THE EFFECT OF CENTRAL DOPAMINE CELL BODY LESIONS ON STIMULATION-INDUCED FEEDING

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ABSTRACT

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The Effect of Central Dopamine Cell Body Lesions
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Animals were implanted with a stimulating electrode in the perifornical area of the LHA and an ipsilateral lesioning electrode aimed at either the zona compacta of the substantia nigra (A-9), the ventral tegmental area of Tsai (A-9.5), or the dorsal aspect of the interpeduncular nucleus (A-10). Animals that ate when stimulated at the hypothalamic site were tested daily until feeding thresholds were stable and then lesioned. Post-lesion testing began 24 hours later and was continued for periods up to four weeks. Animals with ventral tegmental lesions which presumably damaged fibers both from the nigrostriatal system cell bodies (A-9) and the mesolimbic and mesocortical system cell bodies (A-10) had markedly elevated thresholds. Animals with extensive damage exclusively to A-9 cell bodies showed no disruption of stimulation-induced feeding while animals with A-10 cell body damage that spared the A-9 cell bodies did show disruption. While these data cannot rule out the hypothesis that damage to the nigrostriatal DA system is the critical factor causing the aphagia seen in the classical "lateral hypothalamic syndrome", they do suggest it should be re-examined.

ACKNOWLEDGEMENTS

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INTRODUCTION

Textbooks taught for many years that the hypothalamus contained two centers involved with ingestive behavior - a center located in the lateral hypothalamus thought to activate feeding and a center located in the ventromedial hypothalamus thought to inhibit feeding. By the late 1960's, it became evident that the concept of the lateral hypothalamus (LHA) as a feeding "center" (Anand & Brobeck, 1951a) was no longer tenable. Although it had been shown that electrical (Miller, 1957; Margules & Olds, 1962), or chemical (Grossman, 1960, 1962; Miller, 1965; Leibowitz, 1970) stimulation of the LHA would induce vigorous eating in sated animals, and that bilateral lesions in this area would result in severe and long lasting aphagia and adipsia (Anand & Brobeck, 1951b; Teitelbaum & Stellar, 1954), eating can also be influenced by stimulation or lesions of a number of extra-hypothalamic sites. Feeding responses in the rat can be induced by stimulation of the cerebellum (Ball, Micco, & Berntson, 1974) and by stimulation of the periaqueductal gray (Clavier, 1974) while rebound feeding has been observed following stimulation of the hippocampus (Milgram, 1969) and the septum (Altman & Wishart, 1971). Stimulation of the rostral fastigial nucleus of the cerebellum (Reis, Doba, & Nathan, 1973), the ventral tegmental area, the ventro-lateral boundary of the central gray (Wyrwicka & Doty, 1966), and the medulla (Berntson & Hughes, 1974) has been shown to induce feeding in the cat. Robinson and Mishkin (1968) induced feeding

in the monkey by stimulation of the pre-optic region, the substantia innominata, dorsal and posterior hypothalamus, medial hypothalamus, midline thalamus, central gray, anterior internal capsule, putamen, and stria terminalis. Complementing these data, Morgane (1961a) had shown earlier that bilateral lesions of the globi pallidi in the rat produced aphagia and adipsia, and Parker and Feldman (1967) had found a similar deficit with lesions in the mesencephalic reticular formation at the level of the red nucleus. Rice and Campbell (1973) showed that neocortical ablations can disrupt feeding induced by electrical stimulation of the LHA. Booth (1967) found that, while chemical stimulation of the LHA could induce eating, the best sites for eliciting feeding are located outside the boundaries of this area. Taken together, these data indicate that, rather than being a feeding "center", the LHA is a "point of convergence" (Ehrlich, 1964) or a "bridge" (Morgane, 1969) over which pass the fibers of widespread neural circuits which subserve or are crucial to ingestive behavior.

Early work in chemical stimulation focussed on norepinephrine (NE) as the important neurotransmitter involved in feeding. Grossman (1960, 1962) delivered NE through indwelling cannulas directly to the LHA of sated rats. With a latency of five to eight minutes, the animals began eating. The average consumption of dry food was approximately 4.3 grams. Booth (1967) found NE to be

effective in eliciting eating not only when injected into the LHA but also when injected into other hypothalamic sites. Margules, Lewis, Dragovitch, and Margules (1972) also induced feeding in the rat with NE. They related the effectiveness of NE to the circadian rhythm and contended that NE's stimulant effect upon eating was seen in the light but not in the dark cycle. Miller, Gottesman, and Emery (1964) showed that as little as .8 µg of NE in the hypothalamus could elicit feeding, although their optimal dose was 21.8 µg. Recent studies have examined the effects of more physiological doses of NE. Leibowitz (1974) showed that as little as 33 ng of NE in the paraventricular nucleus can elicit feeding. Ritter and Epstein (1974) found that an even smaller dose (15 picomoles) of NE can increase meal size by 180%. Ritter, Wise, and Stein (1975) showed that intraventricular injections of clonodine, (a noradrenergic receptor stimulant) are as much as 100 times more effective than NE itself in evoking feeding. Moreover, the feeding response elicited by NE and clonodine can be blocked by the α-adrenergic receptor blocker phentolamine (Slangen & Miller, 1969; Leibowitz, 1970). Inhibition of NE reuptake by desmethylimipramine or lidocaine can potentiate the NE-induced eating effect (Slangen & Miller, 1969).

Neurotransmitters other than NE do not seem to be directly effective in eliciting feeding. Slangen and Miller (1969) were unable to induce eating with an infusion

of serotonin. They did, however, find a moderate though delayed feeding response to dopamine (DA); this delayed reaction was felt to represent the time required for the conversion of DA to NE. Ritter, Wise, and Stein (1975) found that intraventricular infusions of DA or a DA receptor stimulant (apomorphine) did not facilitate eating. Eating has been reported after cholinergic stimulation (Fisher & Coury, 1962; Myers, 1964; Blanchard & Blanchard, 1966; Coury, 1967; Myers & Sharpe, 1968; Smith, King, & Hoebel, 1970), but this appears to be a rebound effect due to ACH receptor stimulation (Wise, 1972). Thus, the chemical stimulation literature has pointed to a noradrenergic mediation of feeding. Since it was known that major NE fiber systems pass through the LHA in the medial forebrain bundle (MFB) (Ungerstedt, 1971; Lindvall & Björklund, 1974), it was not unreasonable to suppose that lesions of the LHA disrupted feeding because they damaged NE fibers which mediate feeding. Recent evidence, however, does not support this interpretation. Noting that DA fibers project with NE fibers through the LHA and that the lesions of the LHA which disrupt feeding would almost certainly have interrupted both fiber pathways, Ungerstedt (1971) re-examined the view that NE is the major neurotransmitter in the control of feeding. He found evidence for the alternative hypothesis that it is DA and not NE fibers in the LHA that are crucial for feeding. He argued that the aphagia and

adipsia produced by interruption of these DA fibers is similar to that seen in the classical "lateral hypothalamic syndrome" described by Teitelbaum and Epstein (1962) and Epstein (1971) and he proposed that damage to the nigrostriatal rather than to the mesolimbic DA pathway causes the syndrome of aphagia and adipsia.

Ungerstedt (1971) advanced this alternative hypothesis to explain data which emerged from his studies of electrolytic and chemical (6-hydroxydopamine) lesions of sites along the DA and NE pathways. He showed that bilateral lesions that were caudal to the DA cell groups and that interrupted major ascending NE fibers produced no more than one day of hypophagia and hypodipsia, despite almost complete disappearance of NE terminals in the forebrain. In contrast, a prolonged aphagia and adipsia, as well as an inability to maintain exploratory behavior and curiosity, were associated with bilateral lesions of the DA fiber systems. Aphagia and adipsia developed whether the lesions were aimed at the cell bodies of the nigrostriatal pathway located in the zona compacta of the substantia nigra (A-9), at the aggregated DA fiber bundle in the ventral tegmental area of Tsai, or at the LHA where this bundle passes in its projection to the internal capsule.

Ungerstedt's conclusion that the DA, and not the NE fibers, that project through the LHA are the critical fibers for feeding seems to explain a number of obser-

vations that have appeared in the literature. Morgane (1961b) showed that stimulation of the far-lateral hypothalamus elicited basic feeding responses from sated rats. It is in the far-lateral portion of the LHA that DA fibers pass (Ungerstedt, 1971; Lindvall & Björklund, 1974). Gold (1967) compared unilateral to bilaterally asymmetrical lesions and identified a rostral "critical forebrain area" that, if damaged, would result in a prolonged aphagia and adipsia. This area included the ventro-medial quarter of the internal capsule, a far-lateral portion of the hypothalamus, and the medial aspect of the globus pallidus. All these loci are traversed by DA fibers (Ungerstedt, 1971; Lindvall & Björklund, 1974). The work of Albert, Storlien, Wood, and Ehman (1970) also supports the notion that the trajectory of fibers crucial for feeding is through the MFB and toward the internal capsule. They found that knife cuts or procaine injections at various points in the LHA, the internal capsule, or the globus pallidus caused aphagia and adipsia. All these reports have associated hypothalamic and extrahypothalamic loci with feeding and all these loci lie along the trajectory of DA fibers which ascend through the LHA towards the internal capsule.

It is known that fiber pathways other than those of NE fibers (Kreig, 1932; Nauta, 1958; Shute & Lewis, 1967; Ungerstedt, 1971) overlap with the DA pathway interrupted by Ungerstedt's lesions and it may be argued that it is

damage to these non-catecholamine fibers which produces aphagia and adipsia. There is, however, evidence involving the neurotoxin 6-hydroxydopamine (6-OHDA) which indicates that it is specific damage to CA systems that causes aphagia and adipsia. It has been shown that 6-OHDA can be used to selectively damage CA neurons and spare other systems (Ungerstedt, 1968; Breese & Traylor, 1970; Uretsky & Iversen, 1970; Jacks, deChamplain; & Cordeau, 1972; Hökfelt & Ungerstedt, 1973). This finding must be accepted with some degree of caution, however, since it has been noted that a number of factors, including the route of administration and the concentrations employed, affect the selectivity of 6-OHDA's action, and the agent can cause considerable non-specific damage (Poirier, Langelier, Roberge, Boucher, & Kitsikis, 1972; Butcher & Hodge, 1973; Sotelo, Javoy, Agid, & Glowinski, 1973; Agid, Javoy, Glowinski, Bouvet, & Sotelo, 1973). Mindful of these problems Jacks et al. (1972) and Stricker and Zigmond (1974) were able to restrict damage to CA neurons by careful intraventricular infusion of 6-OHDA. Animals so treated became aphagic and adipsic. Jacks et al. (1972) reported extensive bioassay data which showed that the levels of serotonin (5HT), acetylcholine (ACH), aminobutyric acid (GABA), glutamic acid, glutamine, glycine, aspartic acid, taurine, alanine, threonine, and serine were not significantly lowered by the 6-OHDA infusion. Only NE and DA levels were altered and these were

reduced to levels as low as 13% and 16% of normal, respectively. On the basis of these data, it seems safe to conclude that damage to CA systems results in aphagia and adipsia and that Ungerstedt's intracerebral injections of 6-OHDA achieved their effects because of CA depletion and not because of the small degree of non-specific damage which occurs at the tip of the cannula when injecting into tissue. In sum, it would appear that it is a CA system which is critically implicated in the neural control of feeding.

Ungerstedt's (1971) lesion data indicated that, of the two catecholamines, it is DA rather than NE that is involved in feeding. In support of this view, manipulations designed to protect noradrenergic neurons from 6-OHDA by preventing the neurotoxin's uptake into these cells, and, at the same time, potentiating the neurotoxin's effect upon DA neurons by inhibiting monoamine oxidase, indicate that DA is more crucially involved in feeding than is NE. For example, Stricker and Zigmond (1974) pretreated animals with desmethylimipramine (DMI) which blocks uptake into NE, but not DA, terminals (Horn, Coyle, & Snyder, 1971) and with pargyline, a monoamine oxidase inhibitor, and then injected 6-OHDA into the ventricles. The animals became severely aphagic and adipsic and, like "lateral hypothalamic" animals, would have died had they not been supported by intragastric intubation. Earlier findings by Fibiger, Lonsbury, Cooper, and Lytle (1972); Fibiger,

Zis, and McGeer (1973) are consistent with these results. Their pretreatment program included only the use of a monoamine oxidase inhibitor (tranylcypromine) and no special provision for the protection of NE was made. They showed that DA, rather than NE, depletion is necessary if a significant feeding deficit is to occur. There seems, thus, to be good evidence favouring Ungerstedt's suggestion that DA, not NE, fibers are important in the control of feeding.

Ungerstedt further suggested that the aphagia and adipsia consequent upon DA depletion are similar to what is seen in the classical "lateral hypothalamic syndrome" described by Teitelbaum and Epstein (1962) and by Epstein (1971). This syndrome involves an aphagia and adipsia so severe that the animal may die before spontaneous feeding and drinking are resumed. If supported by tube-feeding, these animals can recover and do so in a fixed sequence; first, eating only wet and very palatable foods and, later, regaining the ability to regulate nutritional needs on dry food. Certain deficits persist, however. Weight is maintained at a lower level or "set-point" that that maintained by unlesioned animals (Powley & Keesey, 1970). The "lateral hypothalamic" animal does not recover the ability to regulate water intake and does not respond to hydrational challenges (Epstein & Teitelbaum, 1964); it drinks only to wash down dry food (Kissileff & Epstein, 1969). In addition, it does not

respond, as does the normal animal, to short-term glucopriyation; it does not increase its food intake following injections of 2-deoxy-D-glucose (Epstein & Teitelbaum, 1967).

Ungerstedt (1971) reported that most of his DA-depleted animals became completely aphagic and adipsic and died if not supported by gastric intubation. He made no more subtle comparisons of his animals to the "lateral hypothalamic" animal but others have expanded the comparison. Zigmond and Stricker (1973) found that, if maintained by tube-feeding, even a severely DA-depleted animal could recover; moreover, it would follow the same sequence of recovery that Teitelbaum and Epstein (1962) described for their lateral hypothalamically damaged animals. In addition to the similarity of the recovery sequence, Zigmond and Stricker (1972), Fibiger, Phillips, and Clouston (1973), and Marshall, Richardson, and Teitelbaum (1974) showed that several of the regulatory deficits which persist in recovered "lateral hypothalamic" animals also persist in DA-depleted animals. Thus, both types of animals showed lack of response to short-term glucoprivation, both maintained their body weights at lower "set-points" than did controls, both failed to drink unless food was present (prandial drinking), both failed to increase their water intake following injections of hypertonic saline, and both showed evidence of sensory neglect by failing to orient to smell and touch stimuli.

Marshall et al. (1974) noted two very significant differences. First, the aphagic rats with nigrostriatal damage ate a small quantity of food if sufficiently activated (tail pinch) and, second, they were less finicky and accepted a quinine-adulterated diet more readily than did the LHA animals. Nevertheless, there is good support for Ungerstedt's second proposition that, in most respects, the DA-depleted animal resembles the "lateral hypothalamic" animal.

Ungerstedt argued that, of the two DA pathways it is the nigrostriatal rather than the mesolimbic system which supports ingestive behavior. He showed that an electrolytic lesion or an injection of 6-OHDA placed in the mesolimbic bundle just rostral to its separation from the nigrostriatal pathway resulted in only one or two days of hypophagia and hypodipsia, despite virtually complete and permanent disappearance of the DA terminals in the nucleus accumbens and in the olfactory tubercle. Participation of the mesolimbic system in the support of feeding would seem, therefore, to be ruled out. On the other hand, an injection of 6-8 µg of 6-OHDA in a 3-4 µl volume into the substantia nigra (A-9), the origin of the nigrostriatal pathway, caused a severe and long-lasting aphagia and adipsia. A critical problem with this part of Ungerstedt's study, is that the 6-OHDA probably damaged other DA systems. Consideration of the close anatomical disposition of the DA systems (Ungerstedt, 1971) shows

why this could be so. The cell bodies of the mesolimbic system are dorsal to the interpeduncular nucleus (A-10) and lie only 1 to 1.5 mm away from the zona compacta of the substantia nigra (A-9) where the cell bodies of the nigrostriatal system are located. Fibers from the two cell groups (A-9 and A-10) ascend together through the ventral tegmental area of Tsai and through the LHA. The mesolimbic pathway seems to take a somewhat more medial position, while the nigrostriatal pathway takes a more lateral position. Divergence of the two systems occurs only at the tip of the internal capsule. At this point, the nigrostriatal fibers enter the capsule and spread laterally through the globus pallidus to terminate in the caudate nucleus. The mesolimbic fibers do not enter the capsule but continue rostrally, sending one branch to the nucleus accumbens and the dorsal part of the nucleus interstitialis stria terminalis, and sending a second branch to the olfactory tubercle. In addition, fibers passing with the mesolimbic system have recently been shown to innervate the lateral septum (Lindvall, 1975) and neocortex (Lindvall & Björklund, 1974).

Ungerstedt (1971) directed his bilateral cell body lesions only to the A-9 and not the A-10 group. Only 6-OHDA and not electrolytic lesions were made. The problem with these lesions is that the infusion of the neurotoxin was of such volume (3-4 µl) that leakage to, and consequent damage of, the nearby A-10 cell group could have

occurred. All Ungerstedt's other DA lesions were aimed at the aggregated DA fiber bundle or at the mesolimbic pathway. The effects of damage to fibers arising from A-9, therefore, were possibly confounded with the effects of damage to nearby fibers arising from A-10.

The criticism that systems arising from A-9 and A-10 were both affected by Ungerstedt's lesions can also be made of the lesions of Fibiger et al. (1972, 1973) and of Marshall et al. (1974). These investigators also attributed feeding deficits to nigrostriatal damage produced by intracerebral infusions of 6-OHDA. As in Ungerstedt's studies, the volume of the infusions was large enough to have spread to both the A-9 and the A-10 cell groups. Zigmond and Stricker (1972) assumed that nigrostriatal damage was the important factor in their studies, even though they infused 6-OHDA via the intraventricular route, and would most certainly have damaged all of the DA systems. No previously reported lesion has been clearly restricted to one or other of the two DA cell body groups and, therefore, the results of damage to one DA system have probably been confounded with undetermined damage to the other.

A further problem for Ungerstedt's hypothesis is that not all animals with substantia nigra cell body lesions become aphagic and adipsic. In Ungerstedt's study only 16 out of 30 animals given this lesion showed the deficit. Zigmond and Stricker (1973) and Creese and Iversen (1975)

also reported that some of their animals with 6-OHDA lesions of the substantia nigra did not become aphagic and adipsic even though a 90% depletion of striatal DA and a 70% depletion of hypothalamic NE was achieved. Creese and Iversen thought that the nigrostriatal pathway is not homogenous and that their lesions must have spared some critical fibers in it. An alternative explanation is that their lesions spared the nearby A-10 system, or some crucial part of it, and that it is these fibers, rather than the nigrostriatal pathway, that are involved in ingestive behaviors.

The importance of these criticisms becomes evident when one considers the findings of recent mapping studies which show that DA fibers other than those of the mesolimbic system arise from the area of the A-10 cell group. Thierry, Stinus, Blanc, and Glowinski (1973) and Berger, Tassin, Blanc, Mayne, and Thierry (1974) have demonstrated the existence of DA-containing terminals in the rat cortex. The DA contained in these terminals acts as a neurotransmitter in its own right and does not exist merely as a precursor of NE (Blanc, Glowinski, Stinus, & Thierry, 1973; Tassin, Thierry, Blanc, & Glowinski, 1974).

Using the glyoxylic acid fluorescence method, Lindvall, Björklund, Moore, and Stenevi (1974) also saw DA terminals in the deep layers of the frontal cortex and identified other DA terminals in the anterior cingulate cortex, the ventral part of the entorhinal cortex, and in

the transition zone between neocortex and piriform cortex along the rhinal fissure. The cell bodies of the fibers ascending to the frontal cortex were found to be located in the A-10 area. Lindvall (1975), using the same glyoxylic acid fluorescence method, identified another DA system with cell bodies in A-10 and terminals in the lateral septal nucleus. In view of these findings, a re-assessment of the individual contributions of the various DA systems seems to be important.

The purpose of the present study was to assess the effect upon feeding of small, discrete, electrolytic lesions which would be restricted as much as possible to the A-9 or to the A-10 cell bodies or to the intermediate group of cell bodies and fibers in the ventral tegmental area of Tsai which Ungerstedt (personal communication) has designated A-9.5.

METHOD

A major difficulty in any study involving aphagia and adipsia is the maintenance of a healthy animal after bilateral lesions. A unilateral lesion would, in some measure, avoid this problem. Gold (1966) showed that animals with unilateral lesions of the LHA do develop the classical "lateral hypothalamic syndrome" but on a more restricted scale; the duration of the aphagia and adipsia is reduced to a few days. Three observations in the literature suggest another way in which a unilateral preparation might be used. First, unilateral stimulation of the LHA is sufficient to induce feeding (Miller, 1957; Margules & Olds, 1962). Second, the effects of CA depletion are seen not only on natural feeding but on stimulation-induced eating (Phillips & Fibiger, 1973). Third, the ascending DA pathways are largely uncrossed (Ungerstedt, 1971; Lindvall & Björklund, 1974). If DA cell bodies were lesioned ipsilateral and caudal to that point in the LHA where electrical stimulation induces feeding, then some conclusions could be drawn about the effects of a DA lesion on stimulation-induced eating. In a recent review of the literature, Wise (1974) concluded that in many important respects stimulation-induced behavior is like that aroused by normal hunger. There is justification, then, for extending to natural feeding any inferences made concerning the effects of DA cell body lesions on stimulation-induced feeding.

Subjects were male albino rats of the Sprague-Dawley

strain (Bio Breeding, Ottawa). They weighed 250-350 grams at the time of surgery. The animals were individually housed and had free access to Purina Lab Chow pellets and water throughout the term of the experiment.

