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A COMPARISON BETWEEN CAUDATE, AMYGDALOID AND HYPOTHALAMIC SELF STIMULATION IN SQUIRREL MONKEYS (SAIMIRI SCIUREUS)

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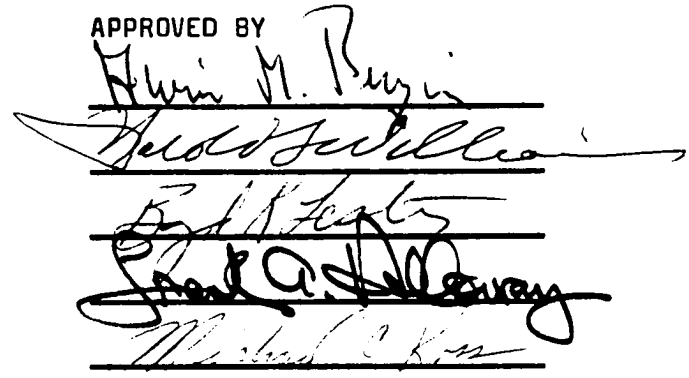

DISSERTATION COMMITTEE

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CHAPTER I

INTRODUCTION, LITERATURE REVIEW, AND STATEMENT OF PROBLEM

Electrical stimulation of various brain loci in humans or animals has produced many types of responses (Doty, 1967). One of the first investigators to describe the elicitation of emotional responses by brain stimulation was Hess (1928; 1936). Stimulation of the posterior portion of the hypothalamus produced rage responses that were integrated and involved direct attack. Later, other investigators (Delgado, 1954; Olds, 1954) demonstrated that brain stimulation had certain "motivational" components. For example, animals could be operant trained to either turn off or turn on brief pulses of current at certain brain sites.

In humans, stimulation within rhinencephalic and diencephalic regions can produce a "pleasurable" and rewarding sensation (Heath, 1960). These terms, however, are somewhat ambiguous since they imply a subjective judgement about a state of consciousness that is difficult to quantify experimentally, especially in animals. Positive reinforcement is a better term than reward because it can be operationally defined as a stimulus (e.g., electric current) which an organism will activate, approach or turn on to then change the frequency of occurrence in some

preceeding behavior (Verhave, 1966).

The following review of literature will discuss certain characteristics of intracranial self stimulation (ICSS) by animals including its generality across several species. Problems associated with ICSS measurement are also discussed. Although many brain sites support ICSS, the lateral hypothalamus is the region commonly used to elicit self stimulation at least in rodents. Thus, the anatomical and histochemical connections of this region are reviewed. Two other brain sites which sometimes support ICSS in more highly evolved animals, the caudate nucleus and the amygdaloid nuclei, are also examined with regard to their neuroanatomy, histochemistry and mediation of motivated behavior.

At one level of ICSS analysis is the work of Miller (1957), Stein (1961) and Olds (1960) which demonstrated that two psychoactive drugs, amphetamine or chlorpromazine (CPZ) influenced rate of ICSS. The hypothesis though (Stein, 1970) that these drugs act on noradrenergic synapses to produce their effects on ICSS has been questioned by several recent studies. In the present dissertation, neuropharmacological data are reviewed which suggest that besides norepinephrine (NE) other neurotransmitters such as acetylcholine (ACh), dopamine (DA) or serotonin (5-HT) are all influenced by the psychoactive drugs and may also influence rate of ICSS.

From this review of the literature a statement of problem is advanced which argues that if ICSS is to be fully understood, it must be analyzed using several parameters of investigations. Two commonly used methods are descriptions of current relationships and influence of drugs on ICSS rate and threshold. Information regarding the presence or absence of ICSS from various brain sites in the squirrel monkey along with the

modification of ICSS by changes in current parameters or drug administration can then be compared with ICSS data collected from other species using similar measurements. At one basic level of analysis comparison of rates of ICSS elicited from different brain sites following the administration of the psychoactive drugs may permit the possible examination of site(s) of action for these drugs. This analysis will be used to test Stein's (1970) hypothesis which asserts that the major site of action for amphetamine or chlorpromazine (CPZ) is mainly within the lateral hypothalamus. If rate of ICSS from the caudate is as equally affected as hypothalamic ICSS following various doses of drugs, Stein's hypothesis would have to then include telencephalic sites.

Phenomenon of Self Stimulation Behavior

In 1954, Olds and Milner first reported that electrical stimulation of various regions of rat brain had reinforcing properties. Using operant training procedures, the animal could be shaped to press a bar to receive a five hundred millisecond train of pulses to either the septum or lateral hypothalamus. Apparently the current served as a positive reinforcement which was similar to more conventional reinforcements such as food or water. ICSS has been demonstrated to have certain functional characteristics in common with the more conventional reinforcers and is sensitive to the manipulation of food, water or sex drives (Olds, 1962). Thus, the "rewarding" effect of central brain stimulation would seem to have implications for drive reduction theorists (Miller, 1958). However, Glickman (1967) has interpreted these reinforcing effects as evidence for a mechanism which insures species-typical responses to biologically significant stimuli. For example, those responses which occur following

activation of neuronal pathways within the brain stem that bring the animal into contact with stimuli relevant to survival are positively reinforcing.

The hypothalamus has been called the head ganglion of the autonomic nervous system because it appears to be the highest center of integration for many visceral functions (Grossman, 1967). Electrical stimulation in the rat hypothalamus not only supports ICSS but a variety of other consummatory, motivated behaviors such as eating (Hoebel and Teitelbaum, 1962), drinking (Mogenson et al., 1967) and copulation (Caggiula and Hoebel, 1966).

The importance of these stimulus bound-behaviors to ICSS and reinforcement is that within the rat hypothalamus the potential may exist for a complex elicitation of many behaviors that may interact with each other. For example, at low current levels, ICSS is facilitated if the animal is concurrently allowed to perform a consummatory response elicited through brain stimulation (Huston, 1972). Perhaps what may become reinforcing via a rechanneling of certain drives is the performance of the motor act.

The potential for self stimulation behavior in other species besides the rat has also been well documented. For example, ICSS has been described in cats (Wilkinson and Peels, 1963), monkeys (Brady, 1961), dogs (Stark et al., 1962), squirrels (Wetzel and King, 1966), chickens (Andrew, 1967), goldfish (Boyd and Gardner, 1962), dolphins (Lilly and Miller, 1962), goats (Persson, 1962), rabbits (Bruner, 1967), and humans (Bishop, Elder and Heath, 1963).

