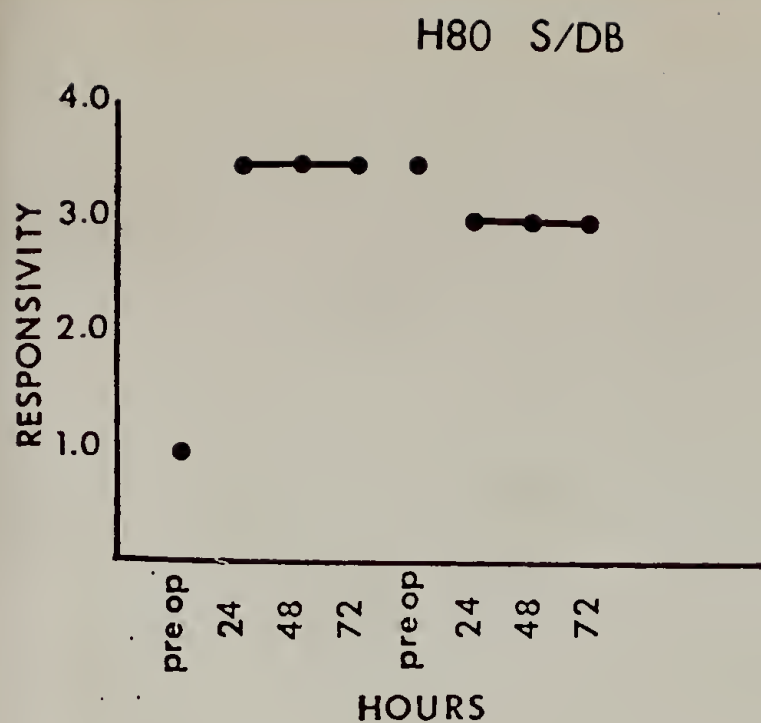


Figure 39. The effects of diagonal band of Broca lesions on septal lesion-induced hyper-reactivity. Subjects H80, H83, H84 and H86 received midline diagonal band lesions 3 days after septal lesions. Pre-operative reactivity scores were obtained before each operation and are indicated as dots.

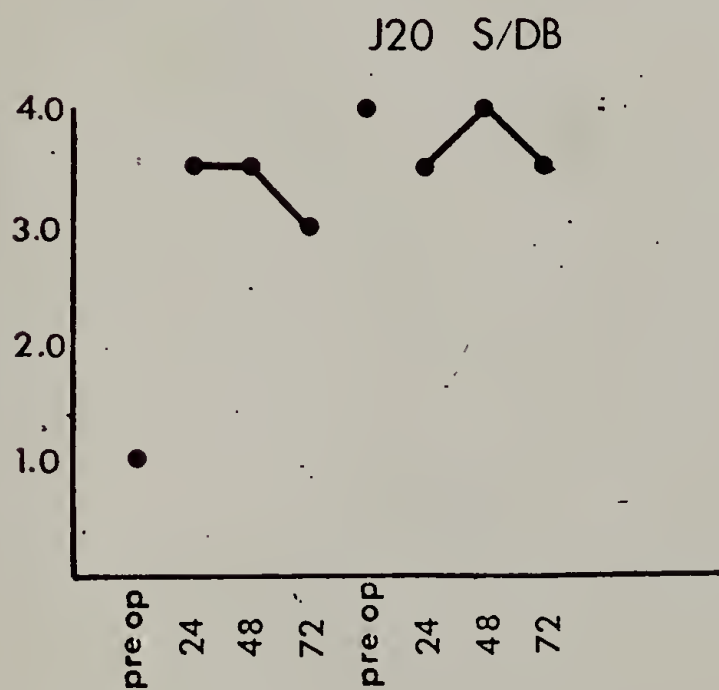
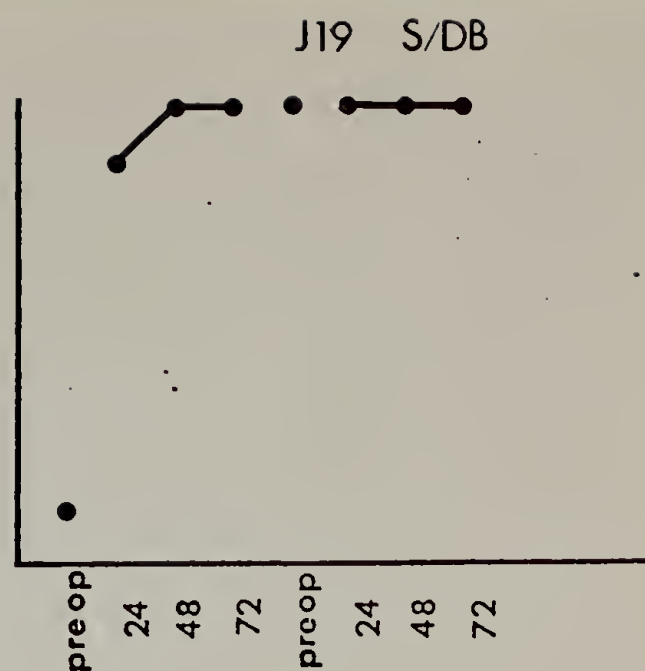
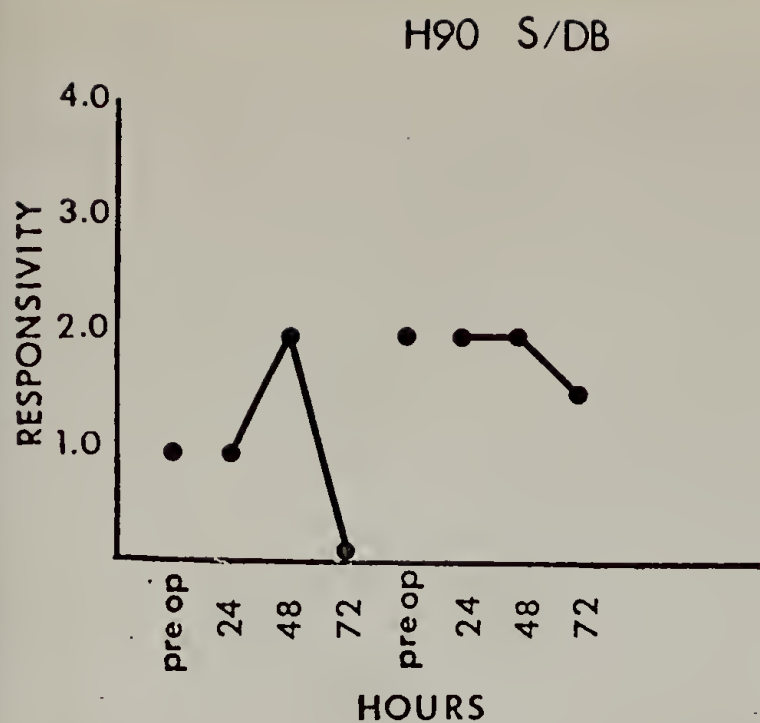


Figure 40. The effects of diagonal band of Broca lesions on septal lesion-induced hyper-reactivity. Subjects H90, J19 and J20 received midline diagonal band lesions 3 days after septal lesions. Pre-operative scores were obtained before each operation and are indicated by dots.