All animals were implanted under barbiturate-chloral hydrate anaesthesia with a bipolar stainless steel stimulating electrode, .01 in. diameter, insulated except at the cross-section of the electrode tip. An indifferent electrode was wrapped around one of the stainless steel screws which anchored the dental-cement assembly to the skull. Stereotaxic coordinates were: .8 mm posterior to Bregma, 1.5 mm lateral to the midline, and 8.5 mm ventral to the skull surface with the incisor bar 3.2 mm above the interaural line. In addition to the stimulating electrode, each animal was implanted with a lesioning electrode made of .01 in. stainless steel wire which was insulated with varnish (Formvar, Canadian General Electric) except at the cross-section of the electrode tip. This lesioning electrode was aimed at one of three areas ipsilateral to the stimulating electrode: the zona compacta of the substantia nigra (A-9: 3.6 mm posterior to Bregma, 2.1 mm lateral to the midline, and 8.2 mm from the skull surface), the ventral tegmental area of Tsai (A-9.5: 3.6 mm posterior to Bregma, 1.5 mm lateral to the midline, and 8.7 mm ventral to the skull surface), or dorsal to the interpenduncular nucleus (A-10: 4.0 mm posterior to Bregma, .5 mm lateral to the midline, and 9.0 mm ventral to the skull surface). In each case, the incisor bar was set

according to the method of Krieg (1932) at 3.2 mm above the interaural line. The notation for the major DA groups was that of Dahlstrom and Fuxe (1965); A-9.5 was used to designate the DA cells intermediate to A-9 and A-10, in the ventral tegmental area of Tsai.

After one week of recovery from surgery, all animals were screened for evidence of stimulation-induced eating. Animals that showed this behavior were retained in the experiment and two measures of the strength of the stimulation-induced behavior were taken. The first measure was a daily eating threshold stated in terms of current intensity. Wise (1968) indicated that, after initial training in the stimulation-induced feeding situation, eating threshold remained very stable over time although it was sensitive to changes in experimental conditions. Threshold was determined according to the method described by Wise (1968) and Soper and Wise (1971). The second measure was the number of trials out of ten in which the animal ate when the current was fixed at 20% above the daily threshold. Fixed current trials have been used by Valenstein, Cox, and Kakolewski (1969) and by Phillips and Fibiger (1973). Subjects were tested in a wooden box measuring 36 x 25 x 38 cm which had a wire mesh floor and a plexiglass observation window along one of the long sides. Food pellets were liberally scattered over the floor. The room was dimly lighted but no special arrangements were made to attenuate noise. Stimulation was delivered by a 60 Hz. sine-wave stimulator on a twenty-

second "on" twenty-second "off" schedule. The animals were tested at about the same hour on successive days. The eating threshold was considered to have stabilized when the variation over 5 consecutive days was no more than 1.5 μa and was not consistently in one direction.

Pilot work had suggested that lesions at A-10, but not at A-9, produce a substantial attenuation of lever pressing for electrical brain stimulation reward. Therefore, the A-10 animals that showed stimulation-induced eating were also tested for evidence of self-stimulation. These animals were shaped to lever-press in a box measuring 34 x 26 x 37 cm, which had a 10 x 10.5 x 37 cm alcove projecting from one wall in which the lever was mounted 7.5 cm from the floor. Each lever-press delivered a .5 sec pulse of 60 Hz. sine-wave intracranial stimulation. Animals were tested daily for 5 min, after a preliminary 5 min "warm-up" period, until the variation in the number of reinforcements earned was no more than 10% over 5 consecutive days and was not consistently in one direction. The score was the number of reinforcements obtained in 5 min. A later squad of animals was tested for 15 min but with the same warm-up period. They, too, were required to meet the same stabilization requirements but, in these cases, the score was the number of reinforcements obtained in 15 minutes.

When an animal's eating threshold and self-stimulation rates both met the stabilization criteria, it was lesioned electrolytically with a Grass D.C. Constant

Current lesion maker, at 1 ma for 10 sec. Threshold testing was resumed 24 hours after lesioning and the two measures of stimulation-induced eating were again obtained. Post-lesion fixed current trials were conducted at the highest level required to elicit eating during the five pre-lesion days of testing at stabilized threshold. Testing for self-stimulation was also resumed 24 hours after lesioning and was conducted in the same manner as in the pre-lesion tests using the same current levels. All animals were tested for at least six days after lesioning. If no return to the pre-lesion eating threshold was seen or if there was no recovery of self-stimulation rates then the testing period was extended up to four weeks.

Upon completion of testing, all animals were prepared for determination of the lesion size and placement according to the Falck-Hillarp method (Falck, 1962; Falck, Hillarp, Thieme, & Torp, 1962). The animals were killed by cervical fracture and the brains were rapidly removed from the skulls. The appropriate frontal section was removed, trimmed, and quickly frozen first in liquid propane then in liquid nitrogen. Sections were freeze-dried for 3 days, then reacted with paraformaldehyde vapour at 80° C for 75 min (relative humidity=70%). The sections were vacuum-embedded in paraffin, sliced at 10 μ, placed on uncoated slides, and inspected under the fluorescence microscope.

Because this experiment was part of a larger series of studies which required that the implantations be on the

plane of deGroot (incisor bar 5 mm above the interaural line), a mapping of the A-9 and A-10 cell bodies as seen from this angle was necessary since Ungerstedt's maps were based on the König and Klippel atlas (1963) and represented the A-9 and A-10 cell bodies in only three frontal sections taken with a level skull. A more detailed mapping of the cell body distribution was obtained by locating the DA cell bodies in a series of sections from unlesioned brains and reconstructing them upon maps prepared by Pellegrino and Cushman (1967), extending from -2.4 to -4.2 mm posterior to Bregma at 20 μ intervals. These maps are presented in Appendix I; they serve as the reference against which lesion extent and locus were assessed.

RESULTS

A total of 25 animals met the stabilization criteria and were lesioned. Four animals lost their skull assemblies before testing was completed and their data were not considered for this study. Three other animals had lesions which were off-target and another did not show any evidence of a lesion. No significant effects were observed in these animals and these data were not analyzed further.

The locus and extent of each animal's lesion was reconstructed on Ungerstedt's (1971) map. Graphs of the day by day pre- and post-lesion eating thresholds and the self-stimulation rates were drawn beside each reconstruction to show the relationship between amount of

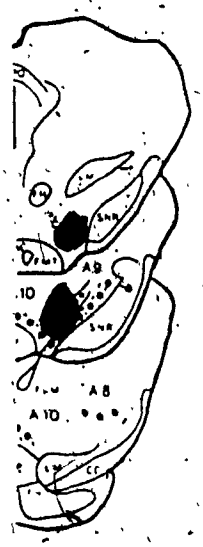
cell body damage and behavioral effects. These data are presented in Figures 1-3. Animals 34A-9, 60A-9, 54A-9.5, 1A-10, 2A-10, 13A-10, 16A-10, 35A-10, 40A-10, 43A-10, and 45A-10 completed the test program after the more detailed maps of the DA cell bodies based on the Pellegrino and Cushman (1967) atlas were prepared. These animals' lesions were reconstructed on these maps as well as on Ungerstedt's maps. The detailed reconstructions are presented in Appendix 2.

Four animals (Nos. 4A-9.5, 11A-9.5, 12A-9.5, and 54A-9.5) had lesions of the ventral tegmental area of Tsai where the fibers from the A-9 and A-10 cell groups aggregate. It should be noted that, although No. 54A-9.5 sustained a moderate loss of A-9 cell bodies, it was classified with the A-9.5 group because the anterior portion of the lesion was medial to the zona compacta; this resulted in considerable encroachment on the mixed DA fiber bundle. The reconstructions and behavioral data from these animals are shown in Figure 1.

Each of these animals had elevated thresholds following the lesion. In the case of No. 4A-9.5, the current was raised to double the level that had induced eating prior to the lesion but no evidence of eating was observed for five days after lesioning. On the sixth day, and for another nine days, eating was seen at current levels near baseline. From this point on, however, the threshold increased. Animal No. 12A-9.5 had a convulsion on post-lesion day 3

Figure 1. Reconstructions of ventral tegmental area lesions and graphs of daily stimulation-induced feeding thresholds for animals 4A-9.5, 11A-9.5, 12A-9.5, and 54A-9.5.

4 A9.5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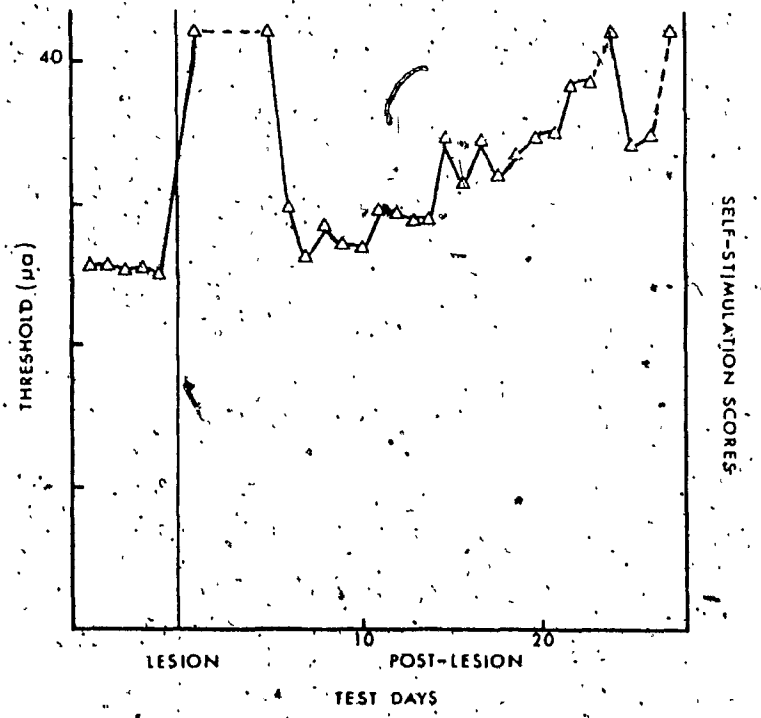
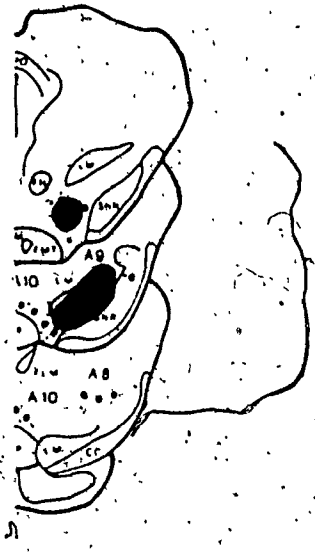
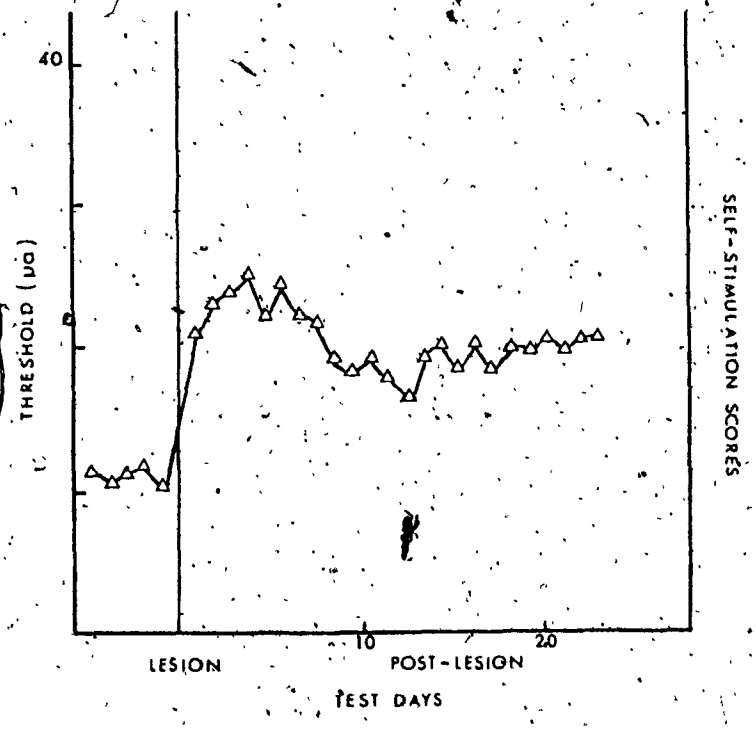
A10
A8


40
THRESHOLD (µa)
SELF-STIMULATION SCORES
10
20
LESION
POST-LESION
TEST DAYS

11 A9.5


A9
A10
A8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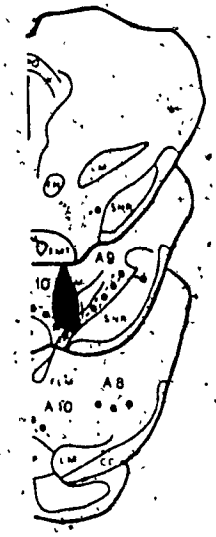
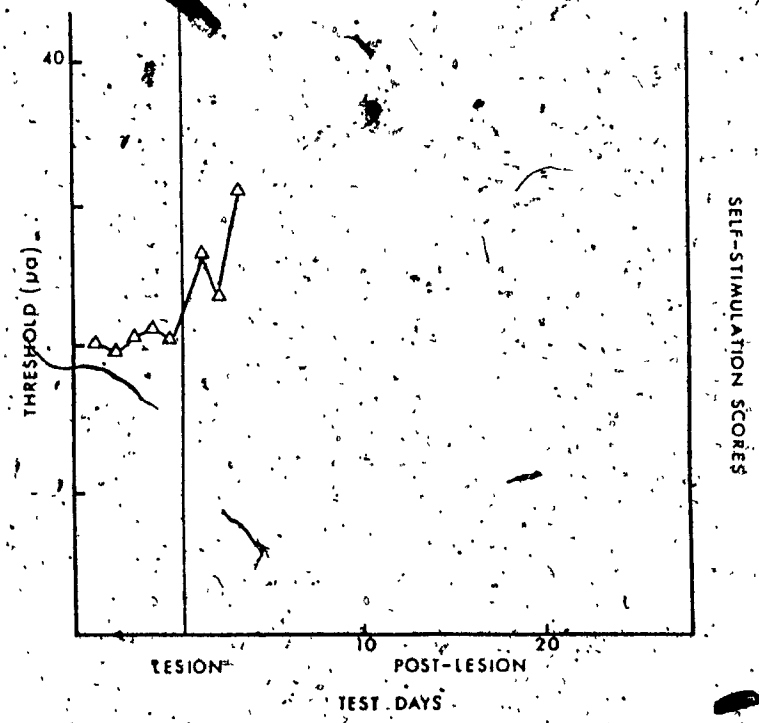
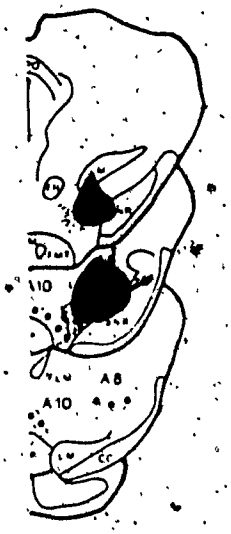
THRESHOLD (µa)
SELF-STIMULATION SCORES
10
20
LESION
POST-LESION
TEST DAYS

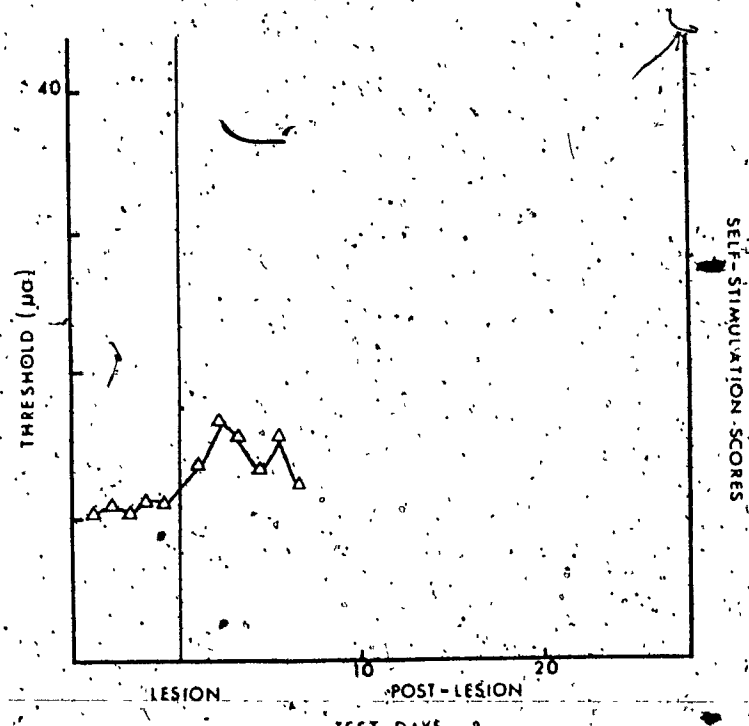
12 A 9.5


A9
A8
A10


40
THRESHOLD (µa)
SELF-STIMULATION SCORES
LESION
10
20
POST-LESION
TEST DAYS

54 A 9.5


A8
A10


40
THRESHOLD (µa)
SELF-STIMULATION SCORES
LESION
10
20
POST-LESION
TEST DAYS

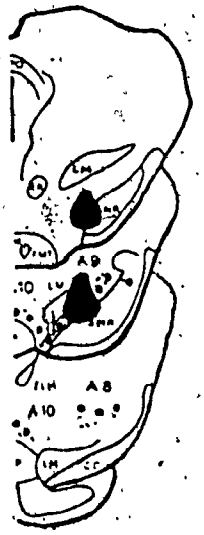
during a series of fixed-current trials and testing was discontinued. However, the data that were obtained indicated a substantial elevation of threshold following lesion.

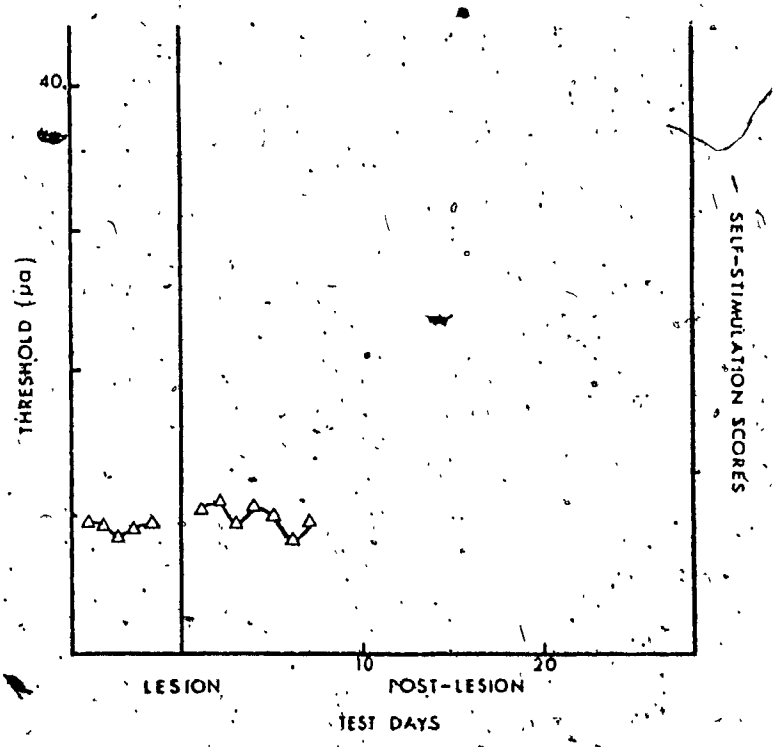
Five animals (Nos. 27A-9, 34A-9, 50A-9, 52A-9, 60A-9) sustained lesions of the A-9 cell group. The data from these animals are presented in Figure 2. Animal 27A-9's lesion was very small and spared about 50% of the A-9 cell bodies. No threshold change was seen. Animals 34A-9 and 50A-9 sustained lesions which were clearly restricted to the area of the A-9 cell bodies and which eliminated virtually all of these DA cell bodies. No change in threshold level was observed in either of these animals. Animal 52A-9's lesion was ventral and No. 60A-9's was anterior and medial to the central cluster of A-9 cell bodies so that encroachment upon the DA fiber bundle might be suspected. Both these animals showed some elevation of threshold.

Variable effects were seen in the eight animals included in the A-10 lesion group (Nos. 1A-10, 2A-10, 13A-10, 16A-10, 35A-10, 40A-10, 43A-10, 45A-10). These data, along with self-stimulation data, are presented in Figure 3. Two animals (Nos. 1A-10 and 43A-10), showed strong lesion effects. Animal 1A-10 was never again observed to eat even when the current was raised to double the level that had induced eating prior to the lesion. After two days, animal 43A-10 began eating in response to stimulation but at

Figure 2. Reconstructions of the lesions of A-9 cell bodies and graphs of daily stimulation-induced feeding thresholds for animals 27A-9, 34A-9, 50A-9, 52A-9, and 60A-9.