Problems of Interpretation Associated with ICSS

During electrical brain stimulation it has been suggested (Roberts, 1968) that it is difficult to specify either which neuronal elements are activated by the current or which elements are responsible for any given behavioral effect. For example, within a small zone of direct electrical excitation, the stimulus current can act indiscriminately on axons of passage, short-axons and local neurone somata all projecting to many brain regions. Electrical brain stimulation may not give a precise indication of the degree of unit or functional interconnections of the neurons mediating motivated responses and, indeed, may not give a clear indication of which neuronal systems are necessary or sufficient to support ICSS.

A variety of investigators have debated which response characteristic best measures strength of positive reinforcement (Hodos and Valenstein, 1962; Olds and Olds, 1963; Valenstein, 1964 and Hawkins and Plishkoff, 1964). The most commonly used measure in ICSS studies has been rate of responding, usually a lever press followed by a positive reinforcer (brain shock). This schedule is called a continuous reinforcement (CRF schedule). Other schedules such as fixed or variable ratios have been used (Olds, 1962) but with less frequency.

It is important to be aware when using only rate of responding that this measure may not always agree with the animal's preference and thus may be a misleading index of reinforcement. Hodos and Valenstein (1962) have demonstrated that at high shock intensities, rate can decline because motor side effects will disrupt press rate and performance. Nevertheless, animals may choose these high intensities over lower

amplitude stimulation that generally supports higher response rates. Plotnik (1972) has argued that if stimulus intensities are low enough so as to not evoke disruptive body movements, rate of response for a given electrode will correlate with reinforcement strength.

Despite these problems of interpretation associated with rate of response as a dependant variable, it was used in this dissertation in order to compare results with those of other drug studies that have used a similar measure.

Current Parameters and ICSS

Generally with a CRF schedule and either Lilly pulse-pair or sinusoidal stimuli, rate of self stimulation is a monotonic function of intensity of current (Lilly et al., 1955; Olds, 1958). In many brain areas, increases in current results in an increase of responding up to some asymptotic point. However, in other regions the stimulus-response relations are more complex with the same current increments producing a complex pattern of rate increases and decreases (Olds, 1962).

When rectangular-wave pulses are used, two other parameters besides intensity, pulse frequency and duration can influence rates of ICSS. For example, when the stimulating electrodes are located in the dorsomedial and posterior nuclei in the rat hypothalamus ICSS response rate is an increasing function of pulse frequency and/or pulse duration at any given pulse train duration (Keesey, 1962).

Stein (1962) manipulated the parameter of train duration and reported that rats with electrodes in the hypothalamus or midbrain prefer shorter train durations while rats with electrodes in the forebrain (caudate or septum) prefer longer durations. These different results

might fit the theory (Olds, 1958) that prolonged trains of stimulation in certain brain sites lose their net reward value through the progressive activation of a second negatively reinforcing system. In the forebrain the reinforcing effect of current may decrease more slowly, possibly as a result of adaptation. That is, with repeated stimulation the longer duration is more likely to activate other closely interconnected pathways and sites. This is more likely to occur within the hypothalamus where there are polysynaptic pathways which project to regions that contain aversive elements.

Neuroanatomical Correlates of ICSS

Although a wide variety of species have been tested for the elicitation of ICSS, a review of the literature indicates more than 80 per cent of all ICSS data have been collected in the rat (Wetzel, 1968). This large preponderance of studies in one animal demonstrates the need to avoid generalization of rat self stimulation data to other species.

Although the primary emphasis has been on the rat hypothalamus as the principal site to elicit ICSS, many other brain sites will support ICSS. These areas include, but are not limited to the frontal cortex (Routtenberg, 1972), the olfactory cortex (Spear, 1962), the olfactory tract (Valenstein, 1966), the optic chiasm (Bower, 1959), the hippocampus (Newman, 1961), the thalamic nuclei (Seward et al., 1960; Spies, 1965), the supramammillary decussation (Olds and Olds, 1963), the locus coeruleus (Ritter and Stein, 1972), and the amygdala (Olds, 1962).

Extensive maps of the occurrence of ICSS have been made in the cat (Wilkinson and Peels, 1963), the rabbit (Bruner, 1967) and the monkey (Plotnik, 1972). O'Donohue and Hagament (1967) have reported that

in the cat positive reinforcement areas include sites in the medulla (reticular formation) pons, midbrain, nonspecific thalamic nuclei, intrinsic thalamic nuclei, hippocampus, amygdala, fornix, caudate, septum and globus pallidus. However, some of these sites, e.g., caudate, globus pallidus, amygdala, do not consistently support ICSS in rats. The hypothalamus appears to be the one area that does support high rates of ICSS in most species.

Anatomy and Histochemistry of the Lateral Hypothalamus

Since the lateral hypothalamus is the site often used to elicit ICSS, the anatomy and histochemistry of this region will now be reviewed.

The lateral third of the hypothalamus is limited medially by the mammillothalamic tract and the anterior column of fornix (Haymaker, Anderson and Nauta, 1969). Rostrally it is contiguous with the lateral preoptic nucleus and caudally with the ventral tegmental area of the midbrain. Laterally the hypothalamus is bounded by the medial edge of the internal capsule. Most of the lateral zone is an undifferentiated mass of cells and fibers (Crosby, 1962).

A major bidirectional pathway that passes through the lateral hypothalamus and supports ICSS is the medial forebrain bundle (MFB). A small part of the MFB originates in the locus coeruleus in the brain stem (Millhouse, 1969). The MFB then ascends to interconnect the ventromedial rhinencephalic areas, pre-optic regions and lateral hypothalamus with the neocortex. The term "bundle" is misleading since the MFB is actually a diffuse group of fiber tracts that interconnect a large portion of the diencephalic and telencephalic regions of the brain.

Descending portions of the MFB originate in part from the

frontal cortex and cross through the preoptic area to go into and through the hypothalamus. The MFB also receives fiber tracts from septum, hippocampus, thalamic nuclei, globus pallidus, and amygdalar nuclei which project into and through the hypothalamus (Nauta, 1960). From the hypothalamus a dorsal and ventral pathway of MFB fibers proceeds caudally to terminate in the paramedian region of the mesencephalic tegmentum and locus coeruleus, respectively.

Several monoamine pathways run through the hypothalamus and proposed transmitters such as NE, DA and 5-HT are contained in the neurons located in these pathways (Dahlstrom and Fuxe, 1964). Recently histochemical fluorescence assays for the monoamines have been developed allowing for the visual identification of NE, DA and 5-HT cell bodies, axons and terminals. It has been demonstrated (Fuxe, 1965, 1968, 1970; Arbuthnott, 1971) that the cells which give rise to ascending monoamine neural pathways are regionally located in the mesencephalon and brain stem.

There are two major NE pathways within the lateral hypothalamus which have been implicated with ICSS (Clavier et al., 1973).