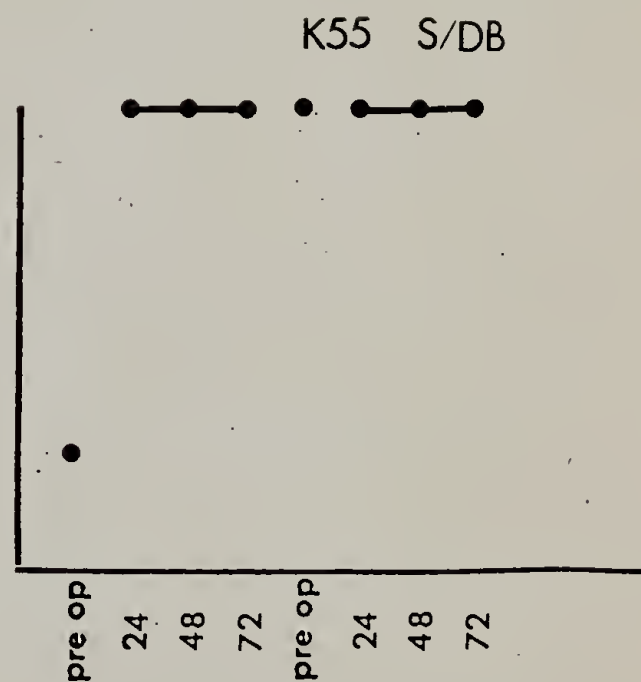
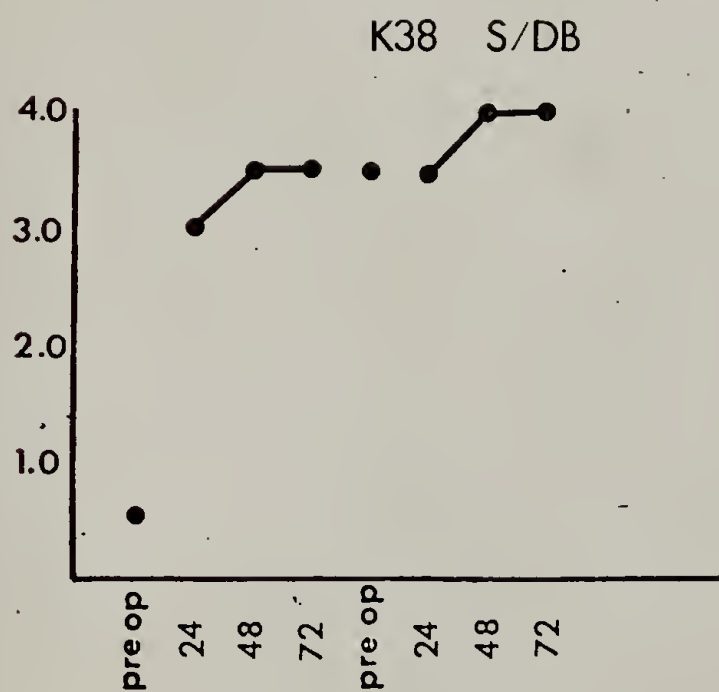


Figure 41. The effects of diagonal band of Broca lesions on septal lesion-induced hyper-reactivity. Subjects H80, H83, H84 and H86 received midline diagonal band lesions 3 days after septal lesions. Pre-operative scores were obtained before each operation and are indicated by dots.

terminalis destruction was, at best, mildly effective in reducing hyper-irritability it was of interest to determine the effectiveness of diagonal band destruction on septal lesion-induced hyper-reactivity.

Method

Nine male mice served as subjects. Procedures were identical to those employed in Experiments 1, 2 and 3 except that on the third experimental day animals received a midline diagonal band lesion (coordinates relative to bregma; anterior +1.0 mm., lateral 0 mm., ventral -4.7 mm.).

The ability of diagonal band lesions to reduce septal hyper-irritability was determined using the following experimental protocol: Normal mice who received a score of 1.0 or below on an initial reactivity test to an air puff received bilateral septal lesions. At 24, 48 and 72 hours they were tested for changes in reactivity. On the third experimental day, diagonal band lesions were preceded by a pre-operative reactivity test. At 24, 48 and 72 hours the mice were again scored for their reactivity. Following the last 72 hour test mice were perfused, brains removed and histological evaluation followed 6 days later.

Results

Figures 39, 40 and 41 present reactivity ratings over the six experimental days for each of the subjects. The order of tissue destruction is indicated by S/DB - septal lesions were followed 3 days later by a diagonal band (DB)

lesion.

Lesions of the vertical limb of the diagonal band had an inconsistent and small effect on hyper-reactivity. These lesions either had no effect on the expression of septal hyper-reactivity (H90, J19, J20 and H84), an enhancing effect (H83) or a transitory attenuating effect (H86). A Sign Test, however, comparing median reactivity scores following septal lesions with median scores following diagonal band lesions revealed no significant difference $N = 5$, $p = .5$). (See Table 4.) Therefore, it was possible to conclude that diagonal band destruction had little effect on the expression of septal hyper-reactivity.

Histology

Individual lesion reconstructions are shown in Figures 42-50. In all subjects but one (H90) interruption of fibers ascending into the septum or emerging from the septum was incomplete, owing to the fact that the diagonal band extends from the hippocampal rudiment to the decussation of the anterior commissure. Single midline lesions did not destroy all fibers of the vertical limb of the diagonal band. At the present level of analysis, however, no correlation could be ascertained concerning the anterior-posterior location of the DB lesion and its effects on septal hyper-reactivity. Nevertheless, it can be stated that diagonal band fibers contribute little to the septal rage syndrome since in one animal (H90) diagonal band destruction was complete yet did

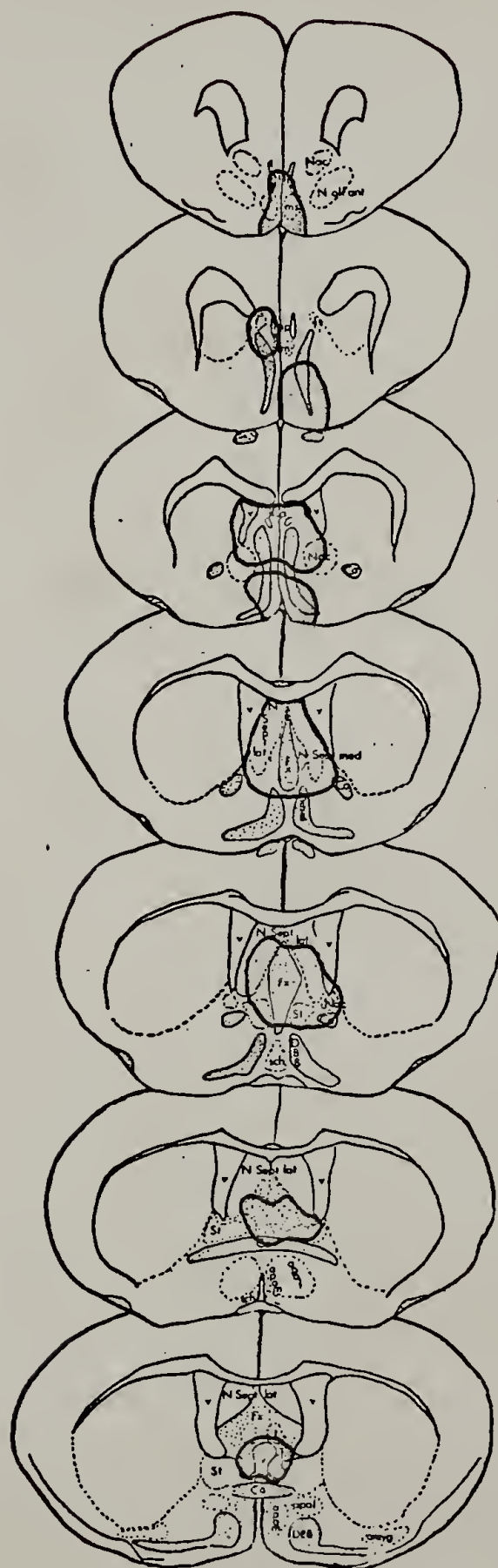
TABLE 4

Subject no.	median reactivity score following septal lesions	median reactivity scores following DB lesions	Sign
H80	3.5	3.0	+
H83	2.0	3.0	-
H84	2.0	2.0	0
H86	3.5	2.5	-
H90	1.0	2.0	-
J19	4.0	4.0	0
J20	3.5	3.5	0
K38	3.5	4.0	-
K55	4.0	4.0	0

N = 9 p = .274 n.s.