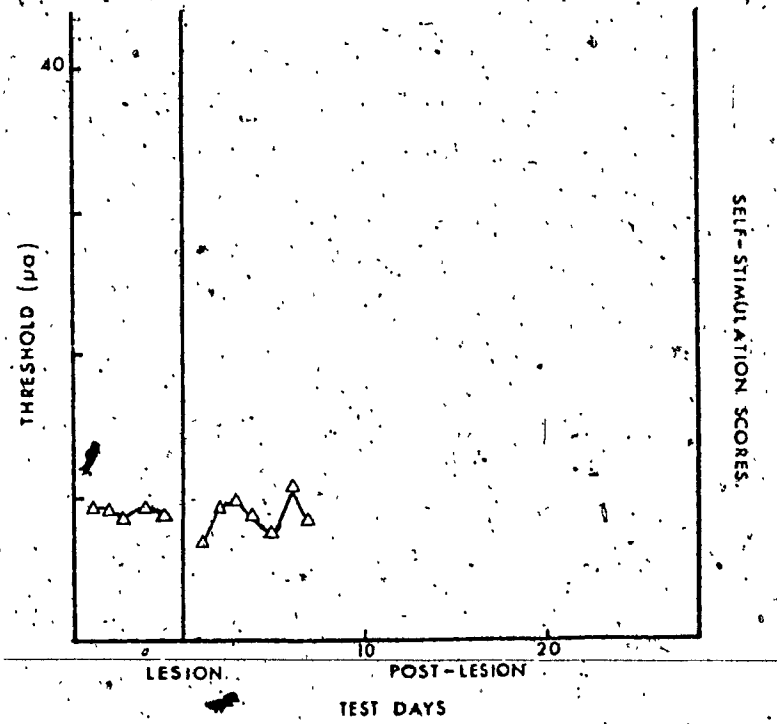
27 A9


LM
A9
A8
A10
LH

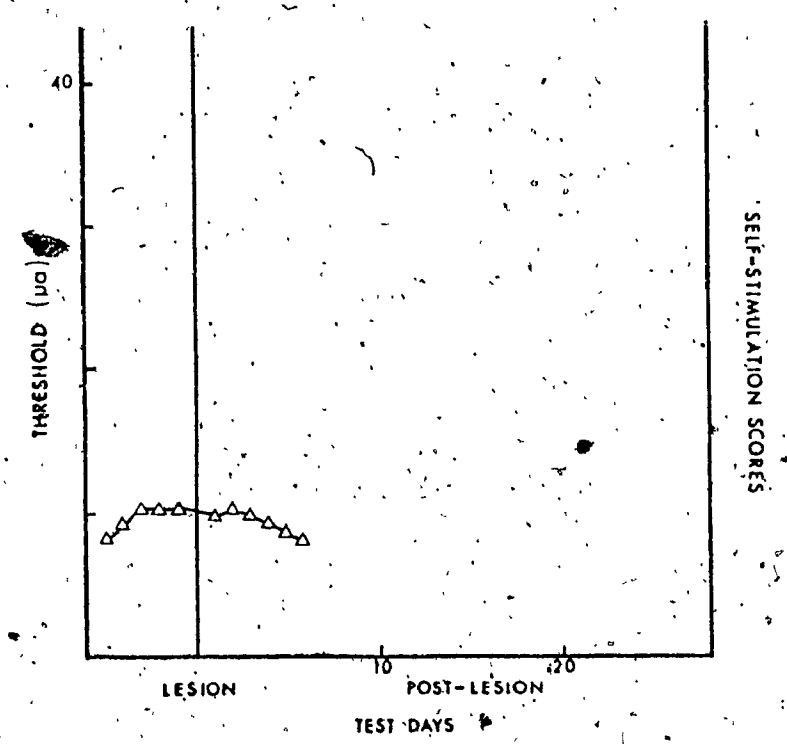

40
THRESHOLD (μa)
SELF-STIMULATION SCORES
10
20
LESION
POST-LESION
TEST DAYS

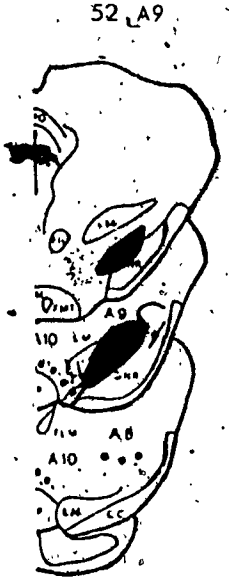
34 A9

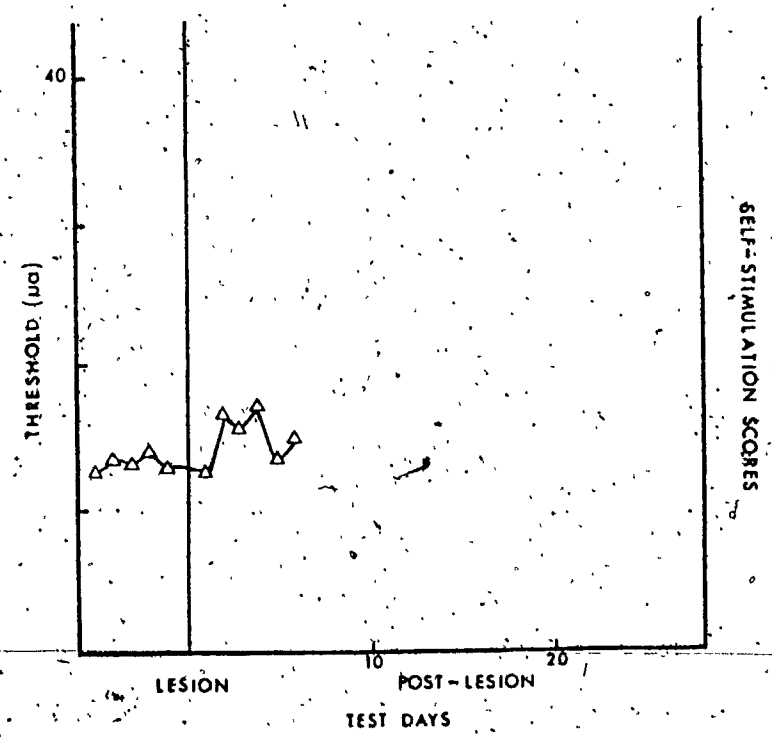

LM
A9
A8
A10
LH


40
THRESHOLD (μa)
SELF-STIMULATION SCORES
10
20
LESION
POST-LESION
TEST DAYS


50 A9
A9
A8
A10


40
THRESHOLD (µa)
10
20
LESION
POST-LESION
TEST DAYS
SELF-STIMULATION SCORES


52 A9
A9
A8
A10


40
THRESHOLD (µa)
10
20
LESION
POST-LESION
TEST DAYS
SELF-STIMULATION SCORES

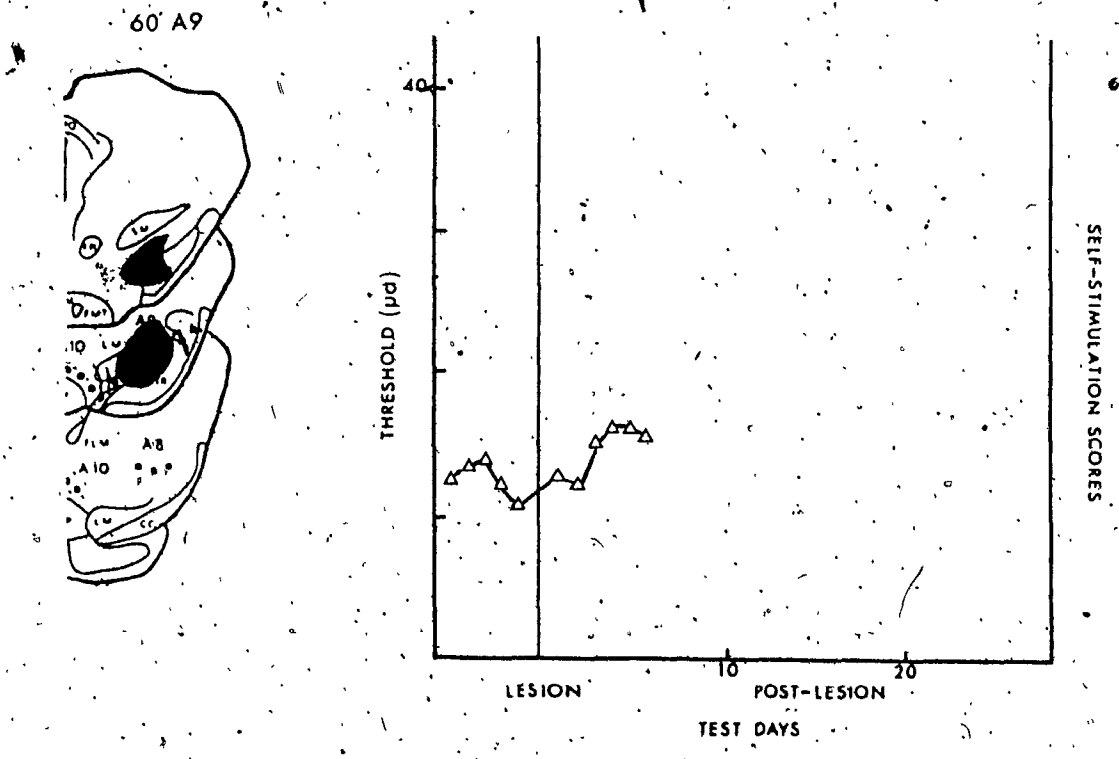
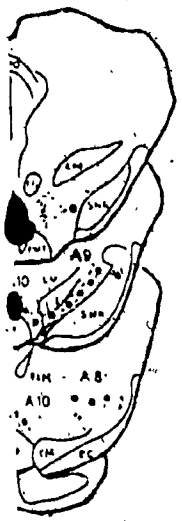
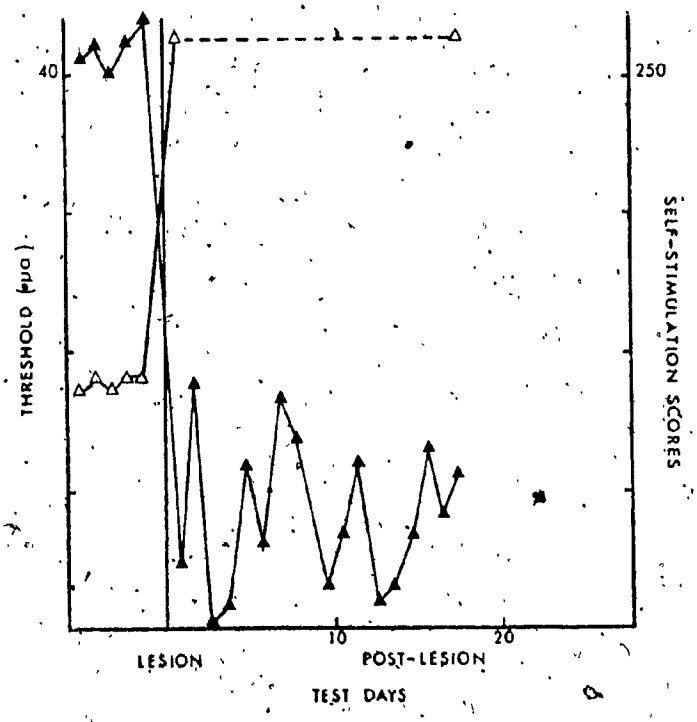

60' A9
40
THRESHOLD (μd)
SELF-STIMULATION SCORES
LESION
10
20
POST-LESION
TEST DAYS

Figure 3. Reconstructions of lesions of the A-10 cell bodies and graphs of daily stimulation-induced feeding thresholds and self-stimulation scores for animals 1A-10, 2A-10, 13A-10, 16A-10, 35A-10, 40A-10, 43A-10, and 45A-10.

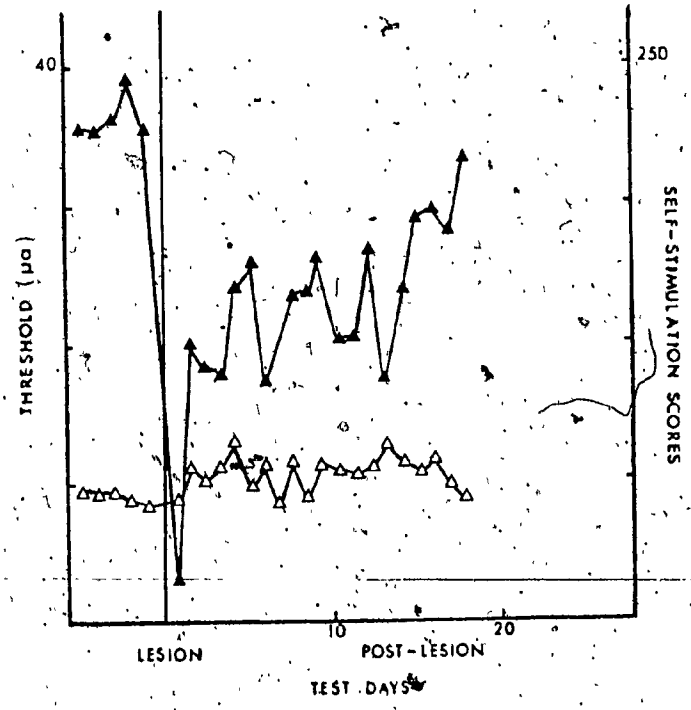
1 A10


AD
FIM
AB
A10
FM
PC

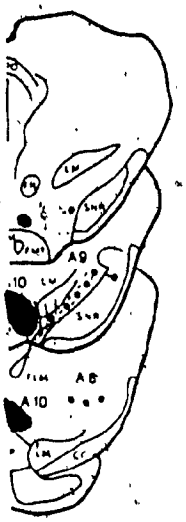

40
250
THRESHOLD (μa)
SELF-STIMULATION SCORES
10
20
LESION
POST-LESION
TEST DAYS

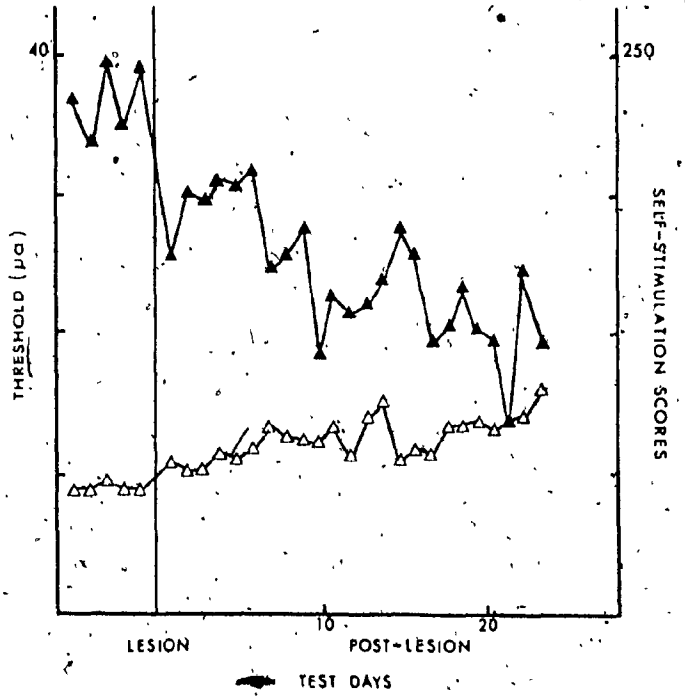
2 A10


AD
FIM
AB
A10
FM
CC

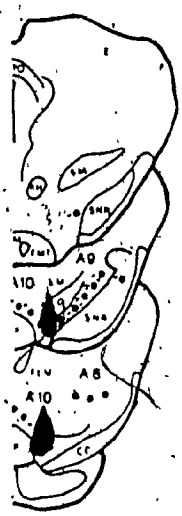

40
250
THRESHOLD (μa)
SELF-STIMULATION SCORES
10
20
LESION
POST-LESION
TEST DAYS

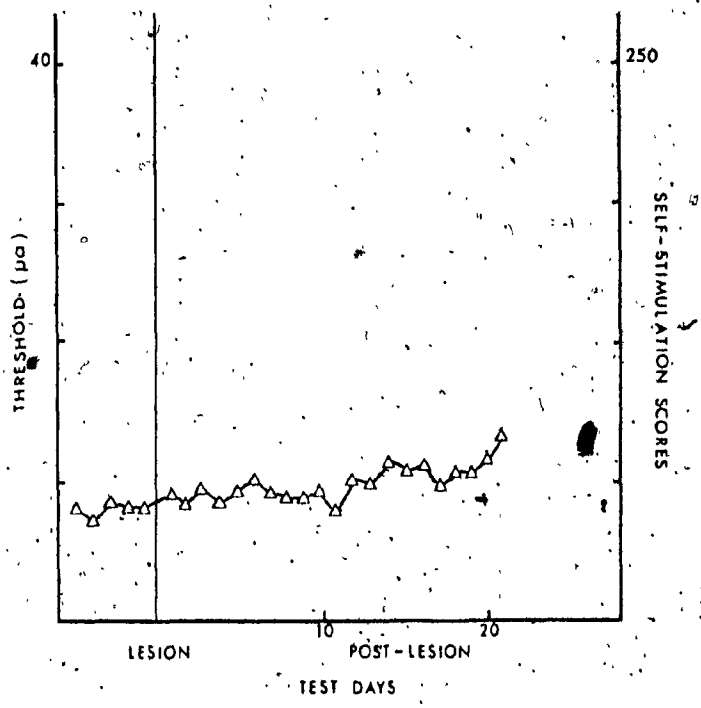
13 A10


LM
SNR
A9
A10
LM
A8
A10
LM
CC

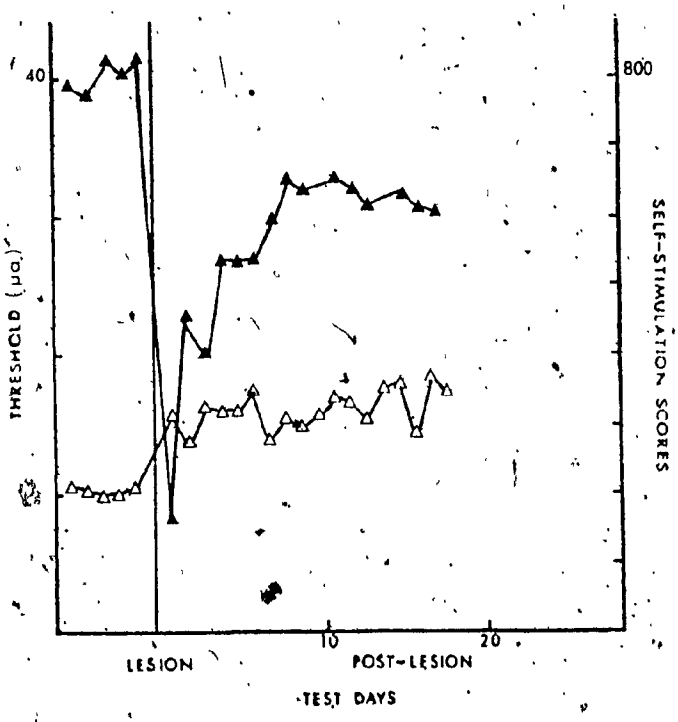

40
250
THRESHOLD (μa)
SELF-STIMULATION SCORES
10
20
LESION
POST-LESION
TEST DAYS

16 A10

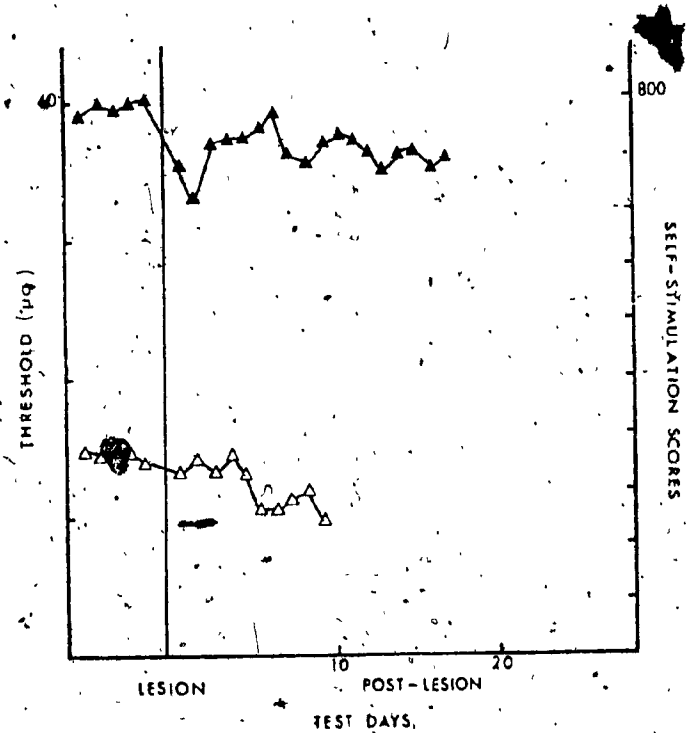

LM
SNR
A9
A10
LM
A8
A10
CC


40
250
THRESHOLD (μa)
SELF-STIMULATION SCORES
10
20
LESION
POST-LESION
TEST DAYS

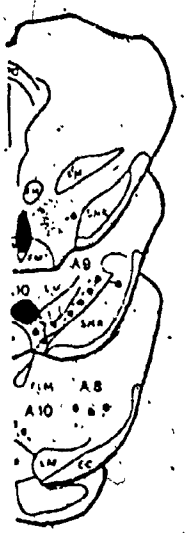
35 A10

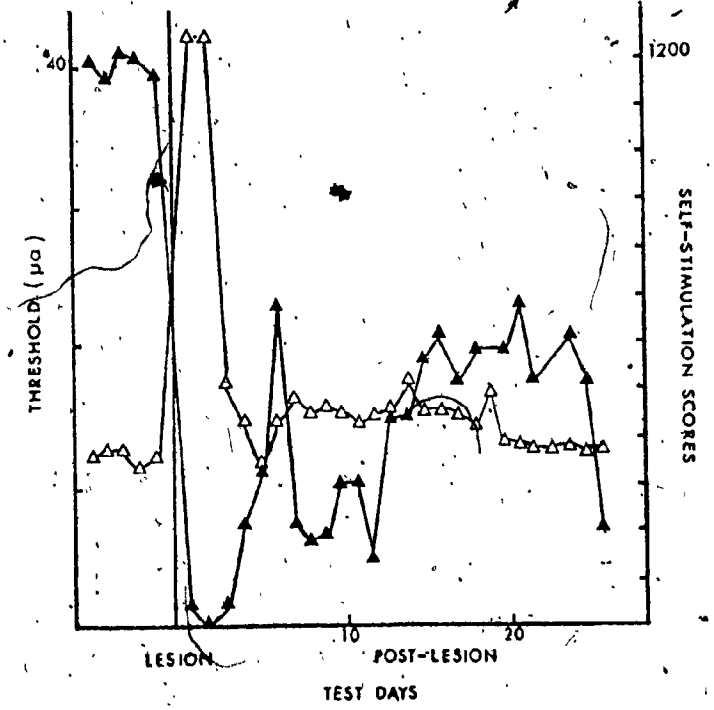

40
800
THRESHOLD (µa)
SELF-STIMULATION SCORES
10
20
LESION
POST-LESION
TEST DAYS

40 A10

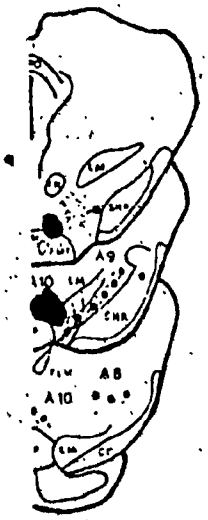

40
800
THRESHOLD (µa)
SELF-STIMULATION SCORES
10
20
LESION
POST-LESION
TEST DAYS

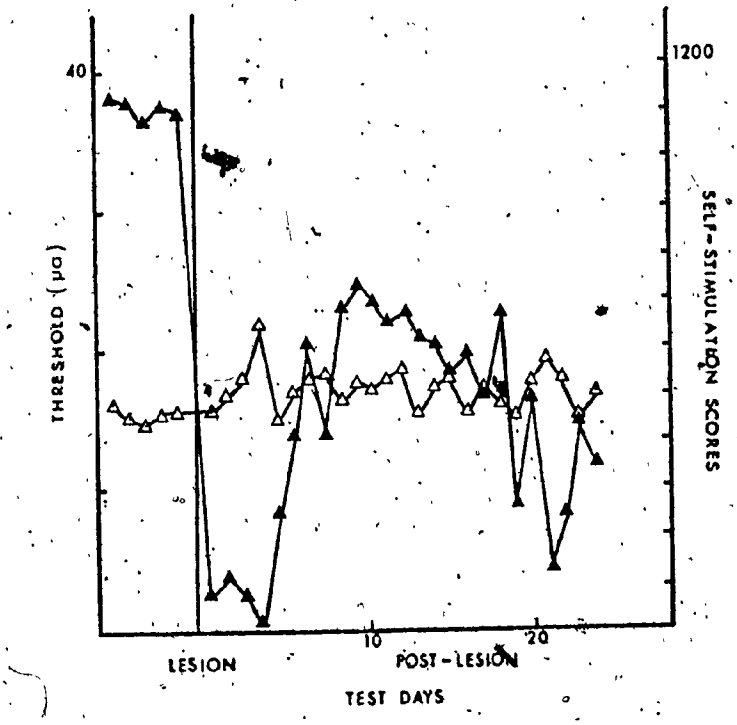
43 A10


A9
A8
A10
CC


40
1200
THRESHOLD (μa)
SELF-STIMULATION SCORES
10
20
LESION
POST-LESION
TEST DAYS

45 A10


A9
A8
A10
CC


40
1200
THRESHOLD (μa)
SELF-STIMULATION SCORES
10
20
LESION
POST-LESION
TEST DAYS

an increased current level. From this point on, the threshold gradually decreased to pre-lesion levels. Self-stimulation rates were markedly reduced in both animals and little or no recovery was seen over the next 20 days. Disruption of stimulation-induced feeding was seen in two more animals (Nos. 35A-10 and 45A-10). Threshold levels were increased over pre-lesion levels while self-stimulation rates were decreased. In another instance (No. 2A-10) the animal's performance became erratic. Before lesioning, the threshold had been very stable, after lesioning it varied considerably from day to day. Two more animals (Nos. 13A-10 and 16A-10) showed gradually rising thresholds and, in the case of No. 13A-10, gradually lowered rates of self-stimulation. Animal 16A-10 did not self-stimulate. No effect was seen in No. 40A-10; in fact, the threshold was somewhat lower following the lesion.