Dorsal NE pathway. This fiber tract originates from cell bodies in the locus coeruleus (Fuxe, 1965) and perforates through the brachium conjunctivum. The tract then passes into the dorsolateral tegmentum lateral to the zona incerta and Forel's field H proceeding into the lateral hypothalamus. When unilateral lesions of the dorsal bundle are made in the mesencephalic tegmentum, degeneration of NE nerve terminals can be traced to the cortex and hippocampal formation.

Ventral NE pathway. This pathway is thought to arise from the

NE cell bodies in the reticularis lateralis nucleus at the level of the superior olive (Fuxe, 1970). The fibers come together in the pons and as they ascend they pass medial to the motor nucleus V and turn medially along the dorsal surface of the medial lemniscus at the caudal third of the substantia nigra. Before reaching the interpeduncularis nucleus the ventral NE pathway turns rostral and reaches the MFB via the ventral tegmentum. Degeneration experiments suggest that many of the axons within the ventral NE pathway innervate the hypothalamus and preoptic area.

Dopaminergic pathways. Besides NE pathways, within the zone compacta and the ventral tegmental area a large pathway ascends into the lateral hypothalamus to enter the crus cerebri and intermingle with the MFB (Ungerstedt, 1971). This nigro-striatal pathway, which contains DA neurons fans out into the internal capsule and globus pallidus finally entering the caudate and putamen.

Axons from DA cell bodies dorsal to the nucleus interpeduncularis ascend together with axons of the nigro-striatal DA system. This second pathway is known as the meso-limbic DA system. It takes a more medial route and ascends in the dorsal part of the MFB. At the level of the anterior commissure one brance of the meso-limbic DA system enters the accumbens nucleus, interstitialis nucleus, and the stria terminalis. Another branch turns lateroventrally to enter the tuberculum olfactorium. The tuberoinfundibular DA system is located within the arcuate nucleus. These DA cell bodies spread out through much of the thalamus including the lateral border of the periventricular nuclei.

5-HT pathways. Cell bodies in the raphe nuclei of the mesencepehalon (raphe dorsalis and raphe medianus nuclei) give rise to seroto-

inergic axons which run in the middle third of the tegmentum (Fuxe, 1965). The majority of these axons lie close to the midline and become aggregated in a bundle between the mesencephalon and diencephalon. Many of these fibers enter the MFB by passing laterally close to the ventral outline of the fasciculus retroflexus and most of them become aggregated close to the lateral surface of the fornix. In the lateral hypothalamus there are two pathways which may originate from the lateral portion of the mesencephalon. One pathway lies beneath the most ventral portion of the crus cerebri. The other pathway lies just dorsal to the lateral part of the optic tract and is a portion of the MFB fiber tract.

Cholinergic pathways. Cholinesterase containing fibers ascend from the brain stem into the forebrain by two main routes (Shute and Lewis, 1963). The dorsal tegmental pathway runs rostral from the midbrain tegmentum and supplies the tectum, pretectal area geniculate bodies and the thalamus. Some fibers may also connect with the globius pallidus. The ventral tegmental pathway arises from the pars compacta of the substantia nigra, subthalamus and hypothalamus. There are direct fibers supplying globus pallidus, posterior and lateral hypothalamus and the lateral preoptic area. Indirectly, there are fibers via the lateral stria bundle and the amygdaloid nuclei. There are cortical radiations supplying caudate, amygdala and accumbens nuclei. The ventral pathway has connections via the lateral preoptic area with the diagonal band and the septum.

Lateral Hypothalamus and ICSS

Olds (1962) and Stein (1970) have postulated that the major site for the occurrence of ICSS, at least in rats, is within the MFB at

the level of the posterior hypothalamus. In this section evidence will be reviewed that supports this view.

In the early studies of Olds (1958; 1962) the more posterior portions of the lateral hypothalamus consistently supported ICSS. The highest rate of ICSS and the lowest stimulation thresholds were found within the hypothalamus.

Using lesion techniques, Ward (1960; 1961) first tested the hypothesis that the positive reinforcing effects from midbrain tegmental ICSS were mediated through the septum, fornix or amygdala. When these structures were removed, however, the rate of ICSS from the tegmentum was not changed. In conjunction with these lesion studies, Porter (1959) reported that lateral hypothalamic ICSS in monkeys did not produce seizures or after discharges in either the amygdala or septum. It appears unlikely according to these results that either the amygdala or septum directly supported tegmental ICSS, or that stimulation recruited other areas.

Later, Olds and Olds (1964) observed that bilateral lesions placed at the posterior boundary of the MFB were more likely to reduce anterior MFB ICSS more than anterior lesions reduced posterior MFB ICSS. Additionally, Olds and Olds (1969) reported that lesions in or near the MFB could impair or block self stimulation behavior in the same pathway with the magnitude of effect depending on the amount of tissue destroyed and the proximity of the destruction to the self stimulating point. Again, posterior lesions were more effective than anterior lesions in reducing ICSS. These studies imply that the MFB at the level of the

posterior hypothalamus might be an essential element in the mediation of hypothalamic positive reinforcement.

In addition to lesion work, mapping studies have revealed that both dorsal and ventral MFB pathways will support ICSS. For example, Routtenberg and Malsbury (1969), Huang and Routtenberg (1971), Crow (1972), and Crow et al. (1972) have shown that at the origin of the dorsal NE system, in the locus coeruleus, high rates of ICSS can be obtained. Dorsal mesencephalic tegmental regions which receive fibers of passage from the dorsal bundle are also positive with respect to ICSS. ICSS is also observed when the ventral tegmental region is stimulated (Arbuthnott et al., 1971).

Extra-hypothalamic Mediation of ICSS

Although Olds (1962) maintains that the lateral hypothalamus and the MFB in rats is the major site for eliciting ICSS, Valenstein (1966) has reported that in the rat no specific structural focus or center exists for self stimulation behavior. Large lesions involving the entire frontal plane of the MFB extending through the preoptic area, the anterior-posterior hypothalamus and the ventral tegmental area of Tsai failed to reduce ICSS elicited from the lateral hypothalamus. Also, Boyd and Gardner (1967) reported that lesions placed in the preoptic area mammillothalamic tract, postcommissural fornix or the interpeduncular and ventral tegmental area did not permanently abolish ICSS. A recovery of ICSS occurred after the initial depression caused by the lesion. These authors concluded that none of these four sites is essential to the maintenance of ICSS. Lorens (1964) also demonstrated that lesions rostral and caudal to an electrode in the MFB at the level

of the lateral hypothalamus failed to reduce ICSS in the rat. In other species such as the cat, Unemoto (1968) has observed that lesions which severed the majority of fibers in the MFB at the level of the septum, lateral preoptic and subthalamic areas caused no impairment of ICSS from the lateral hypothalamus.