Figure 42

H80 S/DB



H83 S/DB

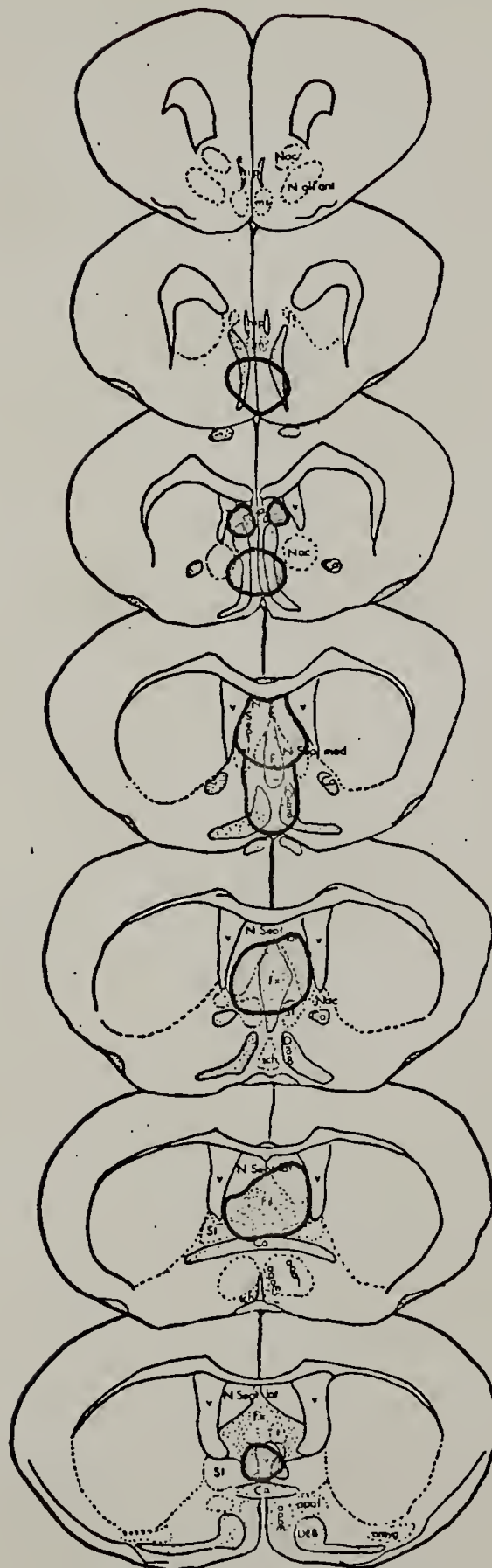


Figure 44

H84 S/DB

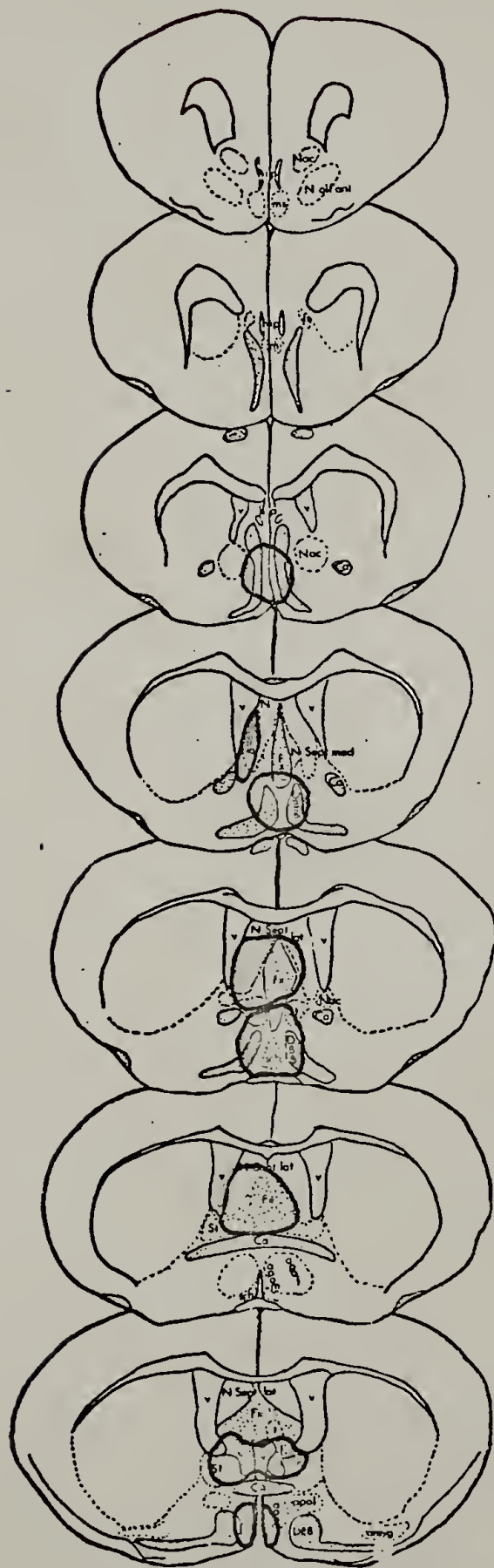


Figure 45

H86 S/D

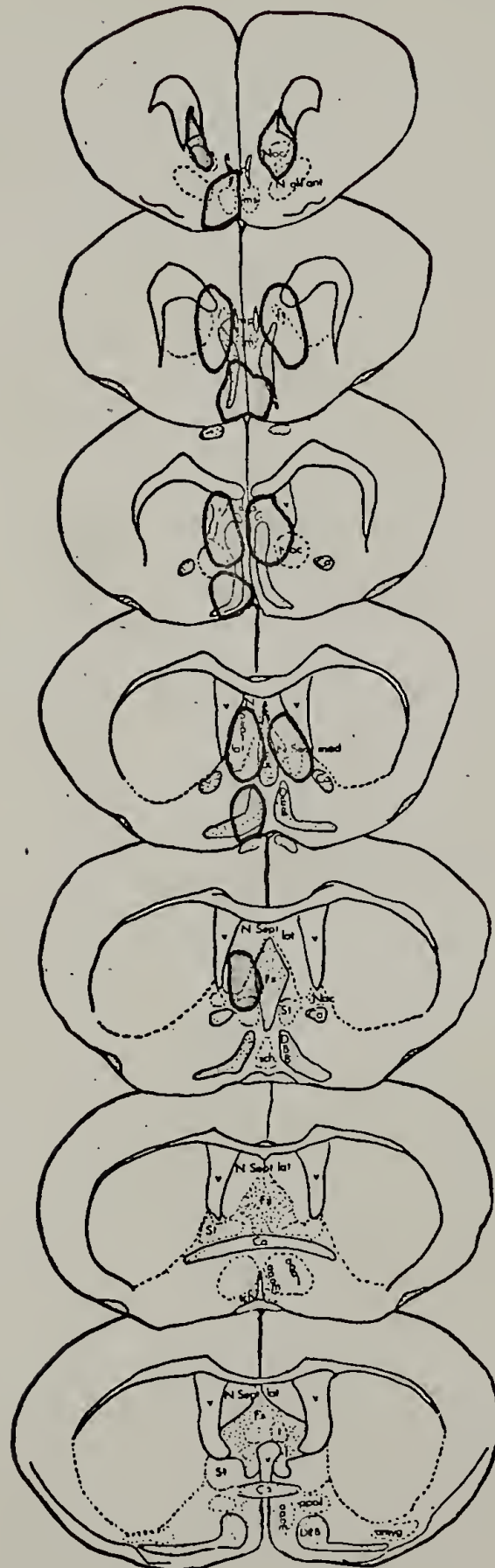


Figure 46

H90

S/DB

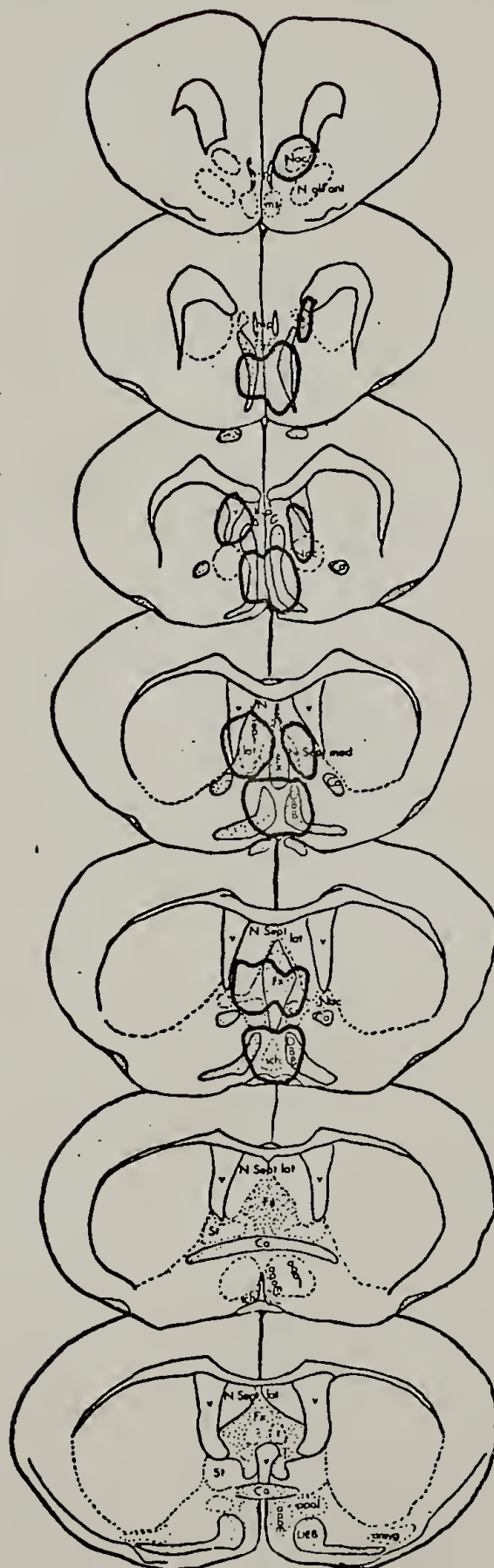


Figure 47

J19 S/DB

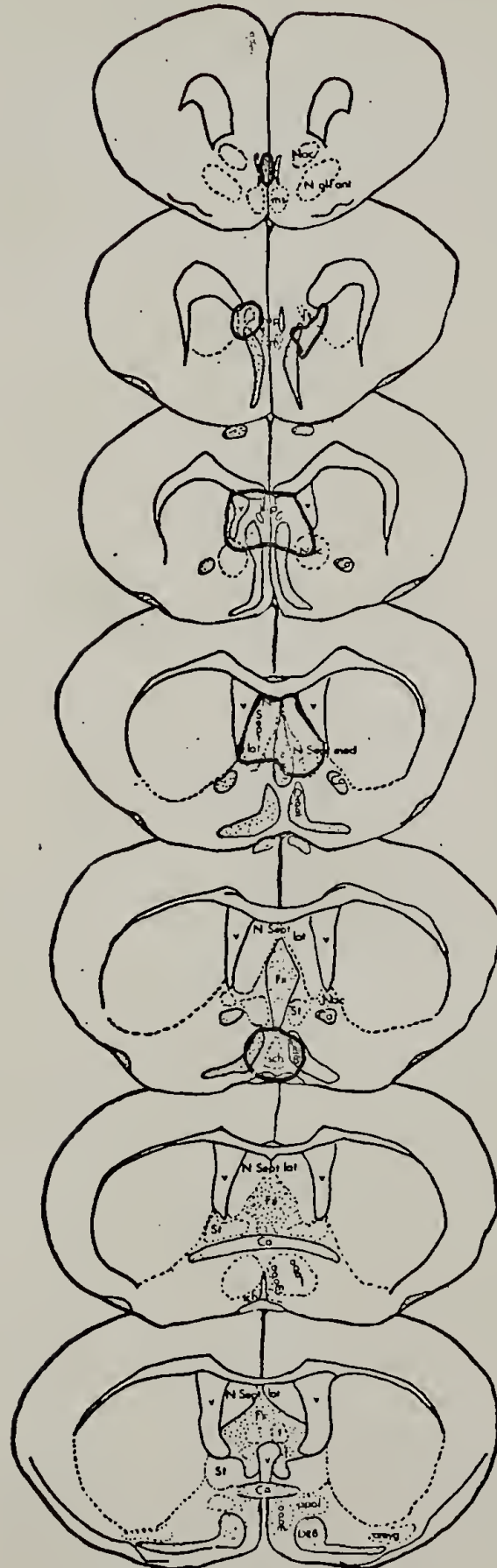


Figure 48

J20 S/DB

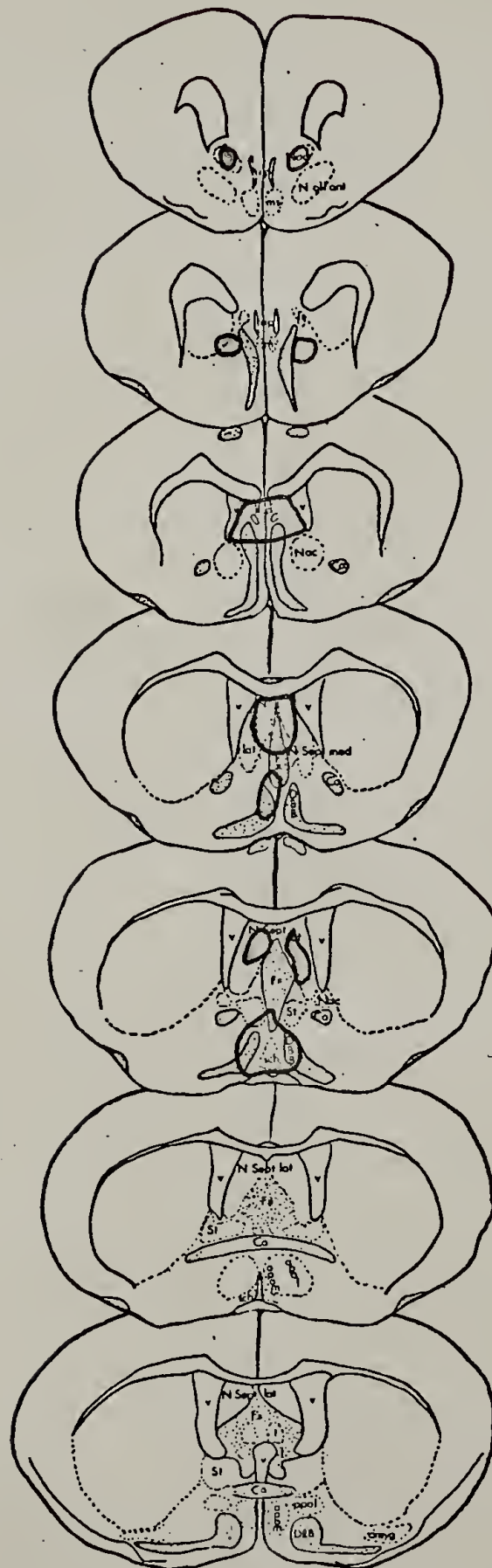


Figure 49

K38 S/DB

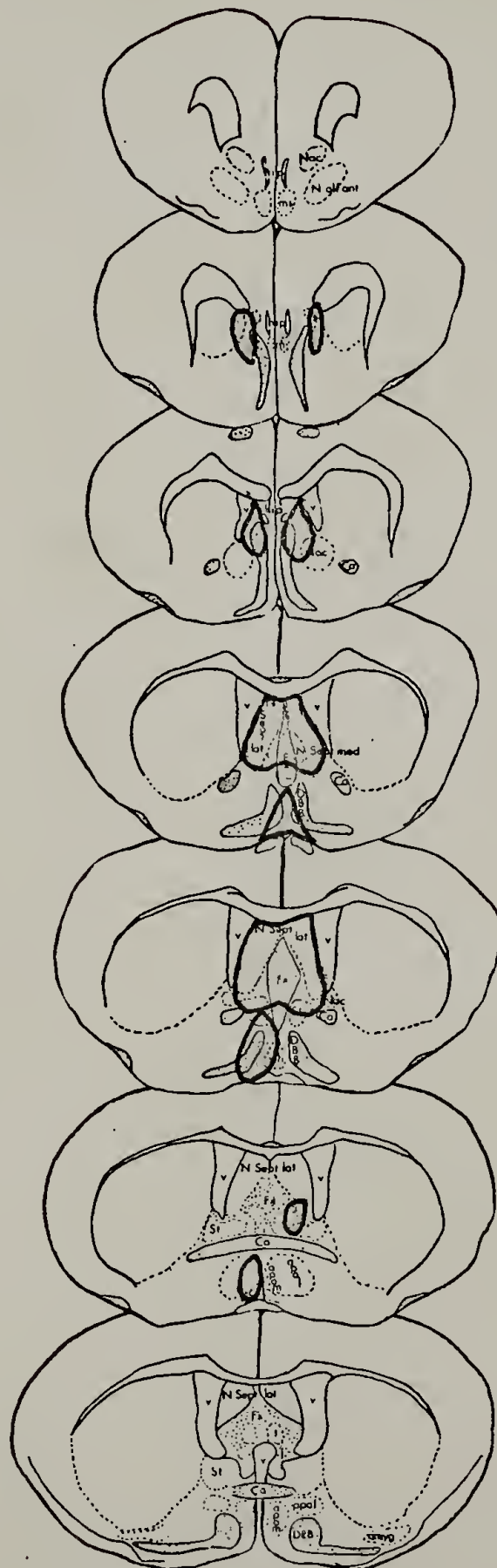
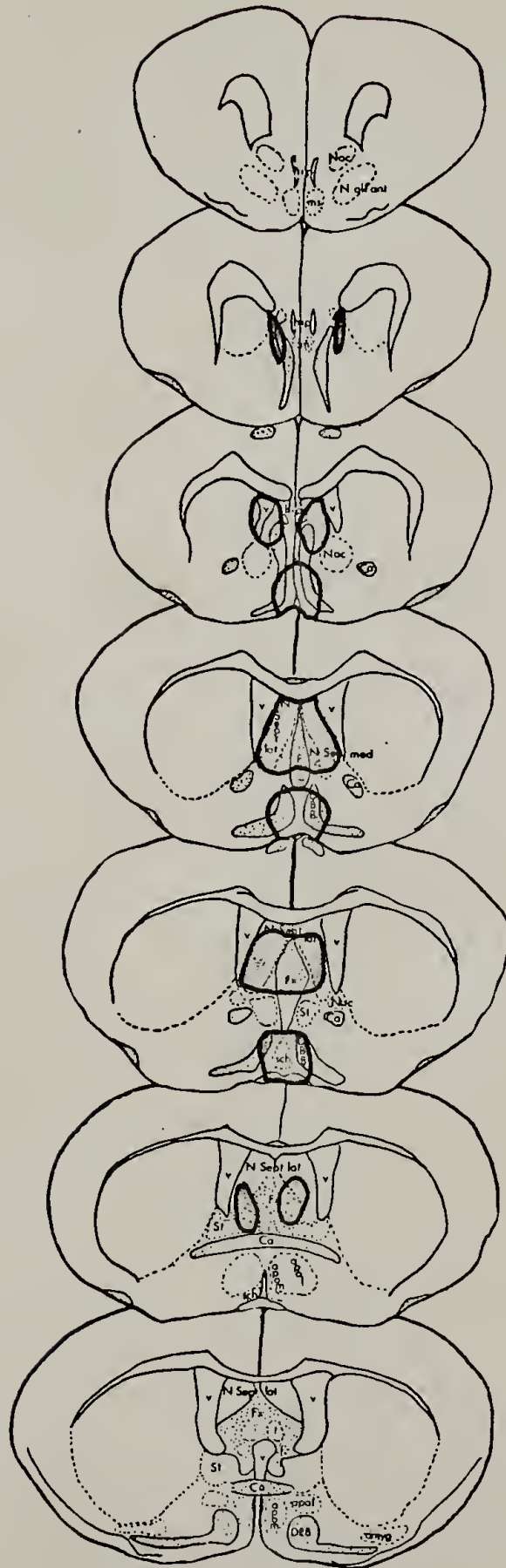


Figure 50

K55 S/DB



not reduce or enhance septal hyper-reactivity.

Discussion

Although diagonal band lesions were found to have no significant effect on septal hyper-reactivity, some animals showed a reduction in reactivity (H80 and H86), while others (H83 and K38) displayed an enhanced response to tactile stimulation as a result of the second (DB) lesion. Lesion location seemed unrelated to these individual differences.

Results of this experiment, in any case, clearly demonstrated that the efficacy of amygdaloid destruction in reducing septal hyper-reactivity is not mimicked by destruction of fibers interrelating the septal area and the amygdaloid complex through the diagonal band.

Since destruction of neither of the two direct routes by which the septum and amygdala communicate was as effective as amygdala lesions in reducing hyper-reactivity two possibilities remain:

- 1) Fibers relevant to the septal rage syndrome are carried by both pathways between amygdala and septum; diagonal band and stria terminalis or -
- 2) The septal-amygdala connections relevant to the septal rage syndrome are not direct fibers but are mediated by some other structure.

This second possibility was investigated in Experiment 5.