In several animals changes in self-stimulation scores tended to be correlated with changes in eating threshold. Thus, a rise in threshold tended to be accompanied by a drop in self-stimulation rates; if there was recovery of the one, there was often recovery of the other.

Figure 4 shows the median scores for fixed-current trials over the five day pre-lesion stabilization period and over six days of post-lesion testing. The results of the fixed-current trials paralleled the threshold change effects. That is to say, the greatest decline in scores was seen with A-9.5 lesions, the least with A-9 lesions, and a decline intermediate to these two extremes was produced by A-10 lesions.

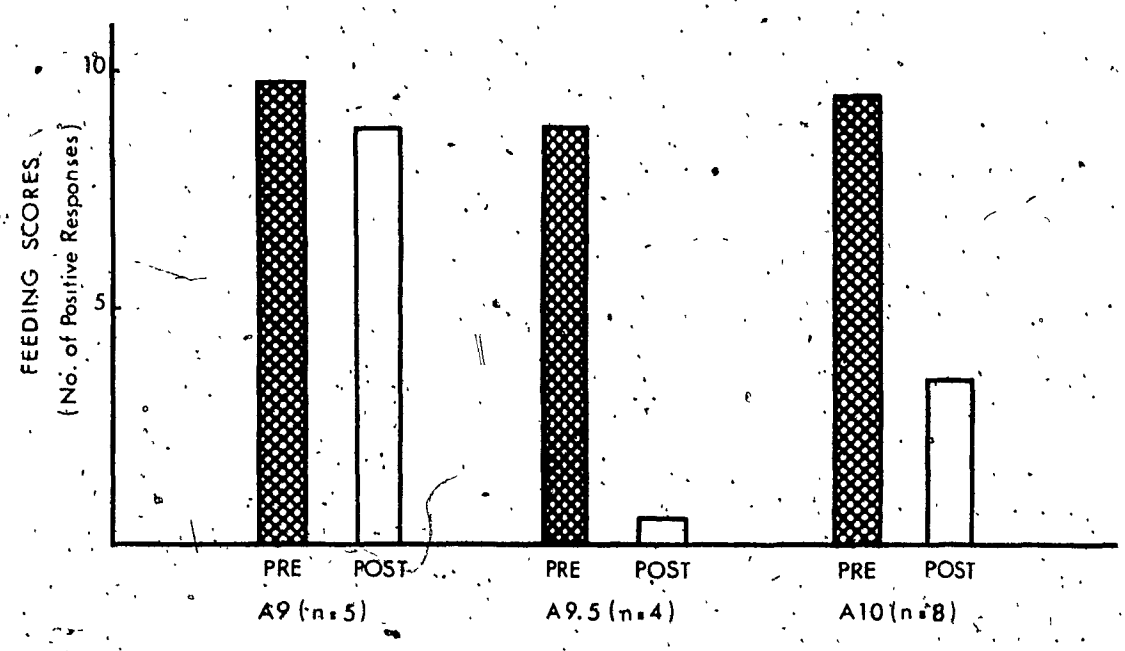


Figure 4. Median fixed-current feeding scores (No. of positive responses out of 10) for pre- and post-lesion conditions according to lesion locus.

DISCUSSION

Three principal findings emerged from this study. First, animals with lesions in the ventral tegmental area (A-9.5) that probably damaged fibers of both the mesolimbic and mesocortical systems, as well as those of the nigrostriatal system, showed a greater disruption of stimulation-induced feeding than did animals with lesions confined to cell bodies (A-10) of the mesolimbic and mesocortical systems or to cell bodies (A-9) of the nigrostriatal system. Second, animals with extensive damage restricted to the cell bodies of the nigrostriatal system showed no disruption whatsoever of stimulation-induced feeding; thresholds were not raised, nor were fixed-current trial scores lower, after lesioning. Third, several animals with damage to the mesolimbic and mesocortical cell bodies and no apparent damage to the nigrostriatal cell bodies and projections failed to eat in response to stimulation unless the current was raised beyond pre-lesion threshold levels. Fixed-current trial scores in these animals were reduced.

Three interpretations of these findings merit consideration. First, overall physical debilitation or specific motor deficit rather than damage to DA systems could have caused or contributed to the observed increases in thresholds and decreases in fixed-current trial scores. This possibility does not seem likely. If simply the insult of being lesioned caused the observed disruptions, then all of the animals should have shown changes in

stimulation-induced eating; clearly, a number of them did not. Several animals lost a little weight after lesioning, but quickly regained this loss and gained weight normally thereafter. Some animals developed a decided contraction of the neck muscles on the right side and this might have interfered with the motor requirements of holding onto the pellet and nibbling. These animals were seen, however, to quickly learn compensating techniques for coordinating these movements and appeared to have no difficulties because of this asymmetry. Moreover, the threshold measure used in this study tends not to penalize an animal for clumsiness. Factors extrinsic to the effects of the DA system lesions, therefore, do not appear to account for the disruptions of stimulation-induced feeding that were observed. Since 6-OHDA causes the same thing, it would seem that the DA system damage was responsible.

A second possibility is that damage to the nigrostriatal system is the crucial factor involved in aphagia; from this position it would be argued that the animals with nigrostriatal damage in the present study were not disrupted simply because the lesion failed to damage a sufficient portion of the A-9 cell bodies. It has been emphasized by both Zigmond and Stricker (1973) and Marshall et al. (1974) that the striatum must be depleted of at least 90% of its normal DA content if aphagia is to be seen. Without bioassay data, it is not known whether the two animals in the present study (34A-9 and 50A-9) with well-restricted A-9 lesions did, in fact, sustain this crucial

level of depletion. The precise boundaries of the ventral aspect of the nigrostriatal system are not known; thus the possibility cannot be ruled out that in these two animals some of the ventral A-9 cell bodies were spared; such a residual could, perhaps, have been sufficient to maintain stimulation-induced feeding. This view would have been quite persuasive if all of the A-10 lesions had been ineffective. However, the finding that half the animals with lesions at A-10 showed disruption of stimulation-induced feeding must be weighed against this. Both Zigmond and Stricker (1973) and Creese and Iversen (1975) noted that a substantial number of their animals did not develop aphagia even when there was a 90% depletion of striatal DA. Thus, it could be argued that it is not that a certain quantity of striatal DA must be lost, but that certain crucial pathways must be damaged if aphagia is to be seen. Striatal DA depletion may be a correlate but not a cause of aphagia. Thus, it is not clear that nigrostriatal damage is the critical factor in causing aphagia, and the question is not resolved by the present data.

A third possibility is that damage to the mesolimbic or mesocortical systems, rather than damage to the nigrostriatal system, causes aphagia. This fits nicely with the finding that four of the eight animals with damage to the cell bodies at A-10 did show clear-cut disruptions of stimulation-induced feeding on one or both of the measures used in this study. Eating in three more animals was disrupted, but not to the same degree or in quite the same

way as in these four animals. One animal was not affected. With this much variability in the data, it is difficult to make a strong assertion that mesolimbic and mesocortical damage is more effective than nigrostriatal damage in causing aphagia.

The present data, taken alone, favours such a view but further work is needed if the issue is to be resolved. The major obstacle to a resolution for this question is that, even with the help of fluorescence histochemistry, the boundary between A-9 and A-10 cell bodies cannot be precisely drawn. Thus, with the present design, it cannot unequivocally be ascertained that an A-10 lesion that disrupted eating spared all of the A-9 cell bodies or, more important still, that an unsuccessful A-9 lesion damaged even the last 10% of the A-9 cell bodies. It was for this reason that testing was discontinued with so few cases. If work with the current paradigm is to be continued, bioassay data is a crucial necessity. The effectiveness of a given lesion could then be confirmed, not only by observing cell body damage, but by comparing the amount of DA depletion in the striatum with the amount of DA depletion in the areas of termination in the forebrain of the mesolimbic and mesocortical fibers. P. Setler (personal communication) has indicated that pre-treatment with an i.p. injection of L-dopa and RO4-4602 increases fluorescence of the A-9, but not that of A-10, cell bodies. This might be used to further refine the capability of the Falck-Hillarp technique for determination of lesion locus

and extent.

Of additional interest is the finding that lesions of the cell bodies at A-10 not only disrupt stimulation-induced feeding but also decrease self-stimulation rates. Moreover, the time-course of recovery of these functions tends to be correlated. That is to say, as threshold levels return to pre-lesion levels, self-stimulation rates also return to normal. This bears on an issue which has received a good deal of interest in the literature. Since the demonstration by Margules and Olds (1962) and Hoebel and Teitelbaum (1962) that feeding responses and self-stimulation behaviour can be induced by electrical stimulation through the same electrode implanted by the LHA, there has been much discussion as to whether drive and reinforcement are subserved by a single neural mechanism (Olds, 1962; Caggiula & Hoebel, 1966; Deutsch & Deutsch, 1966; Mogensen & Stevenson, 1966; Glickman & Schiff, 1967; Milner, 1970; Gallistel & Beagley, 1971; Phillips & Fibiger, 1973, 1974). The present finding that stimulation-induced thresholds and self-stimulation rates tend to recover at the same time fits best with theories of a unitary neural mechanism underlying drive and reinforcement. This is in conflict with conclusions of Phillips and Fibiger (1973, 1974). They found that, rather than being correlated, recovery of stimulation-induced feeding and self-stimulation were dissociated; in their hands, self-stimulation recovered over time while stimulation-induced feeding did not. There are two possible explanations for

the difference between the findings of the present study and those of the Phillips and Fibiger study. First, Phillips and Fibiger infused 6-OHDA into the ventricles and thus caused bilateral damage of both DA and NA systems. Unilateral electrolytic lesions of DA cell bodies were made in the present study. The bilateral depletion of brain DA, or the concomittant depletion of NE, might account for the long-term abolition of stimulation-induced feeding observed by Phillips and Fibiger. Alternatively, methodological considerations might explain the discrepancy between the results of the two studies. The fixed-current trial score in this and in the Phillips and Fibiger studies is very sensitive to small changes in feeding capabilities. The threshold measure is sensitive over a wider range of change. It was observed in the present study that thresholds in several animals (Nos. 2A-10, 40A-10, 43A-10, 45A-10) were at or near pre-lesion levels 21 days after lesioning but fixed-current trial scores were still low. Thus, if fixed-current trial scores alone had been considered, this study might also have suggested a dissociation between stimulation-induced feeding and self-stimulation. A broader perspective of an animal's performance after lesioning may be provided when both methods of scoring are used, and a choice of measures of analogous sensitivity for stimulation-induced eating and self-stimulation may be required if correlation of recovery is to be seen.

To summarize, the present data cannot rule out the

notion that nigrostriatal DA damage is the crucial factor causing the aphagia seen in the lateral hypothalamic and related syndromes. The data do suggest, however, that damage to mesolimbic or mesocortical DA systems or to both may in and of itself produce aphagia. Further work on this question would seem warranted, and the present approach would seem useful if it is combined with regional forebrain assays of residual DA in the lesioned animals.

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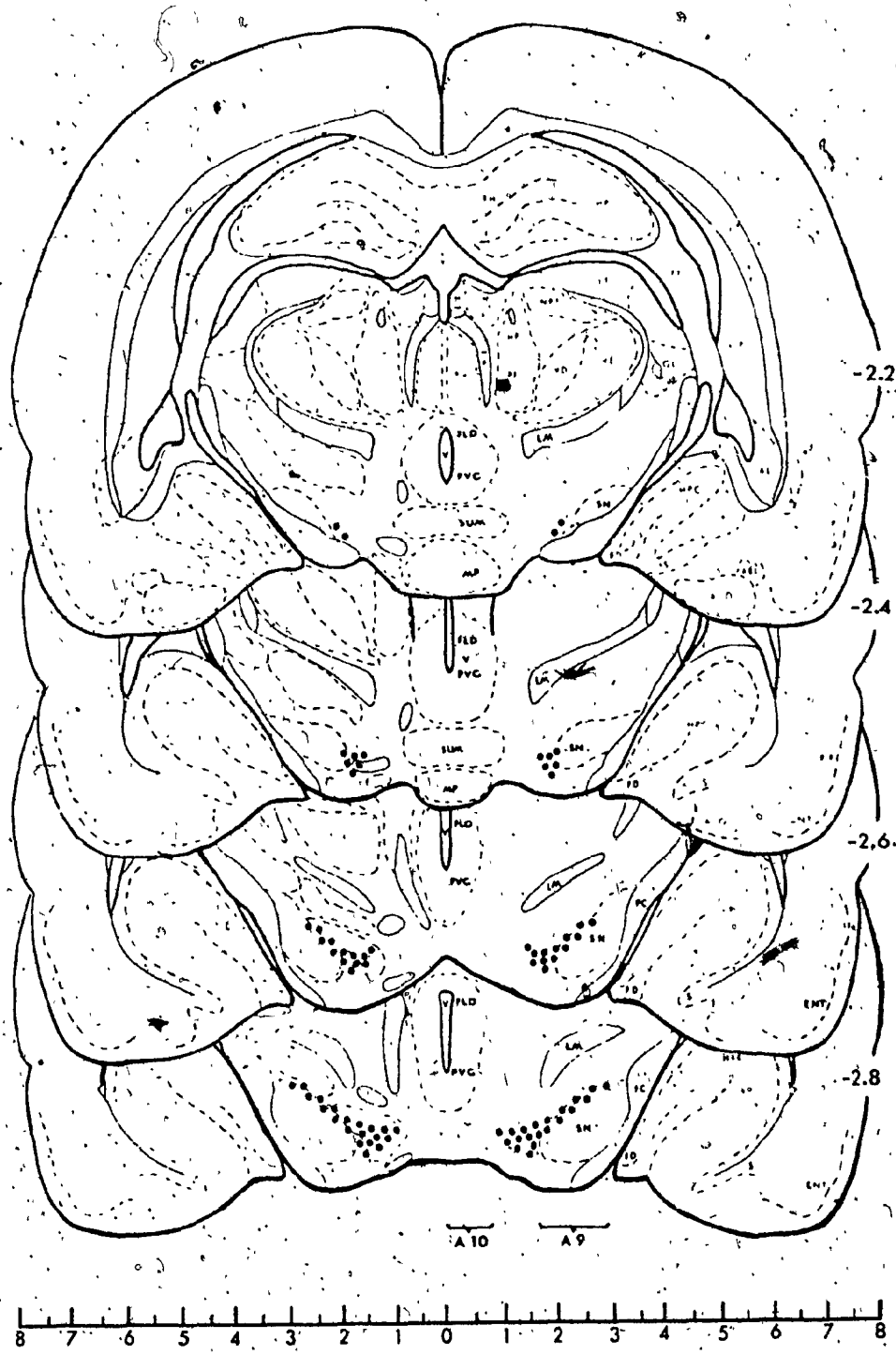
APPENDIX 1