One difference between Olds' procedure and others is in the length of recovery period between lesions and re-testing. For example, in Valenstein's work longer recovery periods were used and possibly other brain sites may have taken over certain functions indicating a degree of neural plasticity within the CNS. Alternatively, a longer recovery period would produce reduction of edema and recovery of function in neural tissue which could influence rate of brain stimulation.

The effect of lesioning one of the origins of the MFB (locus coeruleus) has been studied by Lorens (1973). When lesions are placed in the locus coeruleus, lateral hypothalamic ICSS is not impaired, suggesting some redundancy in pathways supporting ICSS. It is of interest that ICSS continued at high rates despite a forty-two per cent fall in telencephalic NE.

Brown (1973) also presents evidence that there may be several pathways involved with hypothalamic ICSS. Electrical stimulation of the ventral fornix (generally a negative reinforcing region) produced high rates of ICSS. When lesions were placed in the septum, a major projection of the fornix, fornix but not MFB ICSS was reduced. When lesions were made in the MFB, fornix ICSS was still observed. These results would suggest that fornix stimulation may activate neurones independent of MFB influences.

Besides lesion studies, which demonstrated that the MFB is not always necessary for ICSS in the rat, mapping studies have revealed that several of the monoamines may be implicated. Clavier and Routtenberg (1973) have argued that the dorsal NE bundle and the ventral midbrain tegmental dopamine systems are the two major positive reinforcement pathways in the rat. In these studies electrodes aimed at the lateral-hypothalamic MFB, shown to support ICSS, were then used to make lesions. Histochemical fluorescence methods revealed a buildup of monoamines in the ventral NE bundle, the nigro-neostriatal bundle and the mesolimbic fibers as well as the dorsal MFB. No electrode implanted in any portion of the ventral system proved to be positive nor were placements in the ventral system caudal to the origin of the dorsal bundle. These studies suggested that the catecholamine fibers in the MFB which are capable of supporting ICSS might consist in part of the two ascending dopamine systems from the ventral mesencephalic tegmentum as well as the dorsal portion of the MFB.

When comparisons regarding ICSS are made between the rat and primate certain species differences are noted. For example, lateral hypothalamic MFB self stimulation in primates is not as easy to obtain as in rats (Routtenberg et al., 1971). Krieg (1971) has proposed that the MFB may diminish in importance with evolution. However, there is no definitive evidence for this hypothesis. Many investigators have reported though that ICSS in primates is more frequently obtained from regions outside the hypothalamus. These areas include the head of caudate and putamen (Plotnik et al., 1972), the internal capsule (Routtenberg, 1971), the anterior, lateral and central amygdaloid nuclei (Brise and Olds,

1964) and the ventral nucleus of the thalamus and substantia nigra (Routtenberg, 1971).

One important pathway which may mediate ICSS in primates is the stria medullaris (Routtenberg, 1971). This pathway contains fibers from both the hypothalamus and the septal area which projects to the habenula and dorsomedial nucleus of the thalamus. This might be one alternative pathway for ICSS that does not involve the MFB, and projects from the septum to the habenula and then to the interpeduncluar nucleus of the midbrain.

In related work, Briese and Olds (1964) tested 245 sites in cynomolagous monkeys and reported that hypothalamic points did not support ICSS except in two cases whereas the highest rates of self stimulation were derived from the medial portions of the internal capsule. These reported results would suggest that either a species difference exists between the rat and the monkey regarding neuroanatomical correlates of ICSS or as Wentzel (1968) posits, sampling of these regions in rat brain has been inadequate. It should be emphasized again that any final "site" of activation following electrical stimulation of neural tissue is not necessarily at the electrode tip.

Since the amygdala and caudate are sites that will support ICSS in primates, a review of their anatomy follows.

Neuroanatomy and Histochemistry of the Amygdala

The amygdaloid nuclear complex is situated in the depth of the uncinate region just rostral to the hippocampus. Within this complex several nuclear masses have been identified in various mammals (Johnston 1923; Brodal, 1947). These include the lateral, basal (pars medialis,

pars cellularis and pars lateralis magnocellularis nuclei), medial, cortical, and central nuclei, the intercalated masses, the accessory basal nucleus of the lateral olfactory tract, and the anterior amygdaloid region which is located rostral to the amygdaloid nuclear complex.

Kaada (1972) has suggested that the amygdala may be divided basically into two portions: 1) a phylogenetically older anteromedial division consisting of the medial, cortical and central nuclei, the lateral olfactory tract nuclei and the anterior amygdaloid area which receives a definite projection from the olfactory bulb and projects to the septal, preoptic and hypothalamic areas; 2) a phylogenetically younger basolateral portion which has no direct connection with fibers from the olfactory bulb and does not send its fibers to the stria terminalis.

The afferent projections of the amygdala are massive but not well defined except for the olfactory fibers. These fibers reach the amygdala via the lateral olfactory tract and terminate in the corticomedial complex (Kaada, 1972). The lateral and basal nuclei receive olfactory impulses indirectly from the piriform cortex. Other brain regions that send afferent connections to the amygdala include the intralaminar nuclei of the thalamus, reticular formation in the brain stem, the hippocampus and the anterior insular and posterior orbital cortex.

The two major efferent subcortical tracts include the stria terminalis and the ventral amygdalafugal pathway (Lammers, 1972). The stria terminalis originates in the caudal one-half of the amygdaloid complex mainly from the nuclei of the corticomedial division. Short and long axons produce a reciprical relationship between the amygdaloid

complex and the ventral septal area, the medial preoptic area, the medial anterior hypothalamus, the ventro-medial hypothalamus and the ventral pre-mammillary region. The ventral amygdalofugal path is the main projection from the lateral and basal nuclei. The fibers spread medially through the central nucleus to then form a direct connection to thalamic, septal, lateral hypothalamic and preoptic areas. Other efferent cortical connections of the amygdala go from the basolateral complex to the piriform cortex, cingulum and tip of the temporal lobe and insula.

The amygdala contains equal numbers of NE and DA staining neurons (Fuxe, 1965). The location of these catecholamines appears to be at the presynaptic axon or bouton. The nigrostriatal dopamine system as it passes through the lateral hypothalamus and caudate projects, in part, to the central nucleus of the amygdala (Ungerstedt, 1971).