EXPERIMENT 5

Experiments 3 and 4 demonstrated that neither pathway connecting the septal area and the amygdaloid complex was critically involved in the expression of septal lesion-induced hyper-reactivity. Lesions of stria terminalis or diagonal band were ineffective in reducing hyper-irritability. Although it is possible that both pathways must be severed in order to septal irritability to be normalized another possibility exists in which fiber connections between septum and amygdala relevant to hyper-reactivity do not communicate directly between the two structures.

The olfactory system has heavy connections with both amygdala and septum and may be a likely candidate as the intermediary structure involved in septal rage. The major efferent input to the amygdala originates in the olfactory bulbs and projects via the lateral olfactory tract to terminate in the corticomедial division of the amygdala. While the septal area does not receive direct fibers from the olfactory bulbs or anterior olfactory nucleus it is strongly interconnected with the olfactory system through the olfactory tubercle which receives direct fibers from the bulbs themselves. It has been reported (see Vom Saal et al, 1975 for review) that the septum receives fibers from and projects fibers to the olfactory tubercle. Afferent fibers from the olfactory tubercle pass via the medial aspect of the medial forebrain bundle and terminate in the medial septum and the

vertical limb of the diagonal band of Broca. Short efferent fibers pass ipsilaterally from the lateral septal nucleus along the medial aspect of the nucleus accumbens septi to terminate in the olfactory tubercle.

Scalia and Winans (1975), using a Fink-Helmer degeneration stain, have reported the distribution of terminating fibers originating in the olfactory bulbs. They have observed degenerating fibers in the ipsilateral olfactory tubercle, the pyriform cortex (including the periamygdaloid part), ventrolateral entorhinal area, corticomedial amygdaloid area, anterior olfactory nucleus and the hippocampal rudiment.

Because of the possible extensive interrelationships between septum and amygdala through the olfactory pathways it was of interest to determine whether olfactory structures are in any way involved in septal lesion-induced hyper-reactivity. Thus, the purpose of Experiment 5 was to determine the effects, if any, of olfactory bulb aspiration on septal lesion produced irritability.

Method

Six male mice served as subjects. They are designated as animals belonging to the S/OB group, indicating that septal lesions were followed three days later by olfactory bulb aspiration.

The experimental procedure was as follows: each normal mouse which received a pre-operative reactivity score of 1.0 or less received a bilateral septal lesion and reactivity

scores were obtained 24, 48 and 72 hours post-operatively. On the third day of the experiment olfactory bulb aspirations were immediately preceded by a pre-surgical reactivity test. Following nembutal anesthesia, bone and dura overlying both bulbs was removed and the olfactory tissue aspirated under visual guidance. Care was taken to avoid damage to the frontal pole. All neural tissue above the cribriform plate was removed. When bleeding was severe, gelfoam was applied for several minutes then removed before suturing. At 24, 48 and 72 hours reactivity scores were obtained and during the sixth experimental day animals were perfused and brains removed from the skull. Care was taken to leave intact any remaining olfactory bulb tissue.

During frozen sectioning the amount of olfactory bulb destruction was determined by counting the number of 40 μ m sections remaining anterior to the frontal pole. This number was compared to the mean length of the olfactory bulbs in 6 normal mice of the same age and strain. The mean length of intact bulbs was 2880[^] (range: 2720-2960). The small degree of variability in olfactory bulb length in normal mice lent a high degree of reliability to this kind of comparison. Thus, for each subject, tissue remaining anterior to the frontal pole was expressed as a percent of the length of intact olfactory bulbs.

Results

Figures 51 and 52 demonstrate the attenuating action of

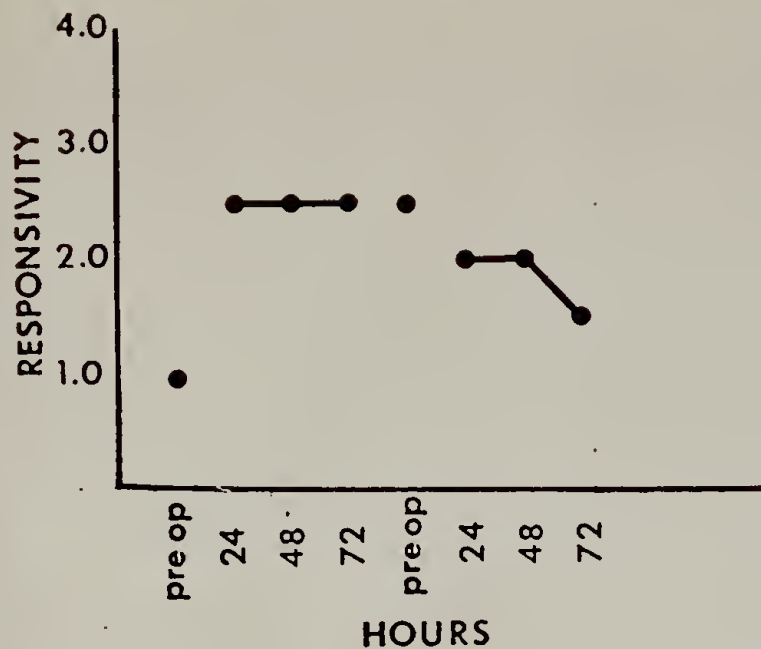
olfactory bulb (OB) removal on septal lesion-induced hyper-reactivity. In each of the 6 mice, olfactory bulb aspiration reduced hyper-irritability. The percent tissue remaining in each olfactory bulb is shown in each individual figure. Olfactory bulb remaining ranged from 0% to 65.28%. Subject J55 who showed the least amount of reactivity suppression also had the least amount of tissue removed from the olfactory bulbs. Septal lesion reconstruction are shown in Figures 53-58.

A Sign Test comparing the efficacy of OB aspirations to reduce hyper-reactivity revealed a highly significant ($p < .001$) attenuation of irritability following this operation. Table 4 presents median reactivity scores after each lesion and the sign assigned to each animal's data. As in the case of amygdala lesions, the ability of OB destruction to reduce septal hyper-reactivity cannot be ascribed to a general malaise produced by this second surgical procedure. Animals appeared healthy and no weight losses were observed. Results of this experiment clearly demonstrate the involvement of primary olfactory structures in the expression of septal rage. Olfactory bulb aspirations were extremely effective in suppressing the effects of septal lesions.

Discussion

The neural mechanisms underlying the suppressant effects of both amygdaloid and olfactory bulb destruction on septal hyper-reactivity may be independent. However, the complex