Maps of the dopamine cell bodies based on the Pellegrino and Cushman atlas (1967).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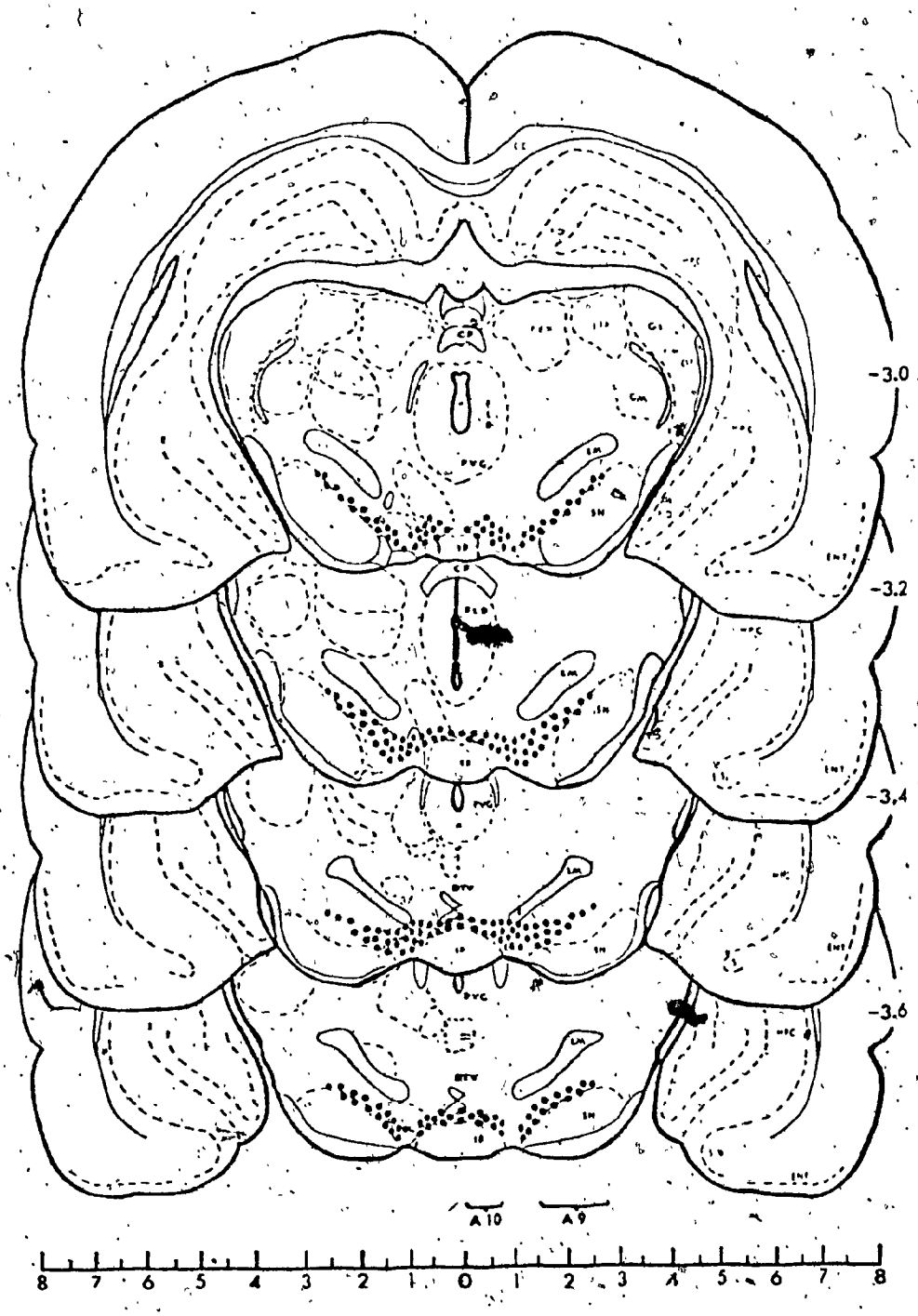
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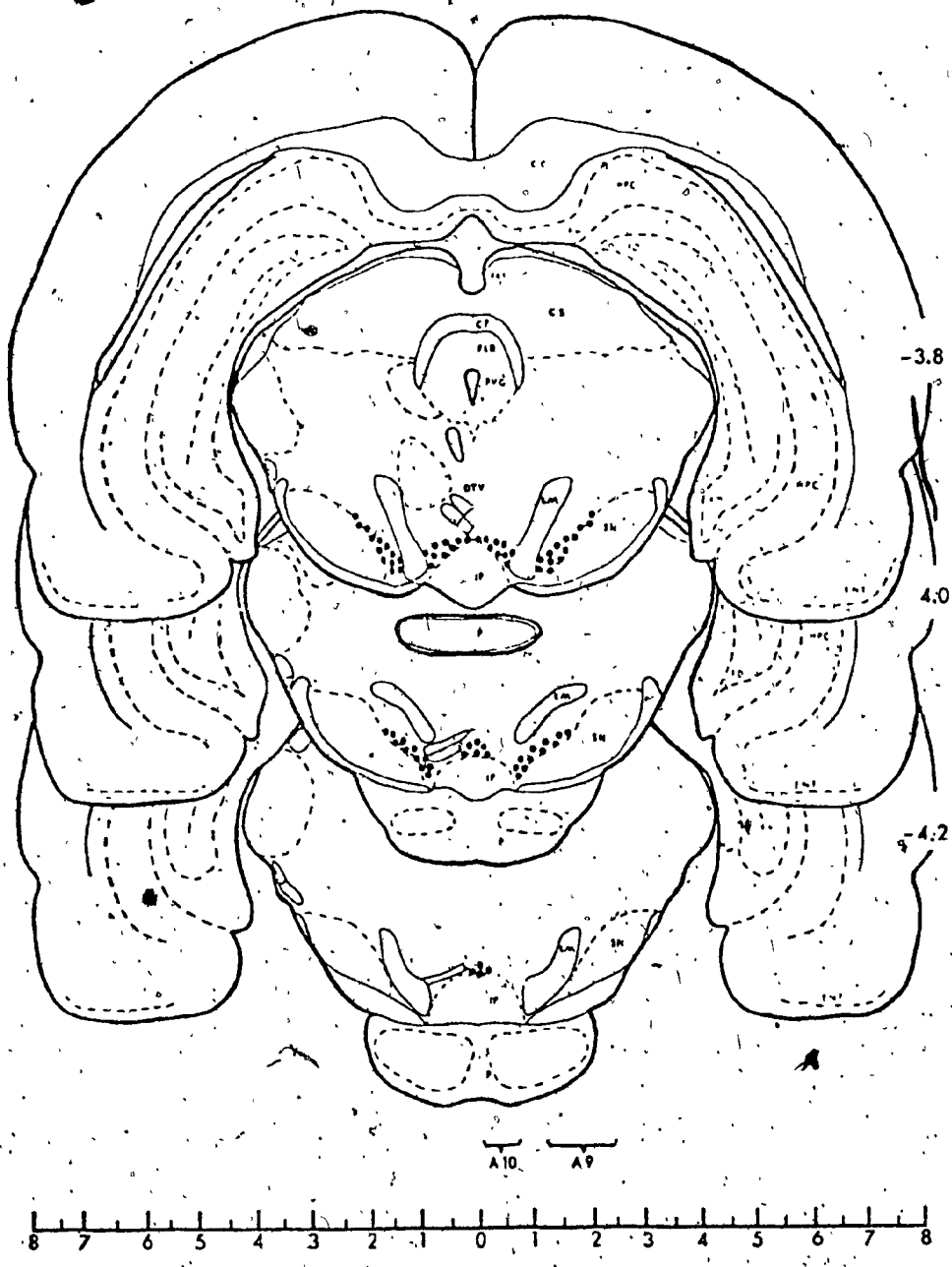
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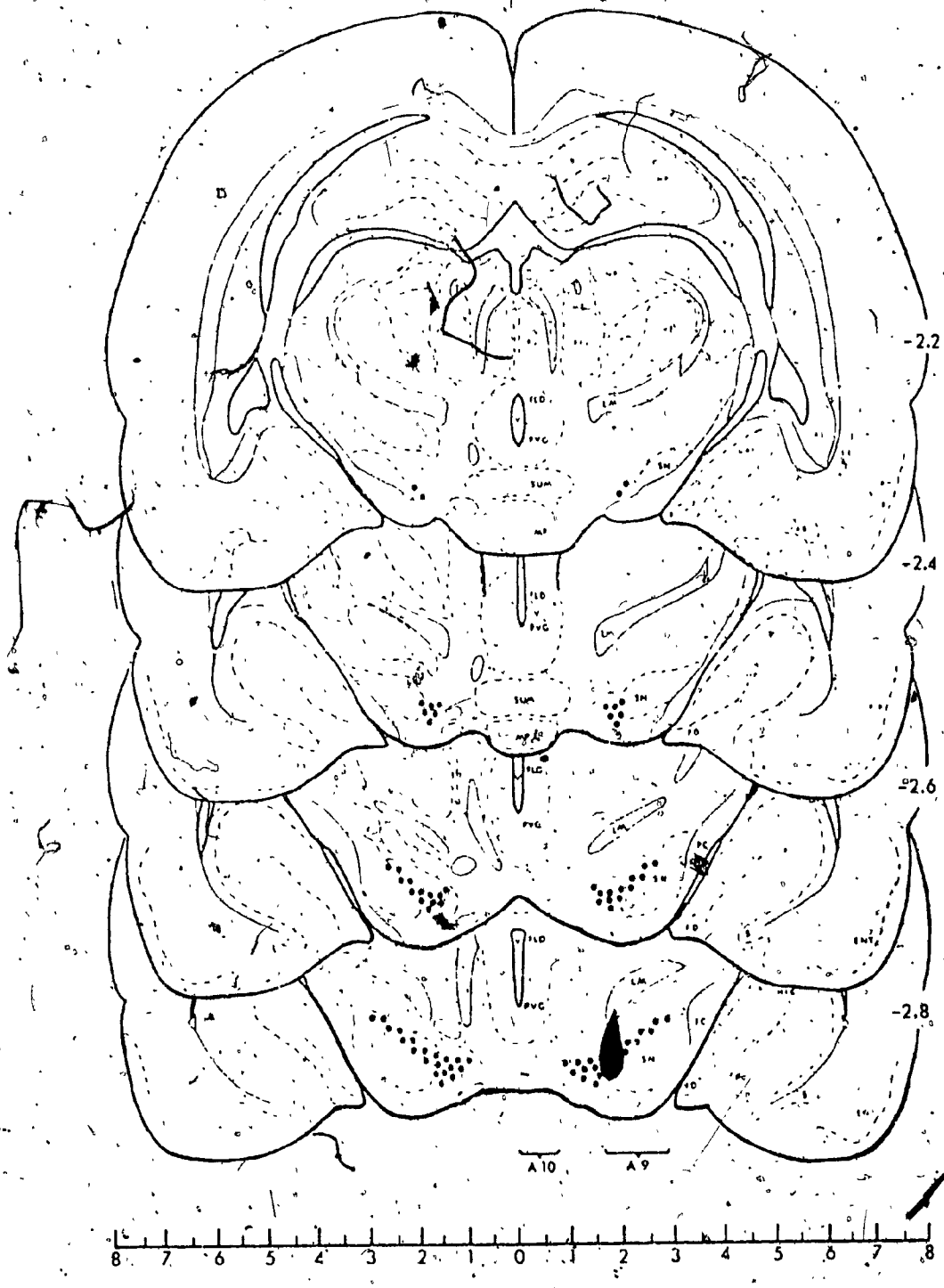
APPENDIX 2

Detailed reconstructions of lesion locus and extent for animals (34A-9, 60A-9, 54A-9.5, 1A-10, 2A-10, 13A-10, 16A-10, 35A-10, 40A-10, 43A-10, and 45A-10).

34 A 9



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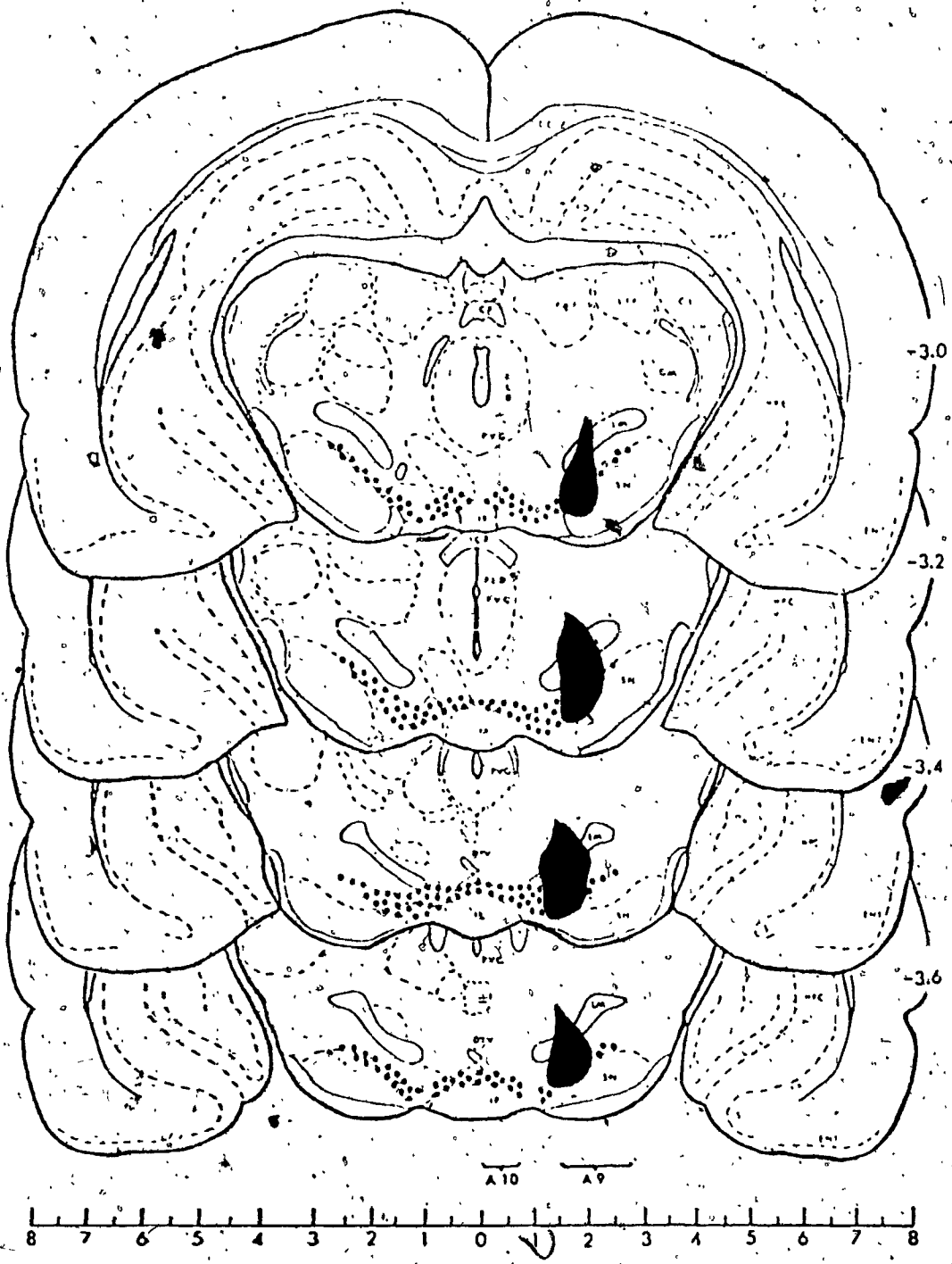

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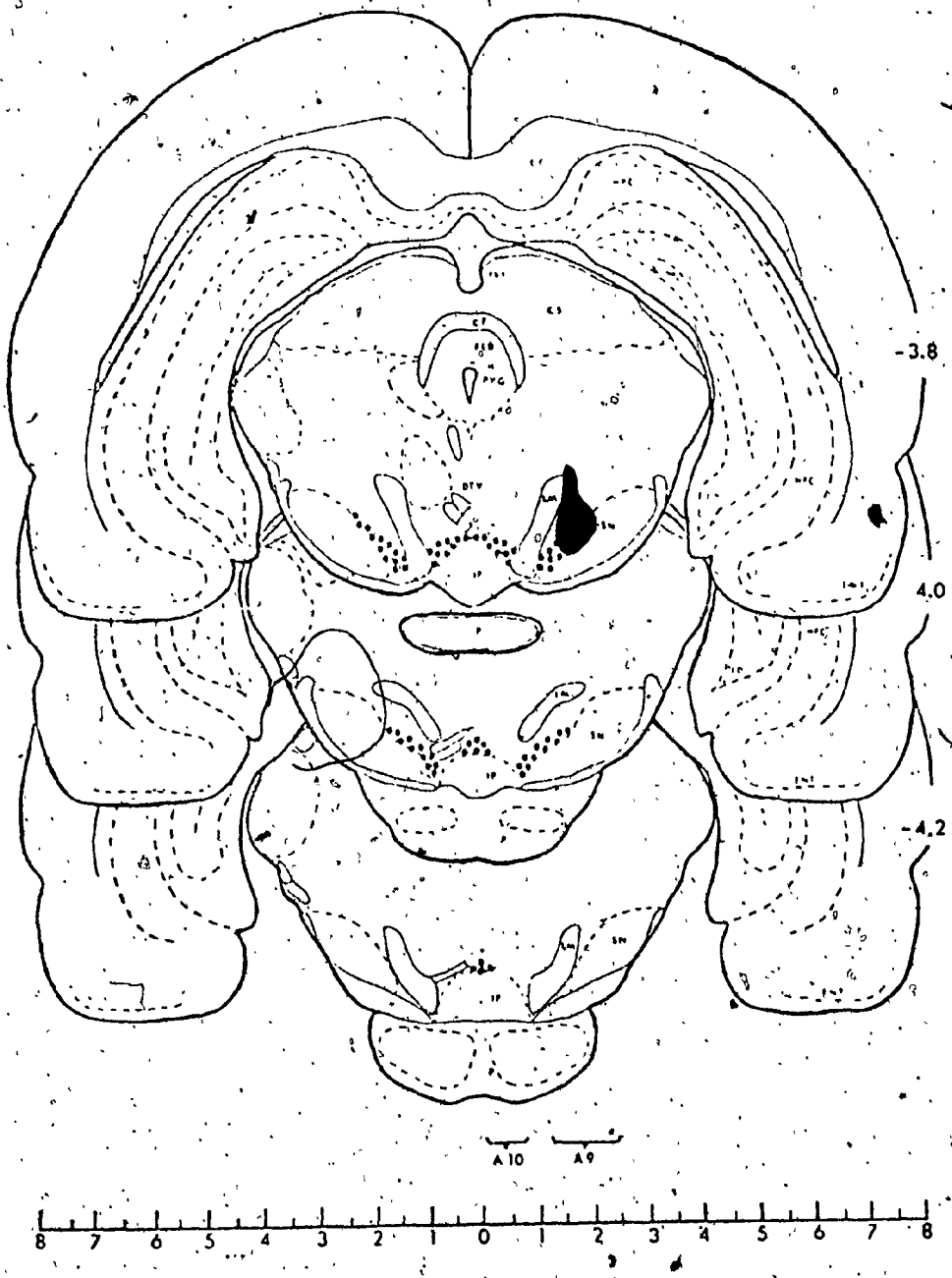

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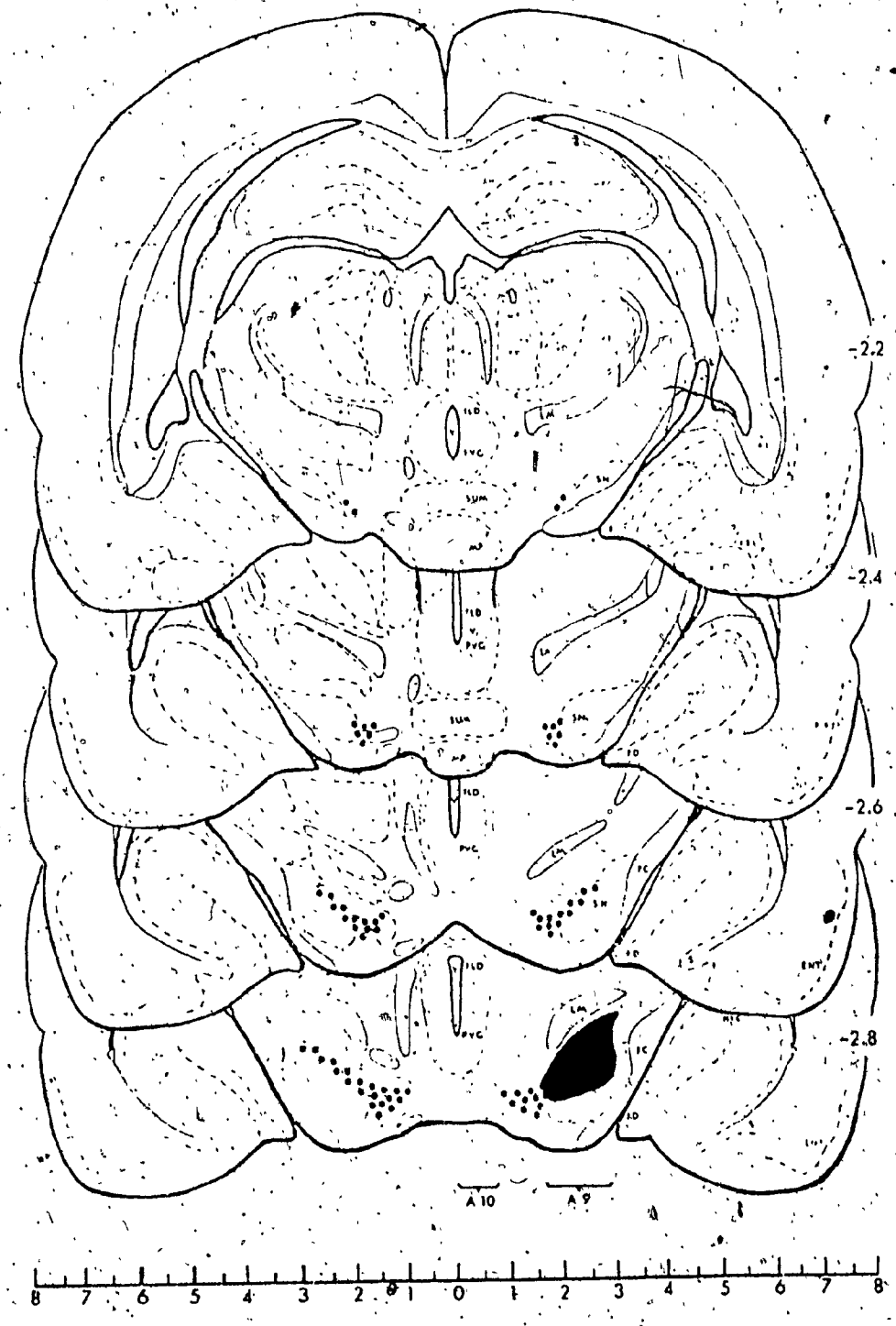

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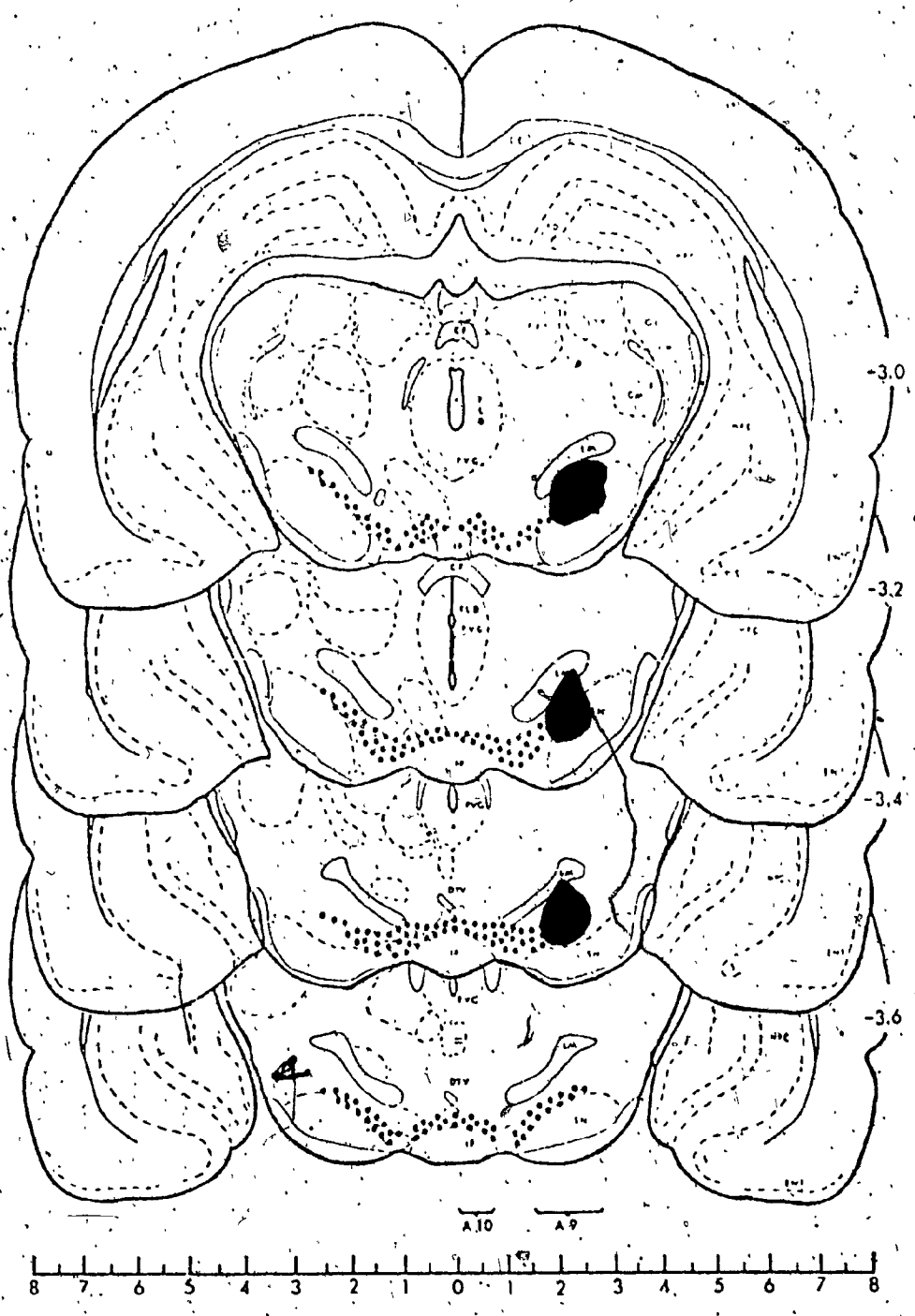