Stimulation of the amygdala in conscious, unrestrained animals, shows that this structure may be involved in a great variety of emotional states and response systems (Hilton et al., 1963). In addition to supporting ICSS, stimulation of the amygdala in cats produces a sniffing, searching and twitching of the face which is often accompanied by turning of the head away from the side of stimulation. Other reaction patterns such as defense reaction, growling, hissing, pilo-erection, salivation, chewing and facial jerks can appear. Many of these behaviors can be localized to various nuclei. For example, Lammers and Magnus (1955) have localized symptoms related to smelling as being elicited from the basolateral nucleus, central nucleus and pre-amygdaloid area. Behavioral responses related to eating, e.g., mastication, licking, swallowing, and vomiting can be elicited from stimulation of the periamygdaloid cortex,

basal nucleus, central nucleus, anterior amygdaloid area and the region around the external capsule. Fear and anger responses can be elicited from central and anterior nuclei.

Neurons in the region of the lateral amygdala are excited when eating, drinking, or positive reinforcement is elicited by electrical stimulation of the lateral hypothalamus (Rolls, 1972). Whether these results mean that the role of the lateral amygdala in many stimulus elicited behaviors is modulatory to or distinct from hypothalamic functions is debatable. Rolls argues that the role is probably modulatory since Olds et al. (1969) have shown that lesions in the amygdala appear to reduce hypothalamic ICSS.

Neuroanatomy and Histochemistry of the Caudate

Johnson and Rosvold (1971) report that the head of the caudate is a collection of nuclei. Thus, prefrontal cortical efferents terminate in different parts of the head of the caudate, those from the dorsal surface of the prefrontal cortex anterodorsally and those from the ventral surface, ventrolaterally. Moreover, cells in different sectors have a characteristic electrophysiological response to thalamic and nigral stimulation. Efferent fibers from different regions of the caudate terminate at different points in the globus pallidus and the substantia nigra.

The caudate projects to the nucleus ventralis anterior and lateralis of the thalamus (Laursen, 1963). It also projects indirectly to the ventromedial nucleus of the hypothalamus (by way of the putamen), subthalamus, zona incerta, red nucleus and the midbrain tegmentum.

Fibers from the intralaminar nuclei of the thalamus, cerebral

cortex and the mesencephalon (substantia nigra) project to the caudate (Crosby, 1962).

In contrast to either the lateral hypothalamus or certain nuclei of the amygdala, the concentration of dopamine in the caudate is twenty to fifty times that of NE (Fuxe, 1965). In most other brain regions dopamine concentrations are much smaller than NE amounts (e.g., hypothalamus one-tenth total catecholamine amount). The activity of acetylcholine forming enzymes is also high as is cholinesterase activity. The concentrations of gamma amino butyric acid, serotonin and histamine are not as high in caudate as hypothalamic regions (Fuxe, 1965).

Lesions in the anterior-ventral portion of the substantia nigra produce a substantial disappearnace of DA nerve terminals in the caudate (Ungerstedt, 1971). Lesions of caudo-lateral nigra induced degeneration in the caput caudatus. These data might indicate a one-to-one topographical relationship between the cell bodies of the substantia nigra and their nerve terminals in the caudate. Uncrossed monosynaptic dopaminergic pathways have been demonstrated in the substantia nigra terminating in the neostriatum (Anden, 1965).

The important of the neostriatum for maintaining normal brain function is evident in the case of Parkinson's disease. Cell loss in the substantia nigra (Hassler, 1955) and a correlated decrease in brain dopamine (Hornykiewicz, 1966) are commonly associated with this disease.

In addition to supporting ICSS, stimulation of the caudate can produce exploratory behavior, eye and ear movements, head turning, freezing, plastic rigidity and circling to the side opposite from stimulation (Laursen, 1963). Many of these behaviors result from extra-

pyramidal involvement (Hornykiewicz, 1966).

To summarize this section on neuroanatomical correlates of ICSS, these data suggest that while the posterior hypothalamus in the rat supports ICSS, its relationship with the rest of the central nervous system (CNS) is diffuse and complex. It is likely that electrical stimulation of the MFB activates numerous brain regions and acts on several brain amines. The lesion work appears to demonstrate that there is both redundancy and a significant capacity for reorganization in the system that subserves ICSS, especially in the posterior hypothalamus. There are discrepancies in the literature, but they are not surprising when one considers that in different investigations, different brain regions were stimulated, lesions varied in size, and recovery periods were sometimes not specified. Histochemical work also suggests a great deal of overlap regarding the relative distribution of NE and DA within and outside the MFB.

As an example, Anand and Brobeck (1951) first reported that lesions in the lateral hypothalamus produced adipsia and aphagia suggesting a role for the hypothalamus in feeding and drinking. Later, Ungerstedt (1971) reported that 6-hydroxydopamine (6 OHDA) which is a catecholamine congener that causes a depletion in both NE and DA levels, produced when placed intracerebrally, bilateral degeneration of neurons in caudate and substantia nigra. Behaviorally, long-lasting adipsia and aphagia occurred. One interpretation of these findings would be that much of the earlier work investigating lesions and their effect on motivated behavior may have caused an interruption of a nigro-striatal DA system although NE involvement cannot be discounted. The striatum would

appear to play a still undefined excitatory or inhibitory role in the control of motivated behavior.

In more highly evolved animals such as primates other regions which differ neuroanatomically, histochemically and produce, when stimulated, different behavioral sequale than the hypothalamus have been shown to support ICSS. Thus, it might be argued that there are several positive reinforcement sites within the brain. This suggestion is in direct opposition to Olds' hypothesis of a single common "pleasure center" located in the posterior hypothalamus. It seems difficult to conceptualize that "pleasure or approach" or positive reinforcement eminate from only one structure since in humans a variety of sensations are perceived when different brain regions are stimulated (Heath, 1960).

The next section of the review of literature will further develop the idea that several amines and brain sites probably support ICSS.

Psychoactive Drugs and ICSS

The self stimulation of the hypothalamus by animals has led to the proposal (Olds, 1954; Stein, 1964) that a neuronal system for "pleasure" exists within the brain. For example, it has been established that systemic administration of the clinically used psychoactive drug amphetamine will influence rate of ICSS (Stein, 1964). Amphetamine also potentiates and blocks catecholamine re-uptake and release (Glowinski, 1965) and when given to humans in very high doses will produce hallucinations. Since the hypothalamus contains high quantities of at least one amine, NE (Hillarp et al., 1966), behavioral investigators (Stein, 1964; 1970) have implicated noradrenergic neuronal projections with

pleasure and affective behavioral states.

The hypothesis that certain aminergic systems may be involved with various affective behaviors is not new. Early investigators (Wearn and Sturgis, 1919) have reported that human subjects that received infusions of epinephrine reported the occurrence of subjective symptoms resembling anxiety. Later, in better controlled experiments, Schacter and Singer (1962) reported that administration of epinephrine increased either moods of euphoria or anger in humans. However, since the blood brain barrier restricts entry of epinephrine into the brain, there is doubt whether a change in any affective state is by a direct central action. Later research has shown that NE or epinephrine administered in such a way as to permit crossing the blood-brain barrier (i.e., intraventriculary or intracisternally) produces lethargy, sedation and depression in various species such as the rat, chicken, cat, dog and man (Mandell and Spooner, 1968). Thus, although interpretation is difficult, it does seem that these amines do act within the CNS.