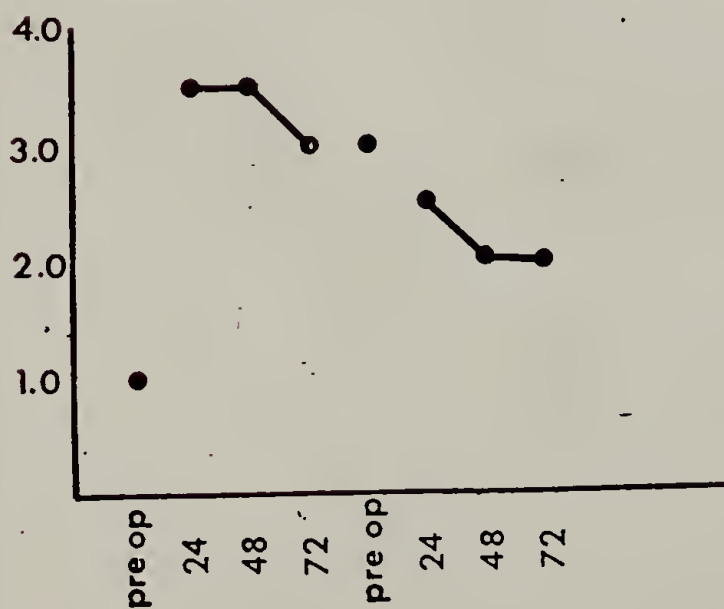
H49 S/OB



J48 S/OB



J55 S/OB



J57 S/OB

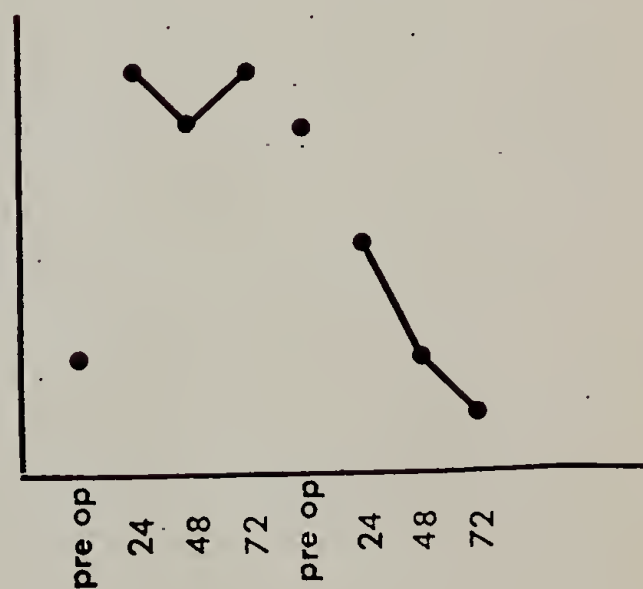


Figure 51. The effects of bilateral olfactory bulb aspiration on septal lesion-induced hyper-reactivity. Subjects H49, J48, J55 and J57 underwent bilateral removal of the olfactory bulbs 3 days after septal lesions. Pre-operative reactivity scores were obtained before each operation and are indicated by dots.

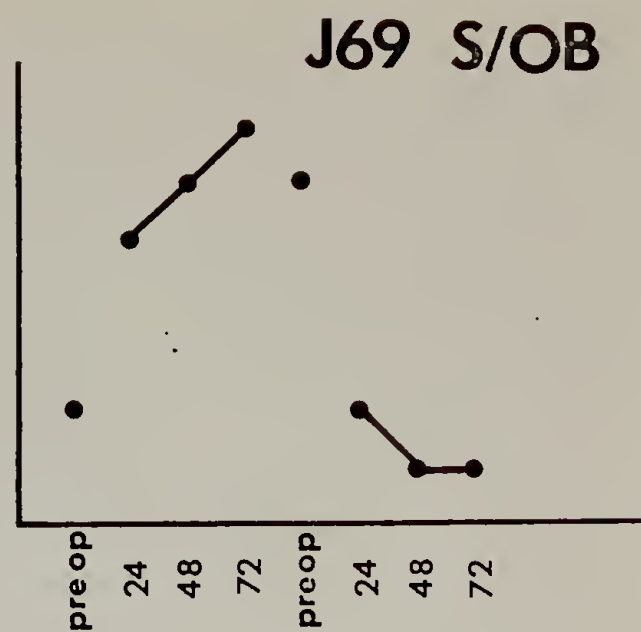
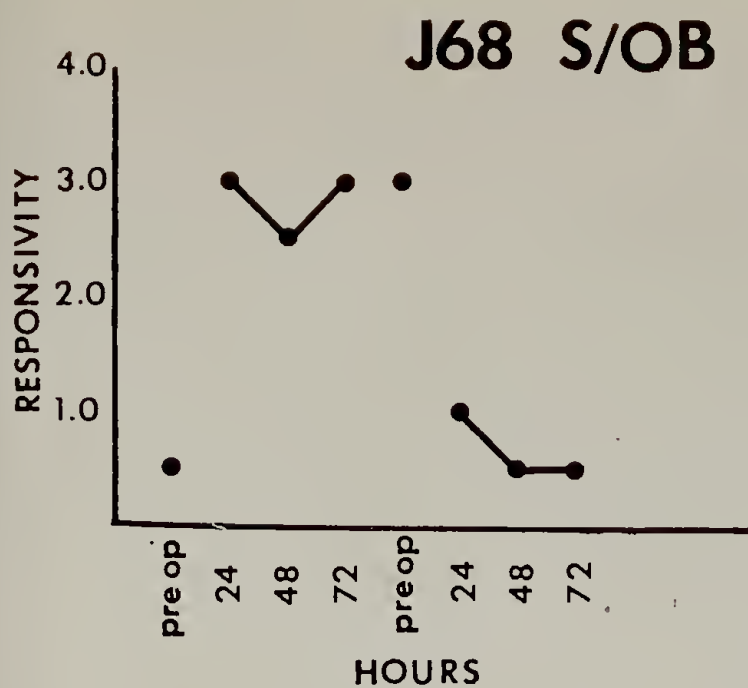


Figure 52. The effects of bilateral olfactory bulb aspiration on septal lesion-induced hyper-reactivity. Subjects J68 and J69 underwent bilateral removal of the olfactory bulbs 3 days after septal lesions. Pre-operative reactivity scores were obtained before each operation and are indicated by dots.