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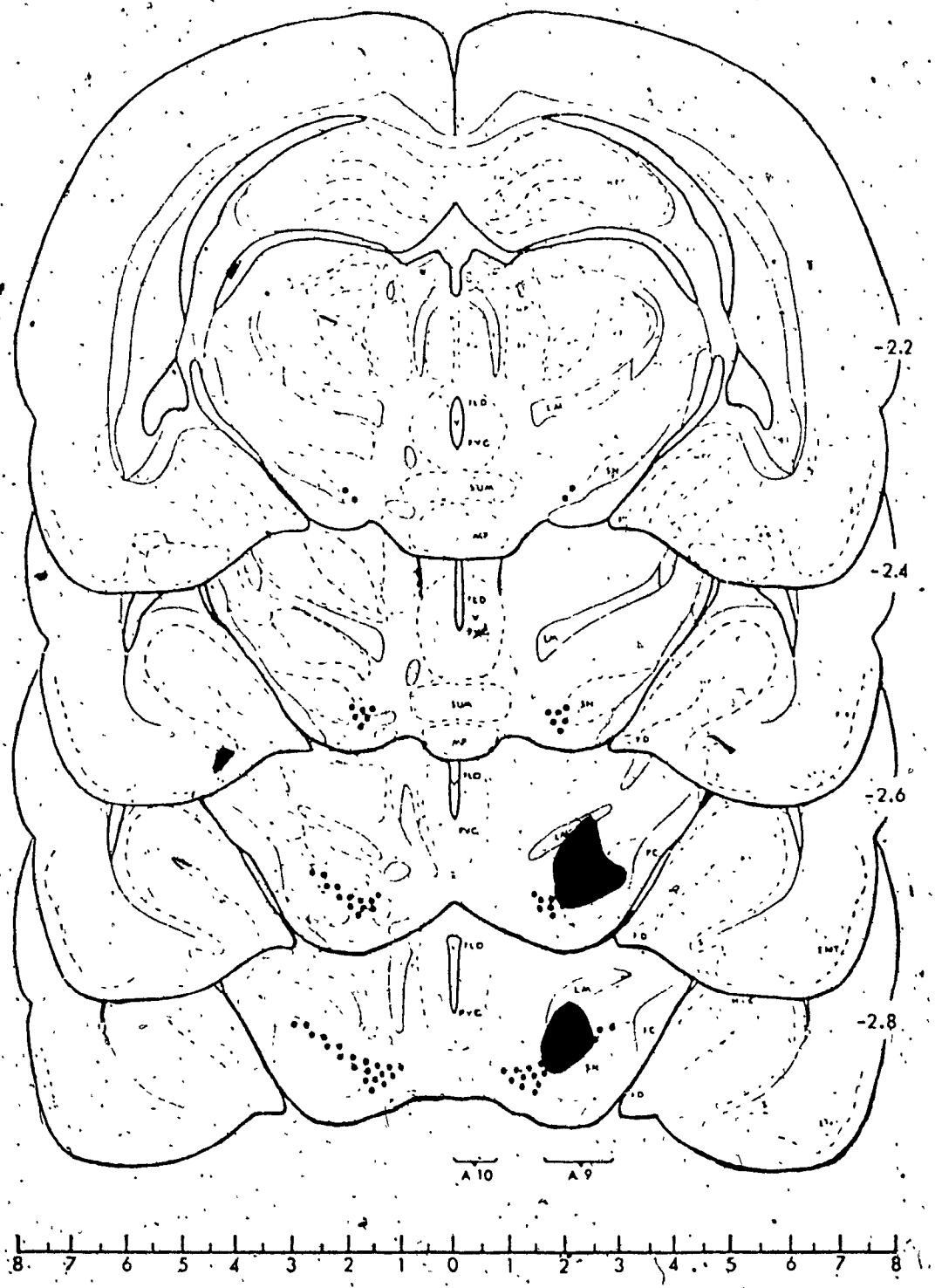

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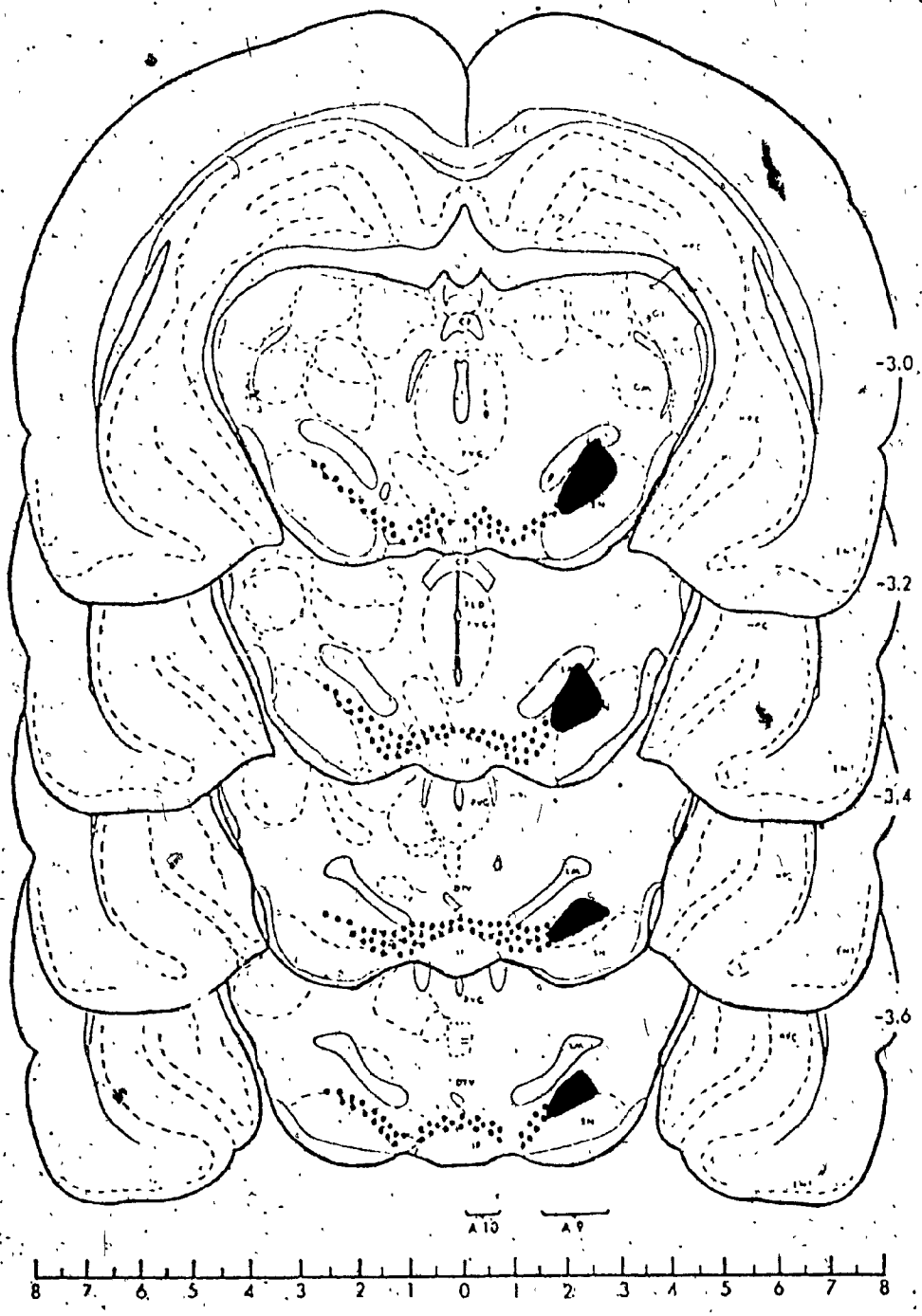

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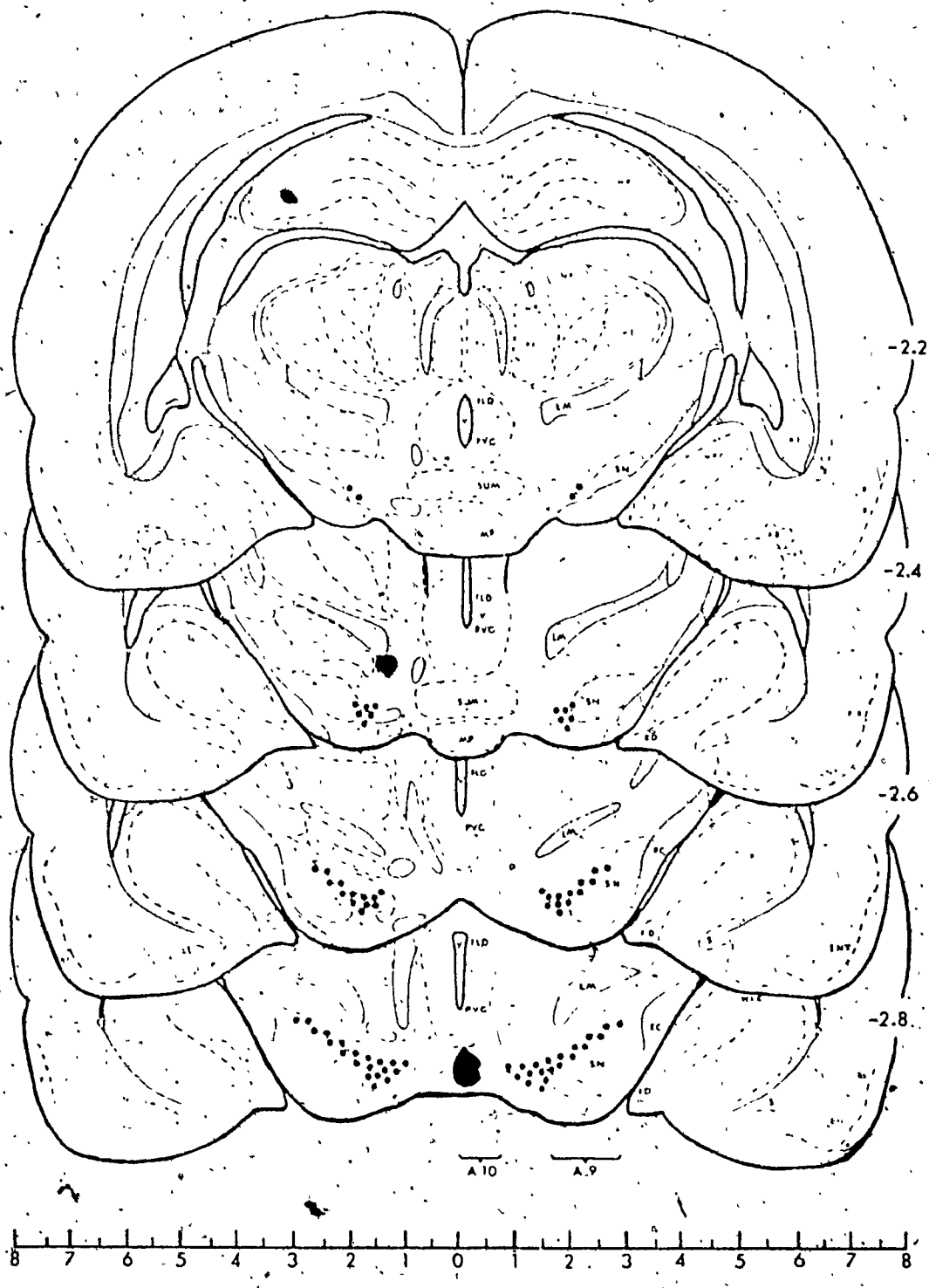

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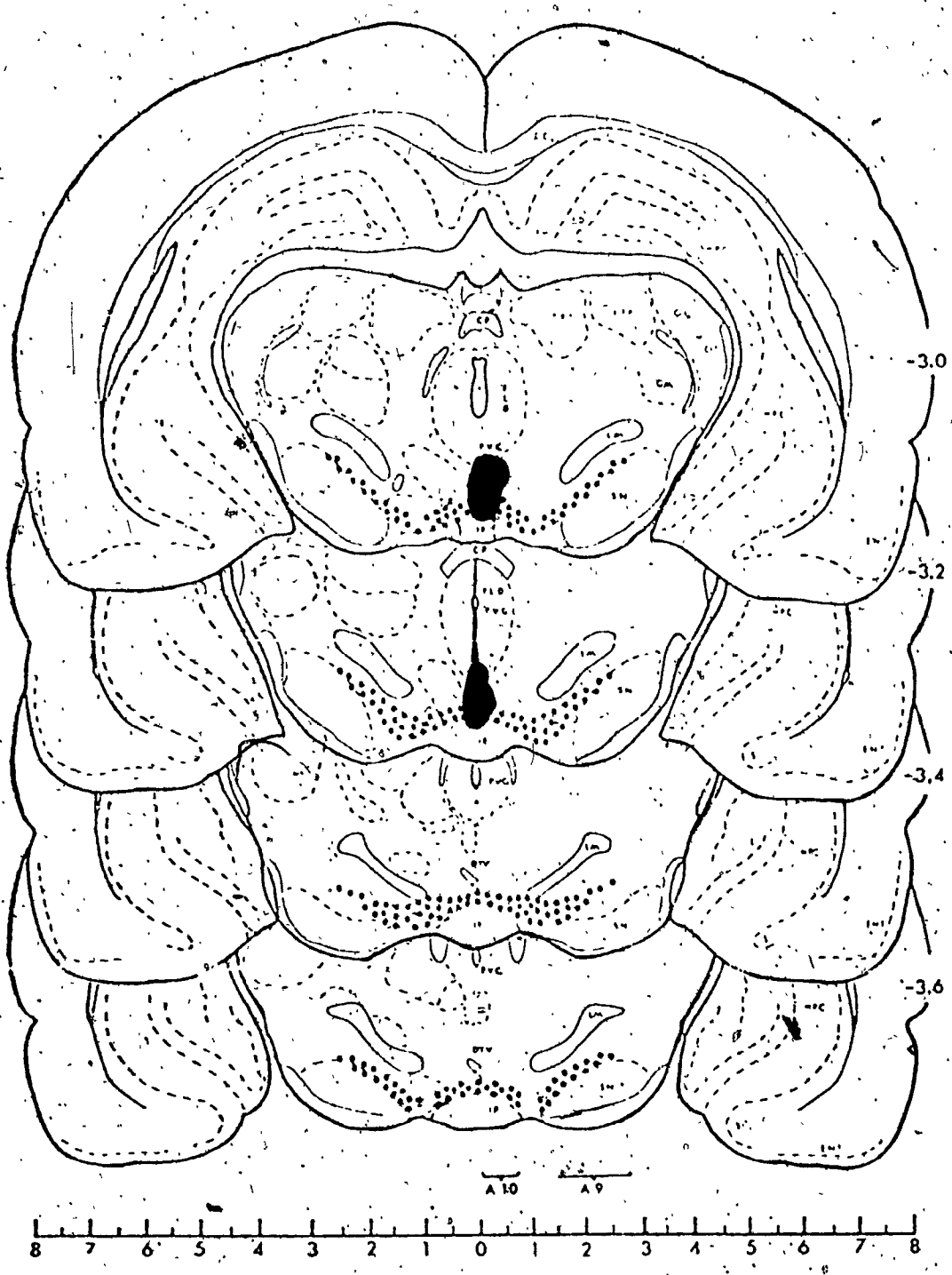

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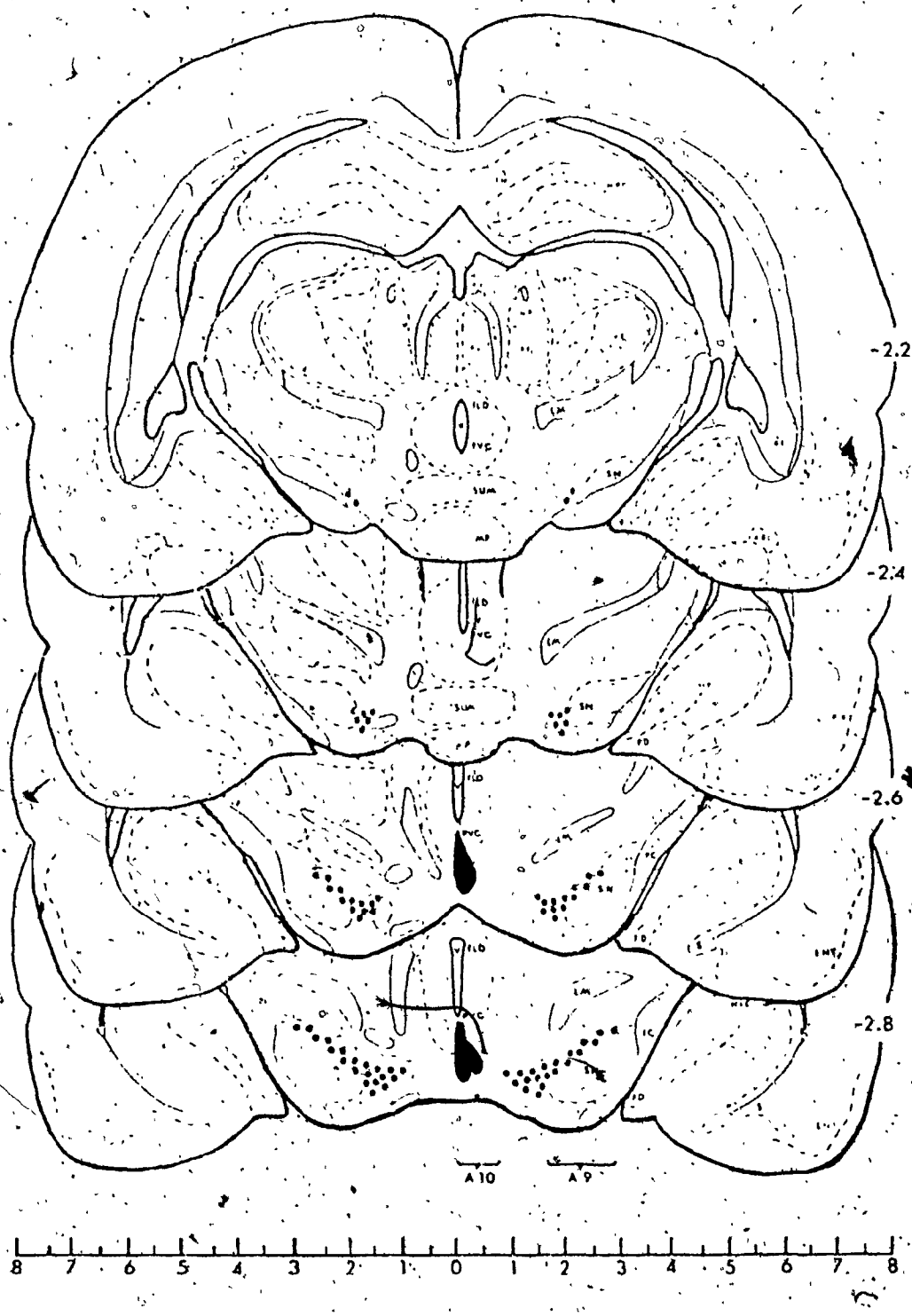

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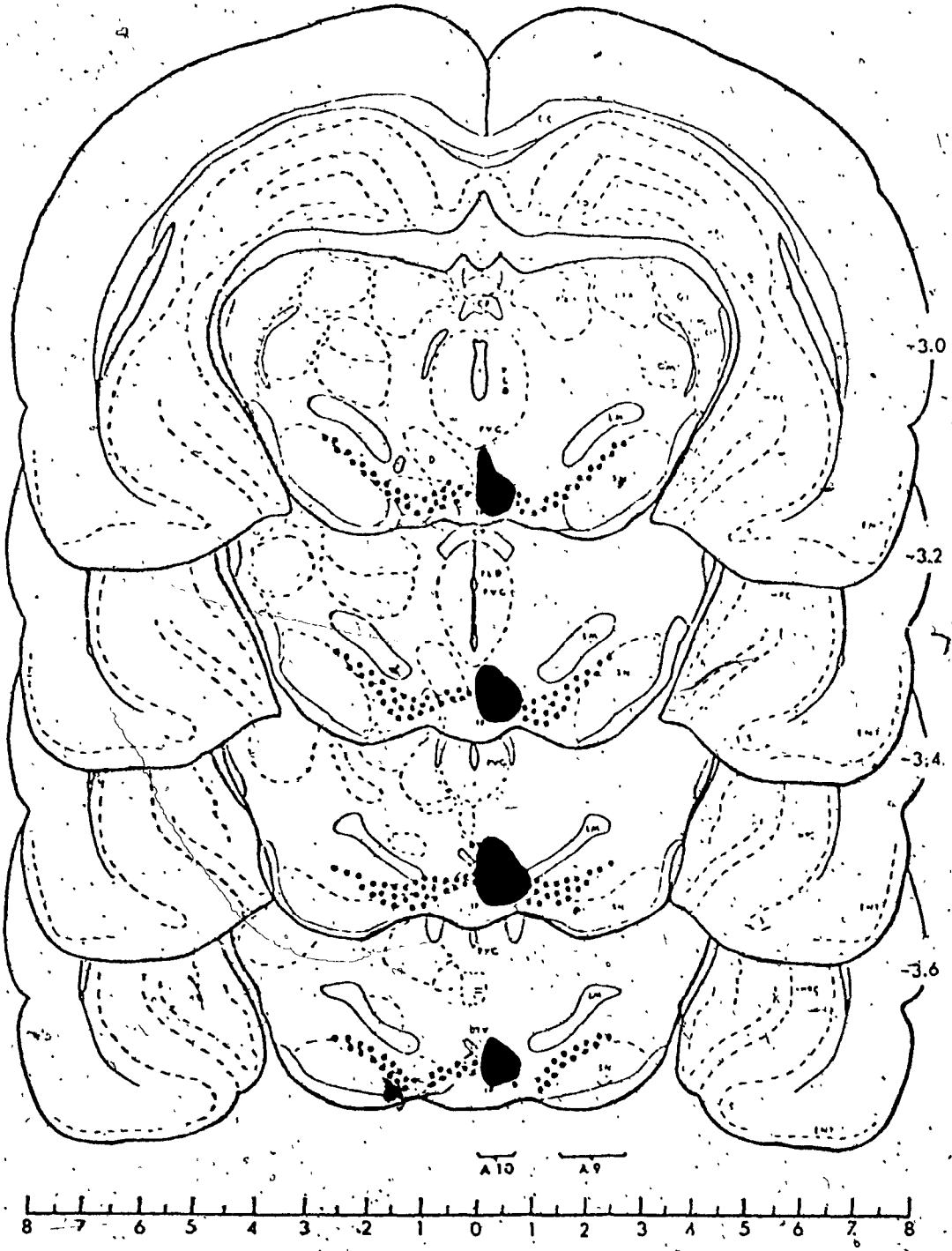