During the past decade, a large number of studies have been devoted to understanding the relationships between the biogenic amines and affective states or behaviors (Bloom, 1968). Both clinical and experimental evidence now suggests that affective disorders may be frequently associated with the dynamics of catecholamine metabolism in the brain (Schildkraut et al., 1967). For example, Moore (1971) suggested that certain moods (i.e., euphoria or depression) may be associated with abnormality in the availability of NE at specific central receptor sites.

If abnormal affective behavior results as a consequence of changes in metabolism or activity of the monoamines, then drugs that

also alter amine metabolism should exert some control over mental function. ICSS has been used in conjunction with the administration of the psychoactive drugs to test this hypothesis by providing a quantifiable model for "affective behavior" in animals. It is realized, of course, that definitive evidence of drug effects on "mood" and emotions will eventually have to come from human experience.

The following review will discuss the mechanisms of action of two psychoactive drugs, amphetamine and chlorpromazine (CPZ) and their effects on ICSS as well as on biogenic amine metabolism.

Amphetamine

Amphetamine is a short-acting stimulant which has been used for many years with variable results in the treatment of depression (Schildkraut and Kety, 1967). Ellinwood (1970) reports that abuse of this drug has increased in dramatic proportions over the last twenty-five years. When given in high doses in humans, amphetamine generally produces psychotic episodes, delusions, visual and auditory hallucinations, hyperactivity, and a fixed compulsive behavior such as repetitive examining, searching and sorting the environment. Stereotypic motor behaviors have also been reported in various species of animals following amphetamine administration (Randrup, 1967).

Neurochemistry. Amphetamine is thought to have multiple actions on the metabolism and functions of the catecholamines in the brain (Glowinski et al., 1966; Axelrod, 1971). Its major site of action is thought to be on the presynaptic axon terminal. Like the tricyclic antidepressants, amphetamine blocks the re-uptake of catecholamines into the neuron, causes their release from storage sites and inhibits monoamine

oxidase. When amphetamine is given in high doses all of these mechanisms can be activated.

The re-uptake process is mediated by several mechanisms (Iverson, 1973). One involves the existence of a specific facilitating or mediating biochemical mechanism whereby both NE and DA are transported from the extracellular space across the axonal membrane of adrenergic or dopaminergic neurons. Another mechanism exists to promote the transfer of free catecholamines from the axoplasm into the membrane bound storage vesicle. Either high doses of amphetamine (10-30 mg/kg) or chronic administration of amphetamine causes a diminution of brain NE levels, whereas 5 HT and DA levels are not as affected (Van Rossum, 1970). In contrast, low doses of amphetamine, which fail to alter the steady state levels of endogenous catecholamines have been reported to increase the turnover rate of both NE and DA in the brain (Kety, 1967). The effect of amphetamine on NE synthesis was reported to be greatest in the mesencephalon.

In addition to acting on catecholamines, amphetamine administration produces an increase in acetylcholine (ACh) release from the cerebral cortex (Hemsworth and Neal, 1968). It has been reported (Pepeu and Bartoline, 1968) that d-amphetamine causes an increase in ACh output from the cerebral cortex and EEG activation in cats transected at the mid-pontine pre-trigeminal level. Further, alpha blocking agents such as phenoxybenzamine do not prevent the action of d-amphetamine on either electrical activity or ACh output. Possibly, either beta receptors or dopamine releasing nerve endings are involved in these effects.

Actions on ICSS. Amphetamine decreases current thresholds for ICSS in the lateral hypothalamus of rats and potentiates ICSS if current levels are low enough (Olds, 1959; Stein and Ray, 1960; Stein, 1961; Stein, 1964). When other stimulant drugs such as strychine or picrotoxin are given, ICSS is not facilitated. Moreover, if the current is turned off during amphetamine facilitated ICSS, rate is decreased. Thus, amphetamine must have a partial central effect as well as peripheral (i.e., the animal is not just pulling the bar due to hyperactivity).

In other species such as primates (Brady, 1961) and cats (Horovitz et al., 1962), amphetamine has been reported to facilitate ICSS from the lateral hypothalamus. However, Umemoto and Kido (1967) have demonstrated that high doses of amphetamine (1.0 to 2.0 mg/kg) reduced ICSS. The discrepancy between these results may have occurred because different operant schedules of reinforcement were used. Alternatively, a more likely explanation is that a biphasic dose effect exists with amphetamine administration. That is, with low doses, amphetamine facilitates ICSS while with higher doses amphetamine decreases ICSS.

When Stein first reported that ICSS of the rat lateral hypothalamus could be potentiated by amphetamine, it was recognized that this psychostimulant had a variety of central effects on a number of different systems. Early theories (Mann and Quastel, 1940) had proposed that amphetamine produced its effect on the CNS by inhibiting monoamine oxidase. This is correct, but the action is extremely weak. It was later found that amphetamine exerted its peripheral effect by a local release of catecholamine (Burn and Rand, 1958). Smith (1965) has proposed that amphetamine acts directly on catecholamine receptors in the

brain by a mimicking action. However, Stein (1964) has reported that reserpine weakens or shortens the facilitatory effect of amphetamine on ICSS while amphetamine-like facilitation was observed after alpha-meta-tyrosine administration. These findings led Stein to an alternative proposal that the behavioral stimulating action of amphetamine was mediated by a local release of a naturally occurring catacholamine. Secondly, while this early work did not specify which catacholamine, NE or DA, was released by amphetamine, Stein argued that serotonin was probably not involved. This hypothesis was based on the observation that alpha-methyltyramine did not facilitate ICSS and that large doses of amphetamine lowered NE content but elevated serotonin. Interestingly, Stein did not take into consideration that the elevation of serotonin may be part of a complex process necessary to produce facilitation in behavior.

Stein (1964) suggests that a phenethylamine derivative such as a catecholamine might be specifically involved with ICSS. Since both amphetamine and NE are derivatives of phenethylamine, Stein proposed that the behavioral facilitating action of amphetamine might depend on its similarity to NE. Amphetamine would enhance ICSS by first causing or facilitating a release of NE, thus producing a high level of free NE at central receptor sites. NE would then cause directly or indirectly "positive reinforcement" neurons to fire within the lateral hypothalamus.

Through the 1960's Stein and his group have attempted to establish the relative contribution of noradrenergic and dopaminergic systems to ICSS. One method was to use the psychoactive drugs amphetamine and CPZ and some of the results of those studies will now be reviewed,

utilizing three different levels of analysis.