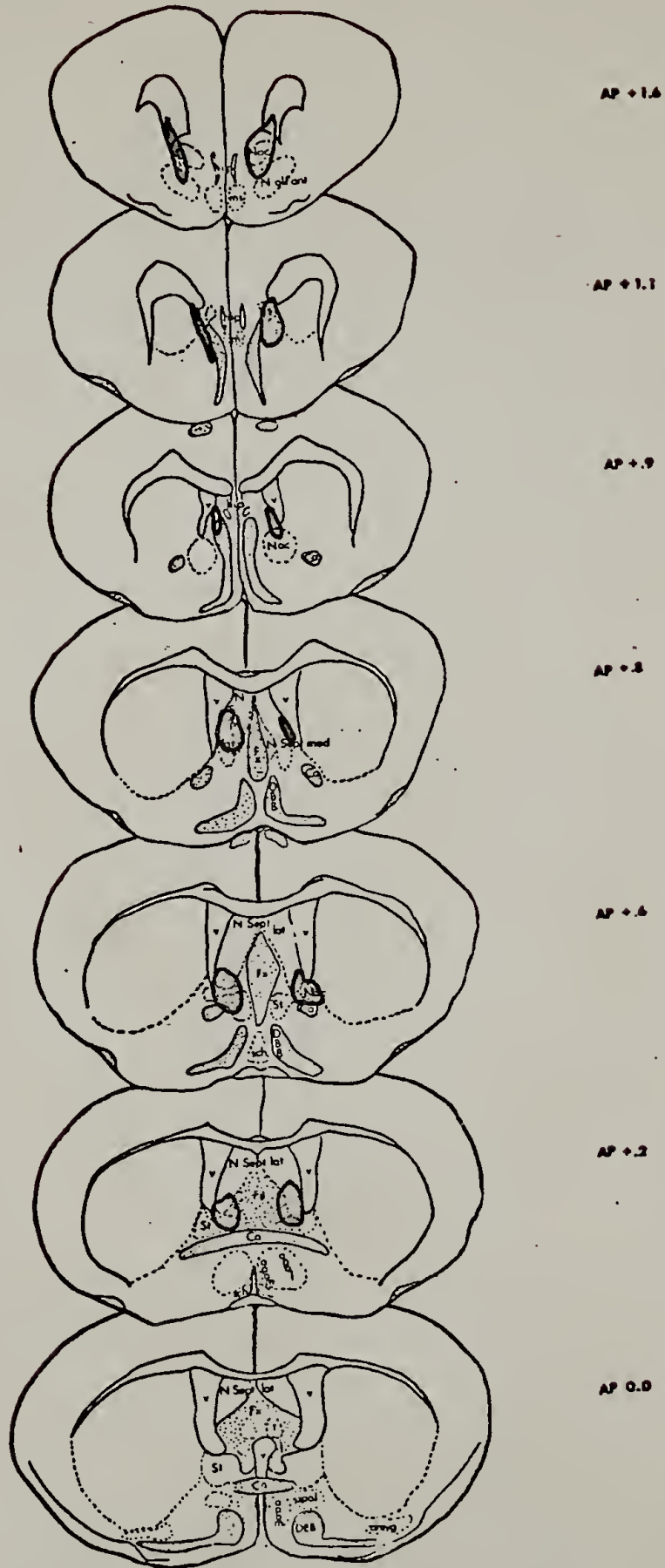
TABLE 5

Subject no.	median reactivity score following septal lesions	median reactivity scores following OB aspiration	Sign
H49	left 65.28% right 47.22%	2.0	+
J48	left 41.67% right 47.22%	1.25	+
J55	left 55.55% right 55.55%	2.0	+
J57	left 0% right 0%	1.0	+
J68	left 21.53% right 13.89%	.5	+
J69	left 6.94% right 6.94%	.5	+

N = 6 $p < .001$

Figure 54

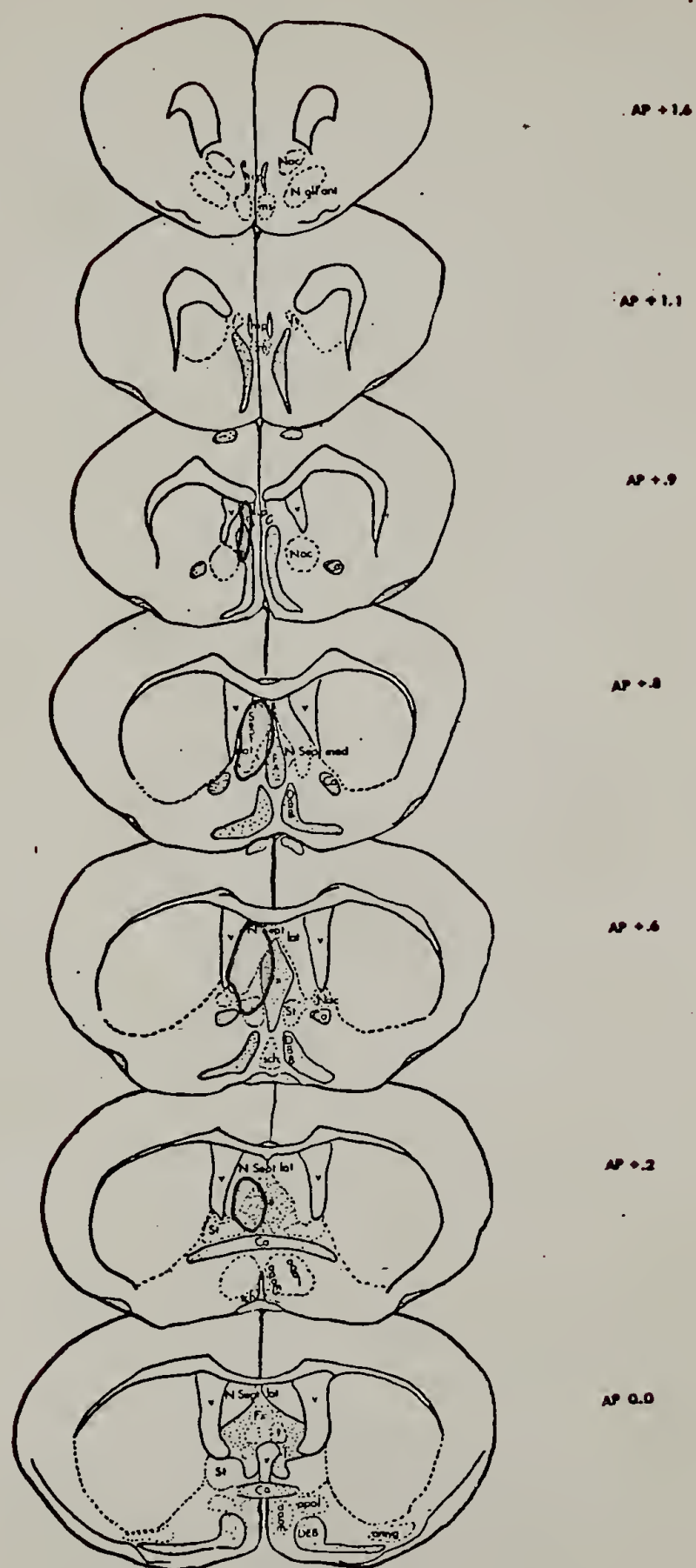
J48 S/OB



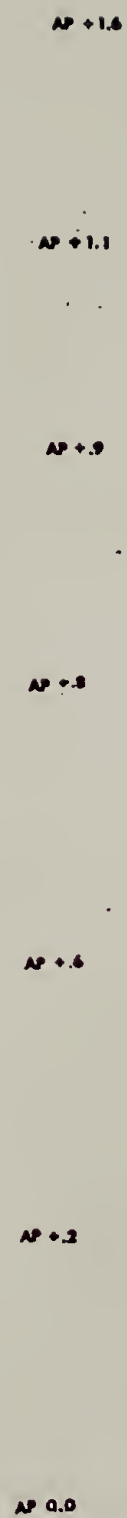
J55 S/OB



Figure 56
J57 S/OB



J68 S/OB



J69 S/OB



neuroanatomical connections interconnecting septum, amygdala and olfactory bulbs argues against such a view.

Since septal lesions enhance olfactory discrimination in mice (Carlson and Vallente, 1974) and in rats (Vom Saal et al, 1975) it was been hypothesized by the latter authors that the septum has an inhibitory influence on the olfactory bulbs. One possible interpretation of the present data might be that septal lesions release the olfactory bulbs from tonic inhibition and thereby allowing the bulbs to have a more powerful excitatory effect on the corticomедial nuclei of the amygdala through the lateral olfactory tract. Thus, septal hyper-reactivity may involve a change in the activity of the amygdala through its indirect connections via the olfactory tubercle.

Olfactory aspiration alone have been reported to produce hyper-irritability in rats (Douglas, Isaacson and Moss, 1968). This observation has not been substantiated in mice in our laboratory however mice with OB lesions appear normal in this regard. Moreover, recent evidence (Cain, 1974) demonstrates that the critical region involved in this type of hyper-irritability lies caudal to the olfactory bulbs within the olfactory penducle or as far caudal as the anterior olfactory nucleus. The results of this experiment demonstrate the existence of another neural system relevant to the hyper-reactivity induced by septal lesions, It is argued that the suppressant effects of both amygdaloid and olfactory bulb

destruction on septal hyper-reactivity are not independent. Furthermore, it is hypothesized that septal lesions disinhibit neurons in olfactory structures whose activity alters amygdaloid function through the lateral olfactory tract. In this way, the amygdala's role in septal lesion-induced hyper-reactivity might involve septo-olfactory-amygdaloid connections rather than direct fiber terminations between septum and amygdala.

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