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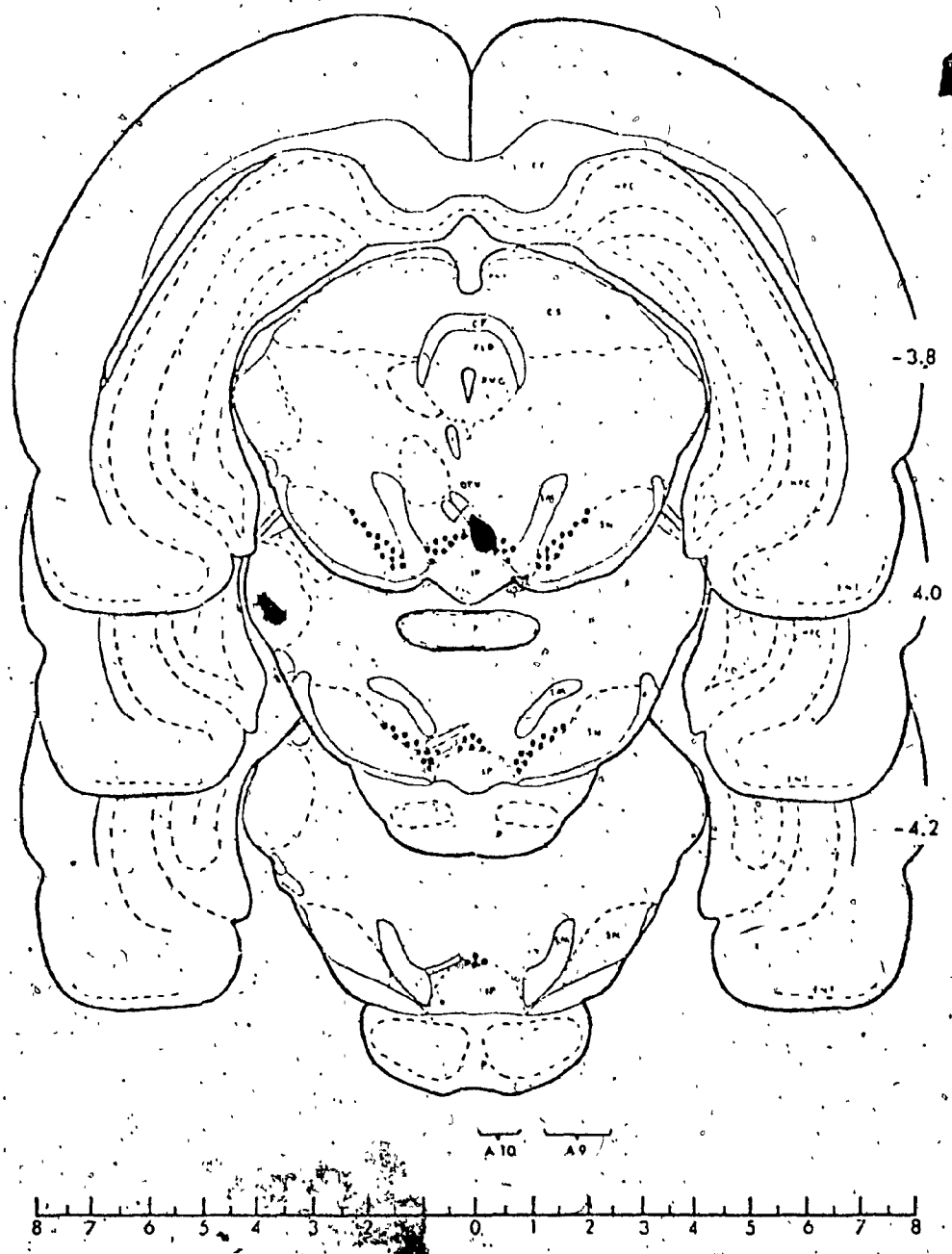

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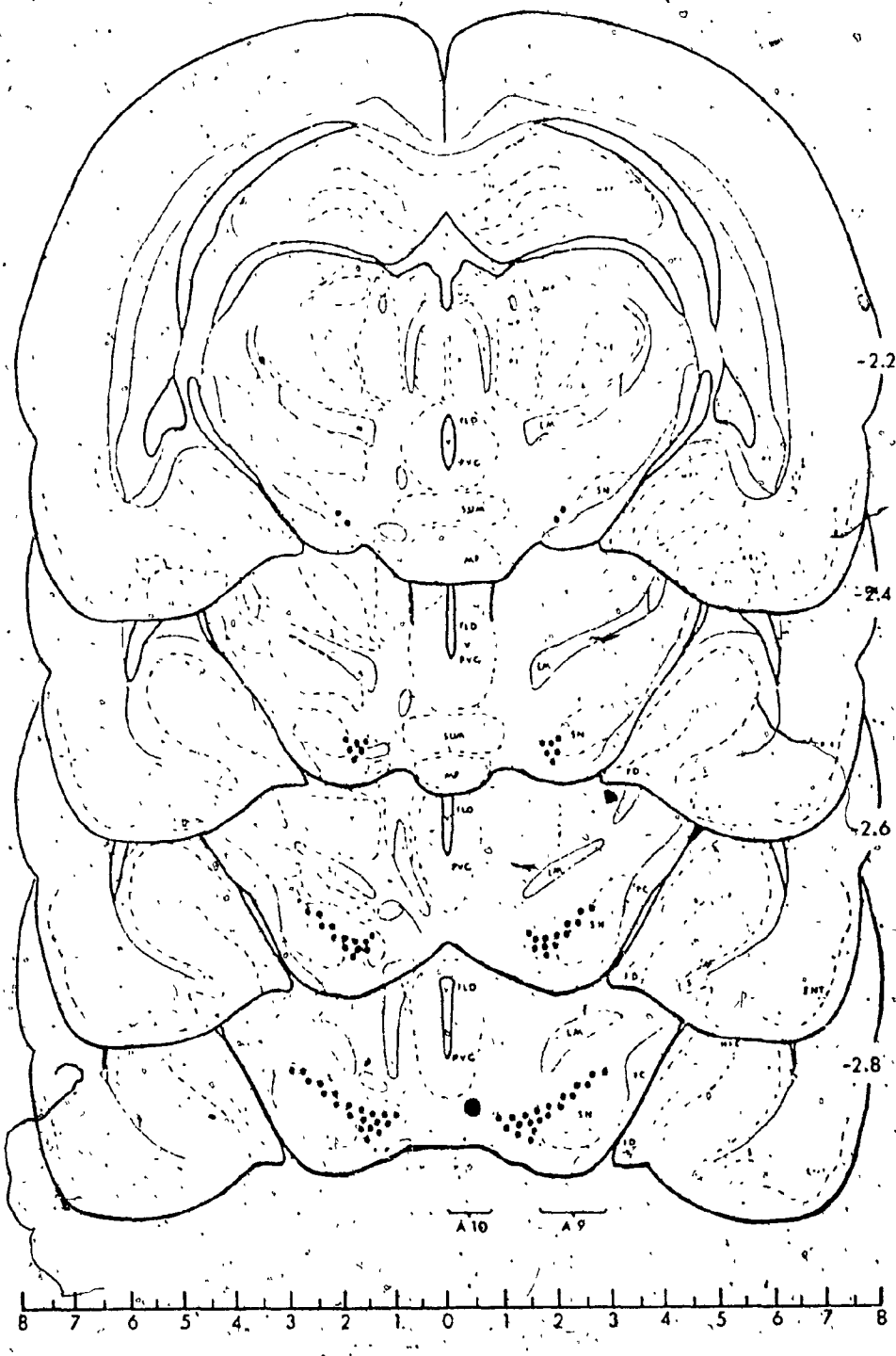

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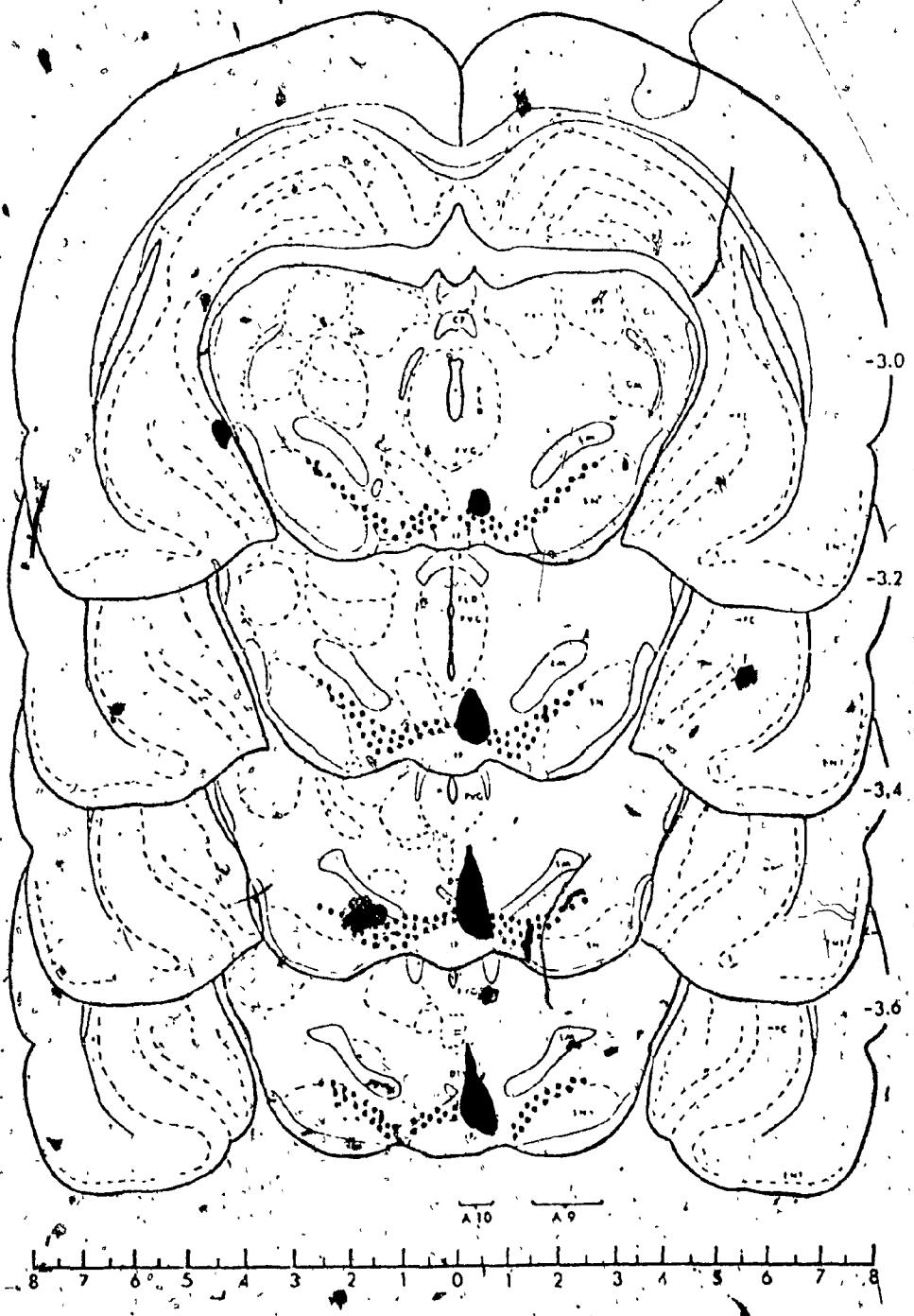

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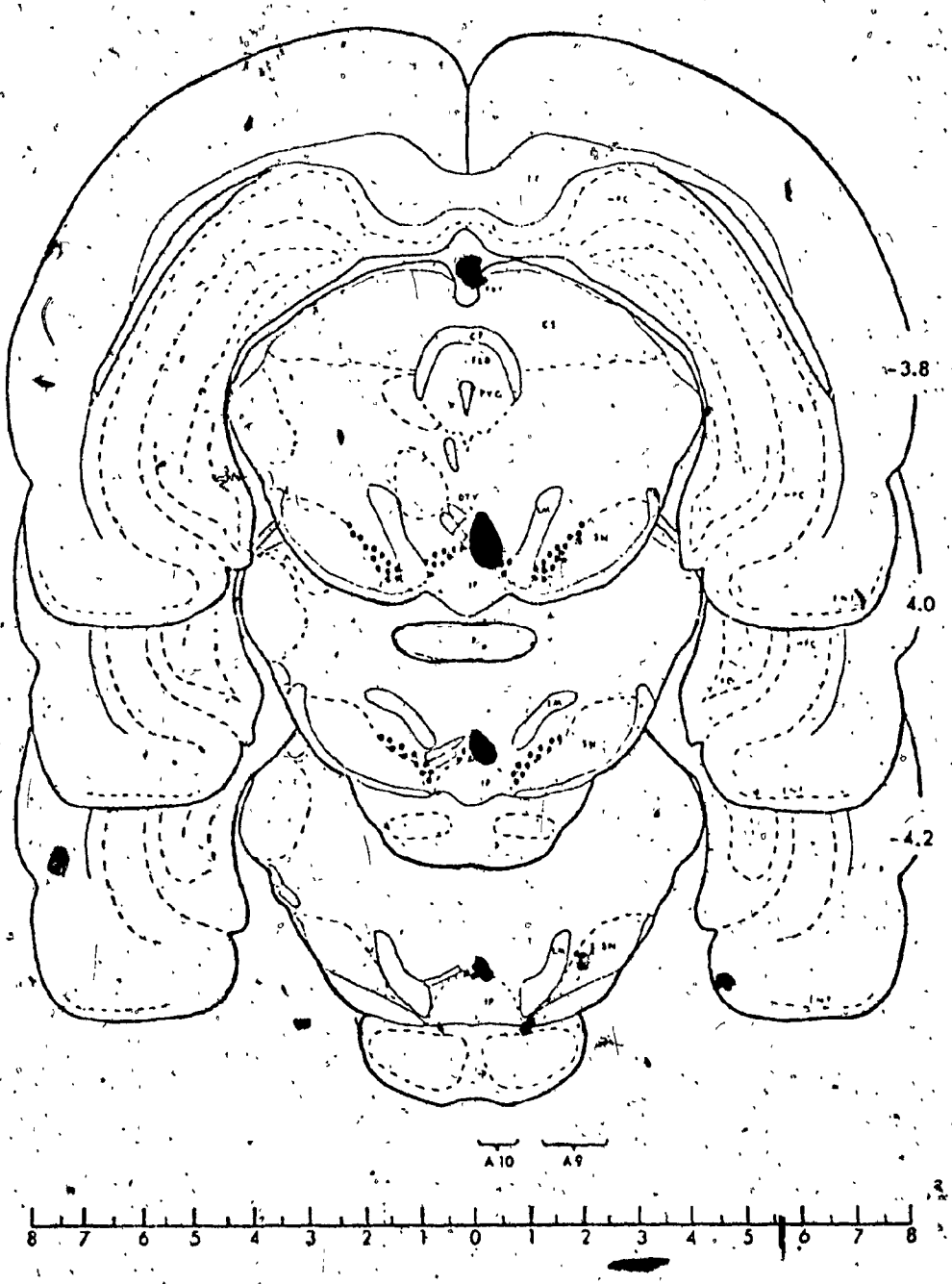

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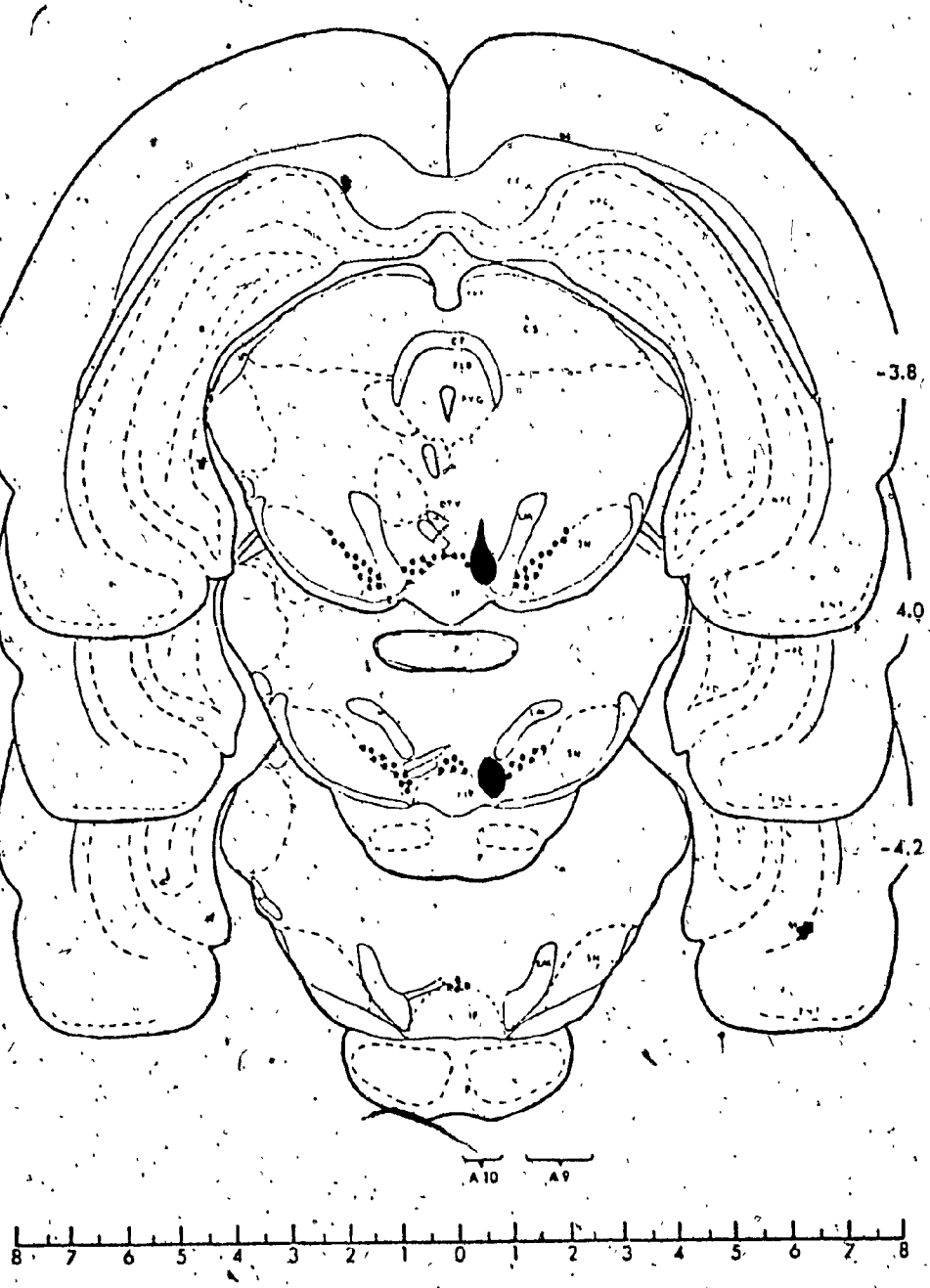

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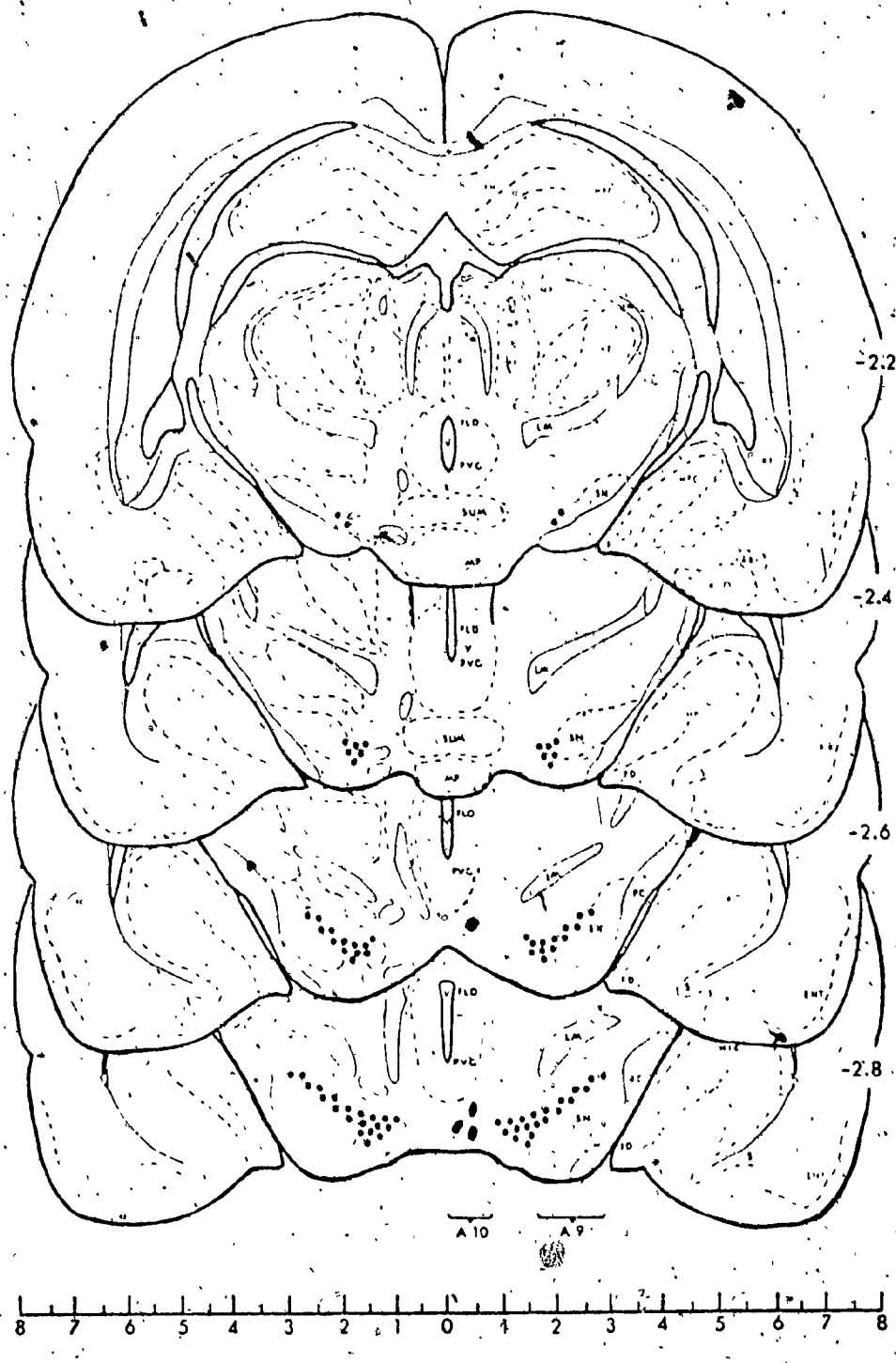