Interaction of amphetamine and dopamine β-hydroxylase inhibitors. Axelrod (1971) and Sedvall et al. (1968) have proposed that NE in the nerve endings is contained in two pools, a small functional one and a larger essentially reserve pool. Stein (1970) has demonstrated that dopamine β-hydroxylase inhibitors can be used to test the hypothesis that the facilitatory effects of amphetamine are mediated by the release of NE from functional pools. Since dopamine β-hydroxylase is the final step in the enzymatic synthesis of NE (Kopin, 1967), presumably inhibition of NE synthesis would produce a decrease in brain NE levels.

Systemic injections of disulfiram or intraventricular injections of diethyldithiocarbamate (DEDTC), proposed inhibitors of NE synthesis, suppress ICSS while intraventricular administration of l-norepinephrine selectively and rapidly reinstated ICSS. Neither 5-HT or DA were capable of reversing the effects of the drugs.

Stein (1970) further speculated that under normal conditions ICSS depends primarily on the synthesis de novo of NE in functional pools. He based this hypothesis on the assumption that inhibition of NE biosynthesis produced failure of ICSS after the small reserve of transmitter in the functional pool was exhausted (Wise and Stein, 1969). His finding (Stein, 1970) that ICSS can be re-instated by the intraventricular administration of exogenous NE again suggests that the critical factor may be the availability of NE in functional pools. When animals were pretreated with inhibitors of dopamine β-hydroxylase, such as disulfiram, and then given a dose of amphetamine that normally produces an increase in ICSS, the result was a complete block of ICSS. However, when l-norepinephrine was injected intraventricularly into these animals,

ICSS was partially restored at 15 minutes, completely at 45 minutes and by 75 minutes the facilitating effect of amphetamine was again observed.

To summarize, it appears that the effects of amphetamine were, for the most part, blocked with peak inhibition of NE synthesis and that the administration of exogenous l-norepinephrine largely reinstated the facilitating effect of amphetamine.

Perfusion studies. The MFB is most commonly used as the neuroanatomical site to elicit ICSS since in the rat at least it is easiest to elicit ICSS from this structure. Stein (1970) has designed a series of experiments that provide a direct test for the hypothesis that the behavioral facilitating effect of amphetamine is mediated by the release of NE from terminals of axons within the MFB fiber tract. These experiments derived from earlier work (Stein and Wise, 1967; 1969) which demonstrated that NE could be released into a brain perfusate by positive reinforcing electrical stimulation of the MFB. The small quantities of NE were measured by a sensitive radiotracer method described by Glowinski et al. (1965). The labeled NE introduced into the brain in this manner mixes with the endogenous store and is used as a tracer.

The same perfusion technique has been used to demonstrate the release of NE into brain perfusates by small intraperitoneal doses of amphetamine. For example, Stein (1970) reports that 3 mg/kg of d-amphetamine sulfate substantially increased the radioactivity of amygdaloid perfusates just as reinforcing electrical stimulation will, but amphetamine will not release NE from the hypothalamus. Stein posits that NE released by amphetamine acts mainly as an inhibitory transmitter which depresses the activity of behaviorally suppressant cell groups in

the forebrain. Amphetamine then would facilitate "behavior" by a disinhibitory action. There is evidence (Margules, 1968; Wise and Stein, 1969) that direct application of NE (but not dopamine or DOPA) to the amygdala produces a decrease in the suppressant effects of punishment (i.e., shock-avoidance), suggesting that NE synapses in at least certain portions of the amygdala are indeed inhibitory. Moreover, in other portions of the mammalian central nervous system, such as the cerebral cortex, pyriform cortex, caudate, olfactory bulb, thalamus, hypothalamus, brain stem, cerebellum and spinal cord, NE when applied microelectrophoretically, produces inhibition in nerve cell responding (Himwich, 1970).

Brain site comparisons of amphetamine's action. Ritter and Stein (1972) demonstrated that amphetamine administration produces a greater degree of ICSS facilitation when electrodes are implanted at the locus coeruleus (L.C.) than at the lateral hypothalamus. This evidence suggests that the L.C. is more sensitive than the hypothalamus to drugs that act on NE releasing neurons. These findings appear plausible since the density of NE neurons is greatest at the L.C. (Fuxe, 1965). Thus, stimulation here would probably release more NE than would stimulation in portions of the MFB fiber tract since the tract fans out and becomes more diffuse as it proceeds rostrally to the cortex.

Chlorpromazine

The phenothiazine tranquilizers, e.g., chlorpromazine (CPZ), are the drugs of choice for a variety of mental disorders (Moore, 1971). They are especially useful in the treatment of psychotic patients because CPZ has a specific antipsychotic effect that is not accompanied by marked

sedation. Some of the central actions also described for CPZ include blockade of vomiting, extrapyramidal, Parkinson-like abnormalities, hypothermia and hypotension.

Neurochemistry. It has been difficult to develop any unifying mechanism of action for CPZ because of its diverse effects on various brain amines (Moore, 1971). The principal and most general mode of action for CPZ is to block amine transport mechanisms at the cell membrane. Possibly as a result of this, post-synaptic amine receptors, principally alpha-adrenergic and dopaminergic, are also blocked.

Actions on ICSS. Self stimulation is inhibited either by the tranquilizer CPZ or the biogenic amine depletor reserpine but not by pentobarbital even with doses that reduce other rewarded behaviors (Olds, Killam and Bach-y-Rita, 1956; Miller, 1957; Olds, Killam and Eiduson, 1957; Olds and Travis, 1960; Stein, 1962; Olds, 1962; Olds and Olds, 1964; Routtenberg, 1971). Most of the other phenothiazines also either increase threshold or lower the rate of responding. When CPZ is given prior to a low dose of amphetamine, the facilitatory action of amphetamine is antagonized (Stein, 1967). This finding suggests that the major central action of CPZ is to block receptors (instead of blocking re-uptake) while amphetamine's major action is to block re-uptake. Thus the accumulation of NE at the synapse resulting from amphetamine's action may be antagonized by CPZ's blockade action at receptor sites.

In the report of Olds (1956), the self stimulation method was used to screen and possibly differentiate CPZ, sodium pentobarbital and reserpine. It was demonstrated that CPZ reduced rates of ICSS to a larger extent in the hypothalamus than in the septal region. Pentobarbital at doses of 10 mg/kg did not reduce ICSS whereas reserpine had

an effect similar to CPZ. Olds concludes that in the rat, ICSS can be used to distinguish tranquilizing agents from other central nervous system depressants. It was proposed that primary drives in various parts of the "positive reinforcing system" might be delineated by the use of different drugs. The hypothesis advanced was that chemicals which successfully control psychotic agitation suppress ICSS.