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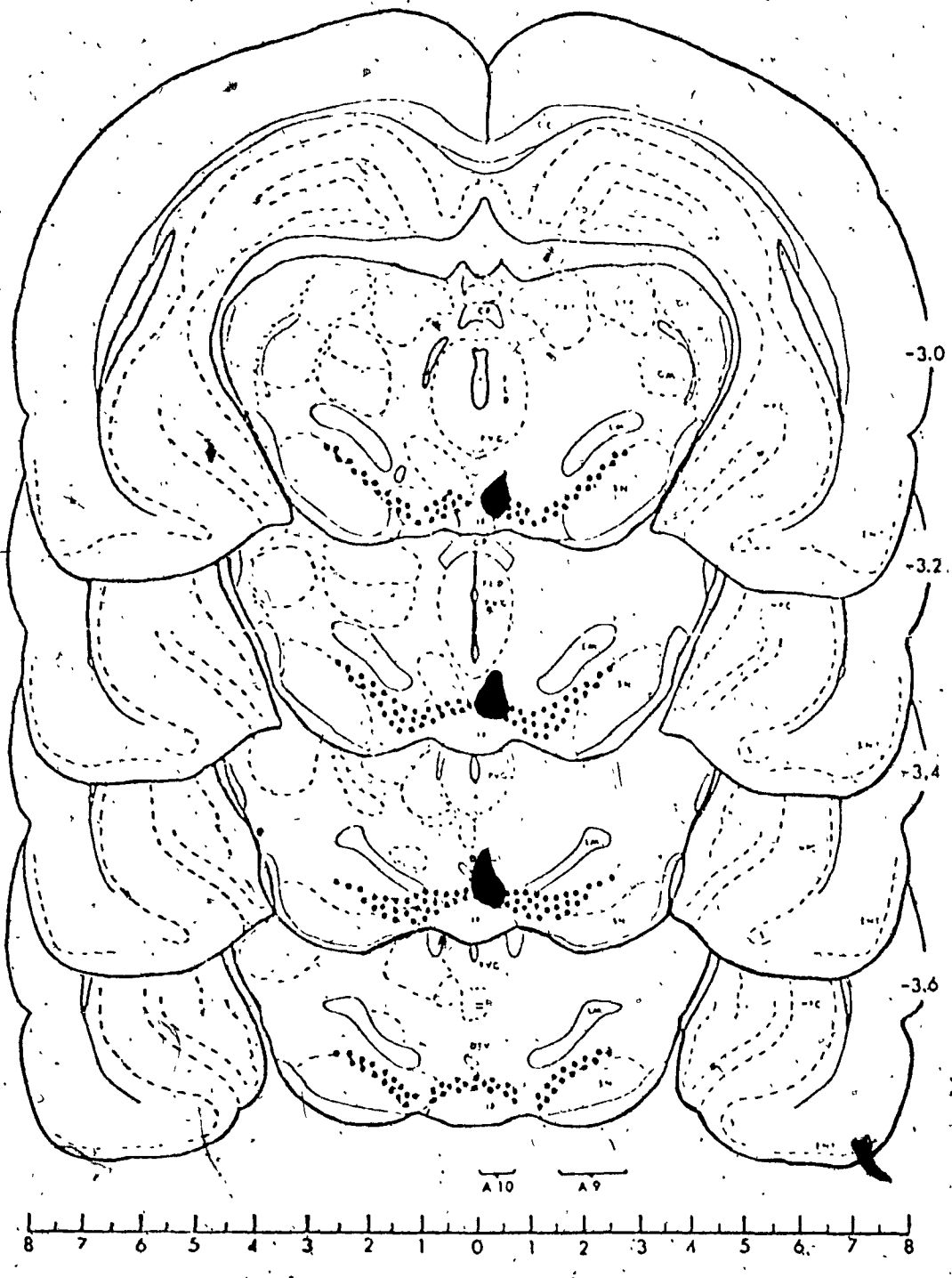

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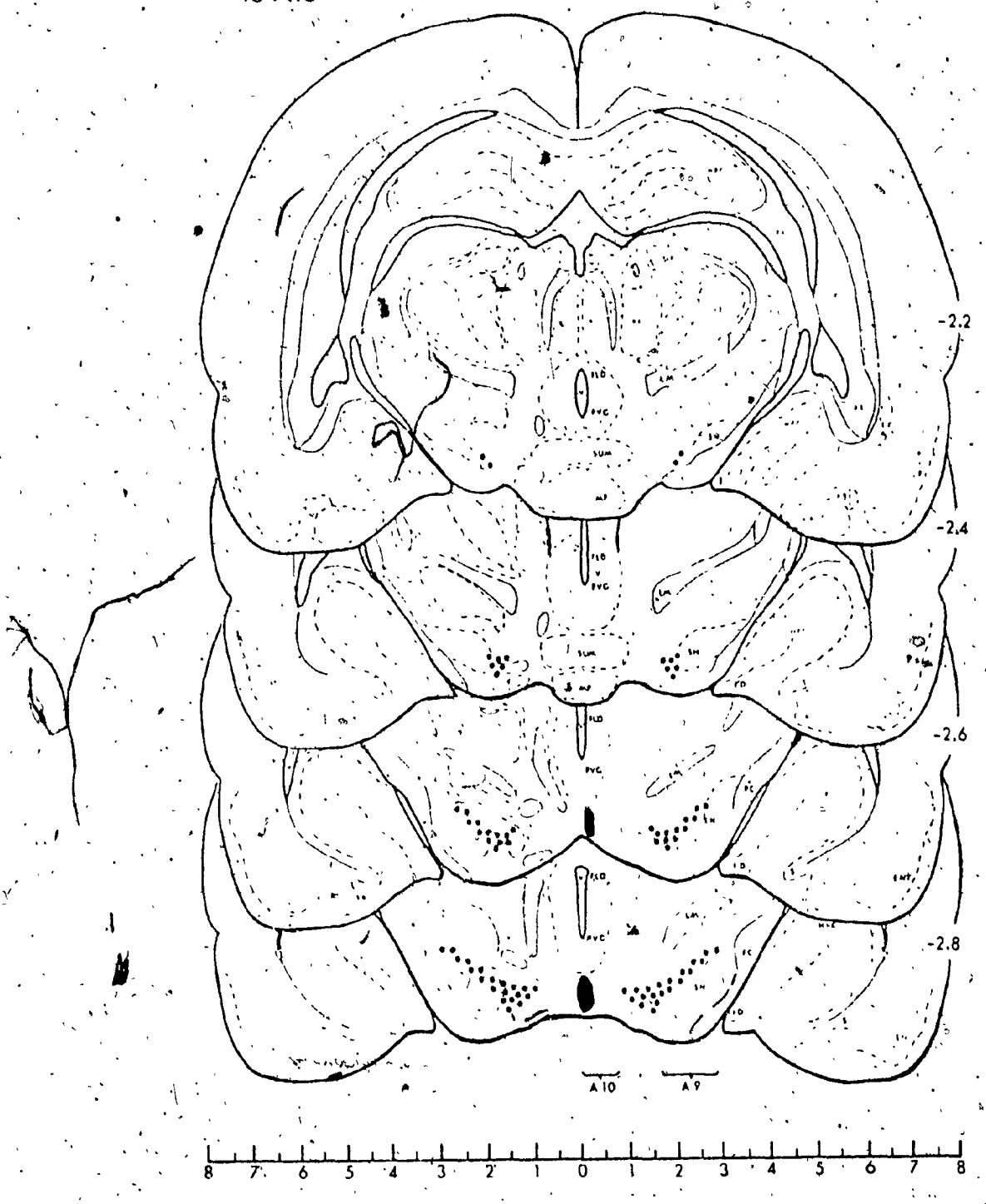

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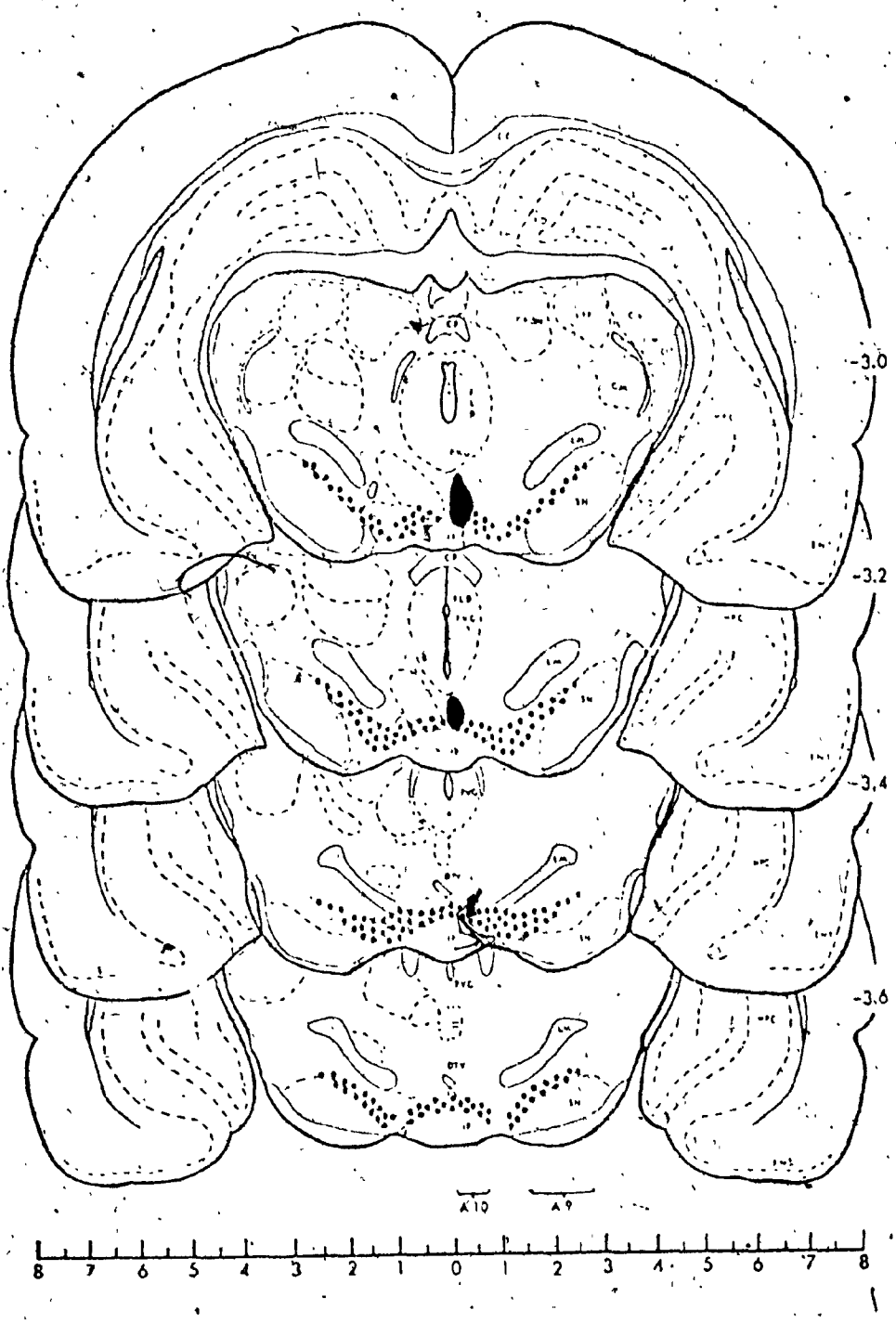

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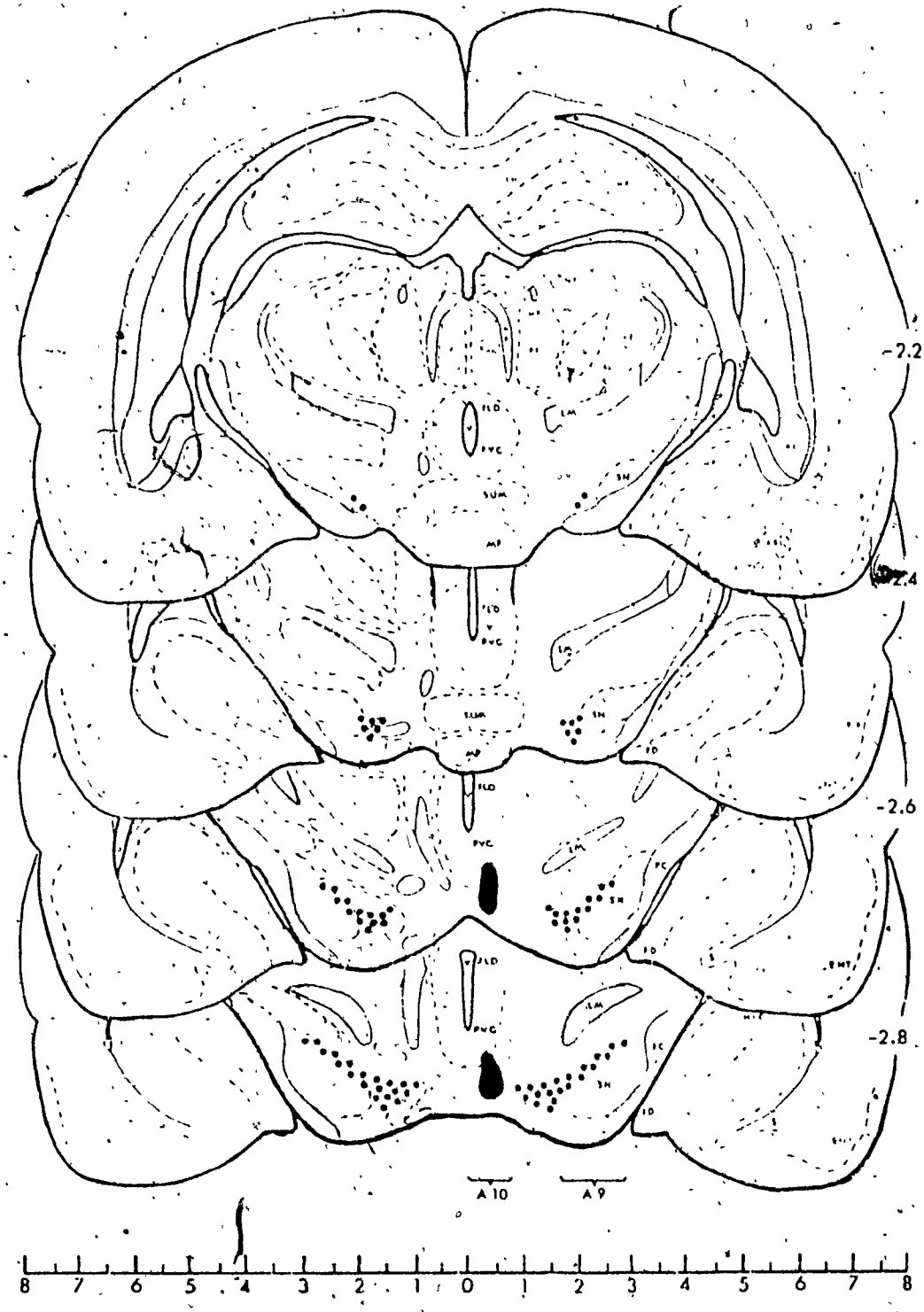

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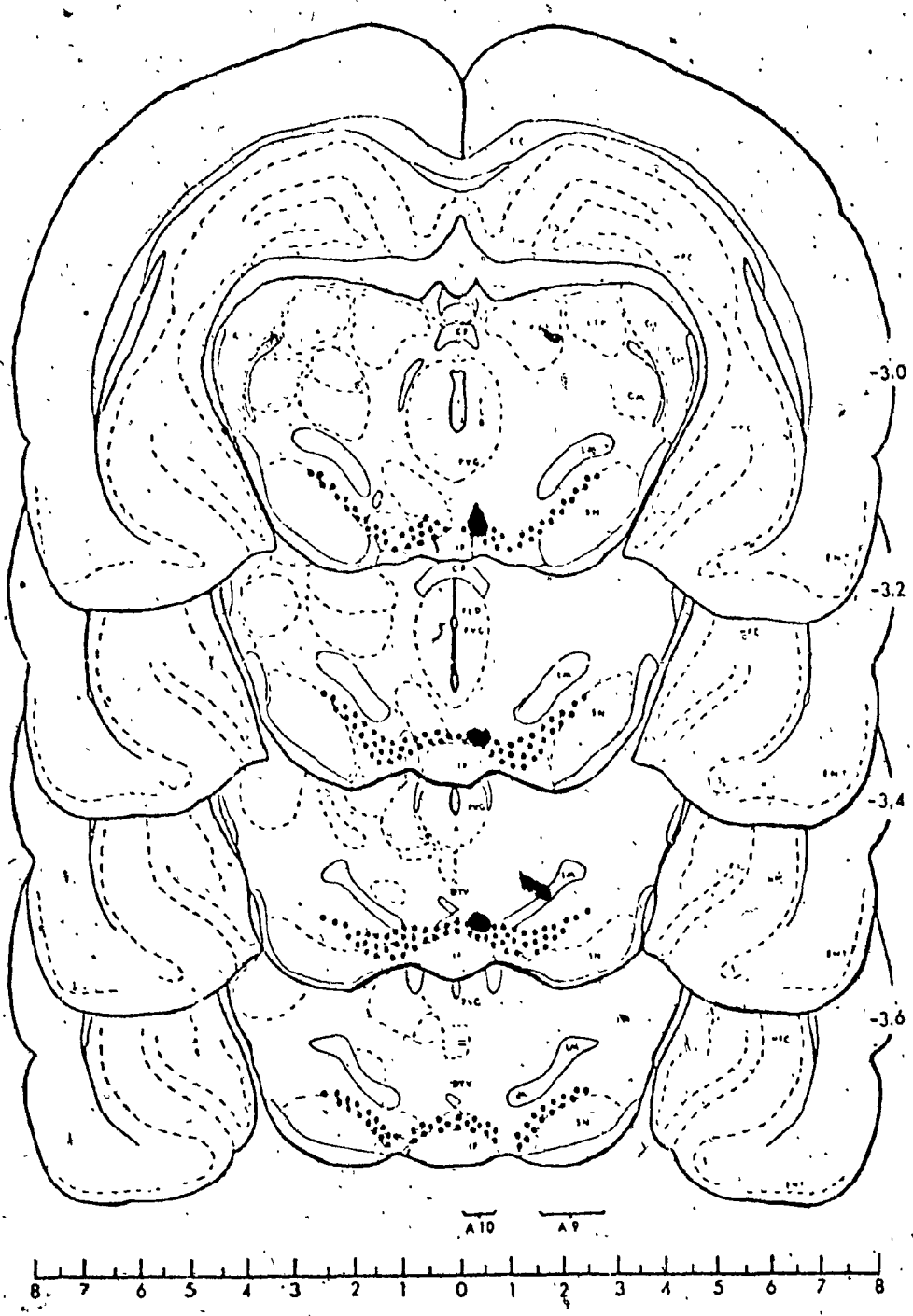

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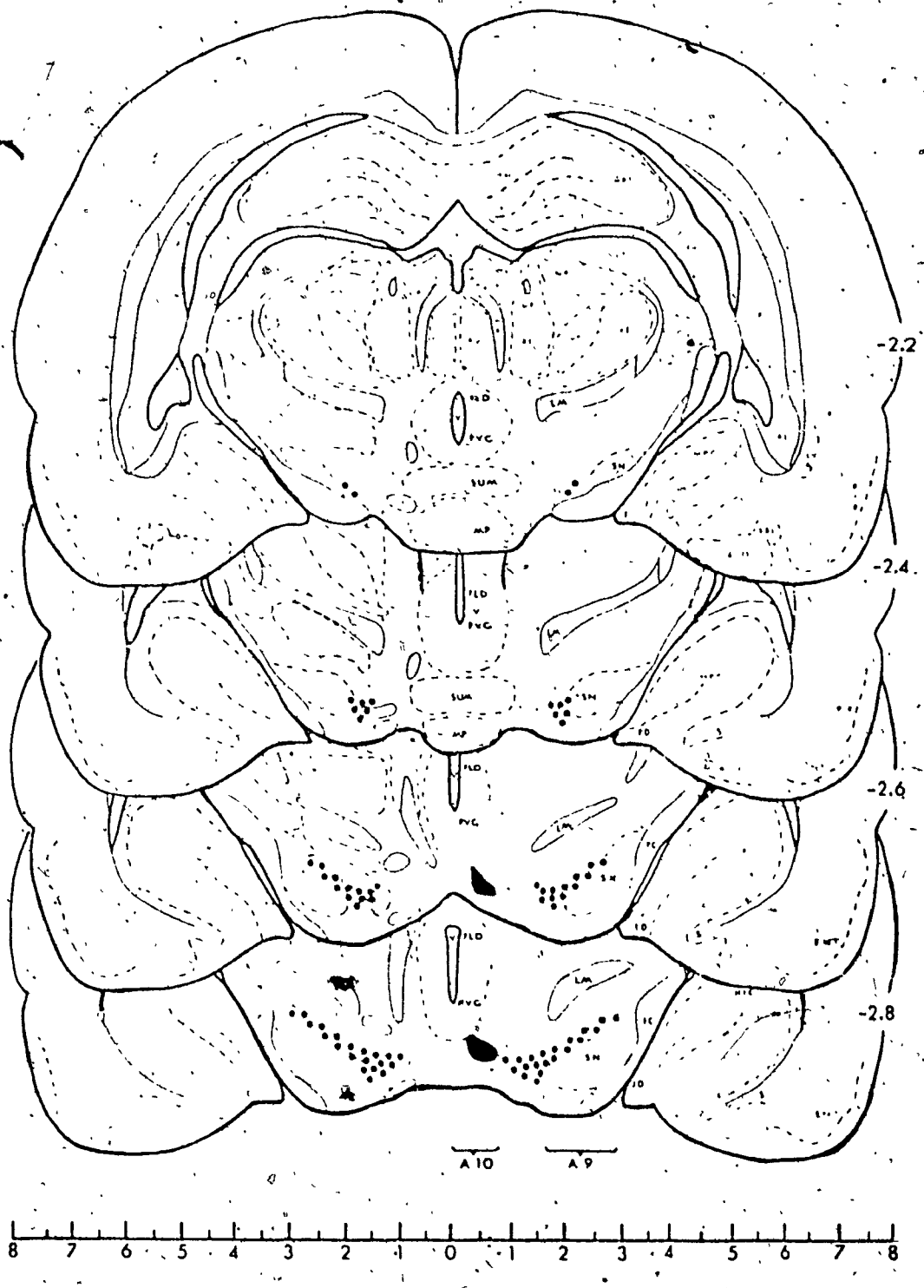

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