Olds and Travis (1960) could not, however, confirm their earlier work. The differential effects of CPZ on different parts of the self stimulation region, e.g., septum or hypothalamus and tegmentum, were not as evident as the general propensity of CPZ to inhibit ICSS when compared with meprobamate and pentobarbital.

Stein (1971) has incorporated the above findings and advanced a biochemical hypothesis of schizophrenia which explains why CPZ exerts an important therapeutic action. Six-hydroxydopamine (6-OHDA), an autoxidation product and metabolite of dopamine, is the proposed aberrant metabolite that produces schizophrenic symptoms. When 6-OHDA is injected intraventriculary into the rat brain, a prolonged and permanent depletion of brain catecholamines occurs with NE depleted more than dopamine. Electron microscopic evidence also shows that NE but not DA nerve terminals in the brain degenerate and disappear after repeated doses of 6-OHDA.

When 6-OHDA is given to rats that are self stimulating for current to the MFB-lateral hypothalamus, all ICSS and other reward behaviors are impaired. The duration of this impairment lasts for at least one week or more. Stein interprets these findings to say that the selective destruction of NE binding sites and terminals of the MFB by 6-OHDA over long periods of time (assuming 6-OHDA is formed endogenously) causes progressive and at least partial irreversible damage to the "reward

mechanism". Such damage, according to Stein, could produce the primary symptoms of schizophrenia.

When rats are given CPZ pre-treatment one week prior to the 6-OHDA administration, ICSS is not reduced. Furthermore, while 6-OHDA ordinarily depletes brain NE, pre-treatment with CPZ prevents this effect. Thus, CPZ prevents destruction of NE terminals and exerts its therapeutic action by blocking 6-OHDA entry into the NE terminals.

Stein's Hypothesis for the Major Site of Action for Psychoactive Drugs

Stein (1970) has now proposed that the ascending adrenergic pathway to the cortex is the central site of action of amphetamine and CPZ. Stein has accepted much of Olds (1962) evidence which argued that the MFB was the principal pathway for "positive reinforcement". In support of this hypothesis, histochemical evidence (Fuxe, 1965; Ungerstedt, 1971) demonstrates the existence of a system of catecholamine containing neurons whose cell bodies originate in the ventromedial part of the mesencephalon or the locus coeruleus. These fibers ascend within the MFB and terminate at various adrenergic synapses in much of the diencephalon and telencephalon. The sites of origin and distribution of this "adrenergic" system are found within parts of Nauta's limbic midbrain circuit (Nauta, 1958).

According to Stein's hypothesis (1970), electrical stimulation of the MFB at the level of the hypothalamus activates adrenergic synapses in the neocortex and amphetamine administration facilitates operant behavior by the release of NE whereas CPZ inhibits behavior by blocking adrenergic transmission at these same sites.

Evaluation of Stein's Hypothesis

Stein's hypothesis that the psychoactive drugs have their major site of action within the hypothalamus needs closer analysis because of evidence (Moore, 1971) that both amphetamine and CPZ have multiple effects on behavior and brain amines. This section will review that literature and suggest that the psychoactive drugs act on regions of the brain that contain dopamine as well as NE containing neurons.

Amphetamine

Behaviorally, amphetamine's effects are complex. Domino (1970) has argued convincingly that one important action of amphetamine is the biphasic effect mentioned earlier in this review. Not only do low doses facilitate operant behavior and high doses markedly depress behavior but a single low dose of amphetamine will depress high rates of the same behavior (Drew, 1958). Stein (1970) suggests, however, that with increases in MFB neural activity (i.e., ICSS), there is increased turnover and release of NE. If to this high release, amphetamine is added, causing more NE release, the "high dose" effect of the drug is seen at lower doses.

Amphetamine produces in a variety of species a type of hyperactivity and stereotyped behavior such as biting the wires of a cage, walking backwards and excessive sniffing (Randrup, 1967). Many of these drug induced behaviors are eliminated by lesions within areas that have high dopamine content, e.g., neostriatum (Ernest, 1969). This type of stereotyped behavior could be due to the blockade of re-uptake of DA at the nerve endings in the caudate. Further, Carr and Moore (1970) have reported that high concentrations of amphetamine perfused through the cerebral ventricles increased the concentration of DA in the perfusate.

Systemic injections of amphetamine into rats with unilateral lesions of the nigro-striatal pathway produced turning behavior similiar to that produced by direct unilateral stimulation of the pathway (Anlezark, Arbuthnott, Christie and Crow, 1971). Thus, amphetamine would appear to act on dopamine as well as NE receptors.

Additionally, Taylor and Snyder (1970) have reported that the d- and l-isomers of amphetamine can be used to assess their different actions on regions of the brain that contain DA or NE. For example, the corpus striatum contains high concentrations of DA and low concentrations of NE while the hypothalamus contains high amounts of NE. Earlier work by Coyle and Snyder (1969) had shown that in the hypothalamus, d-amphetamine was ten times as potent as l-amphetamine in inhibiting catecholamine uptake by synaptosomes while in the corpus striatum the two amphetamine isomers were equally active. In the Taylor and Snyder study, male rats were injected subcutaneously with 10 mg of either d or l (3H) norepinephrine labeled for radioactive tracer analysis. Following decapitation, the concentration of tritiated and endogenous NE was determined in various regions of the brain. In the hypothalamus, d-amphetamine produced a marked reduction in the accumulation of (3H) norepinephrine as well as reducing endogenous NE. L-amphetamine had no effect. In the corpus striatum both d and l amphetamine caused a marked reduction in the accumulation of (3H) NE but had no effect on endogenous NE. In behavioral related studies, d-amphetamine was almost ten times as potent as l-amphetamine in enhancing locomotor activity but only twice as potent in eliciting a compulsive gnawing syndrome. These studies led to the conclusion that behaviors such as locomotion involve NE while

dopamine neurons play a more important role in compulsive gnawing.

The above findings have now been extended to show that d- and l-amphetamine produce different effects on ICSS depending on the brain site stimulated (Phillips and Fibiger, 1973). The d isomer was seven to ten times more effective than the l-isomer in facilitating ICSS at the hypothalamus whereas both isomers were equipotent for substantia nigra electrodes. The significance of these findings is that both noradrenergic and dopaminergic systems in the brain are differentially responsive to the isomers of amphetamine in terms of brain neurochemistry as well as behavior (i.e., activity, ICSS). Probably, both DA and NE systems subserve reinforcement as previously suggested by histochemical work (Clavier and Routtenberg, 1973). It should be noted, however, that stimulation of substantia nigra could, by current spread through habenula, activate NE fibers as well as DA fibers (Anden et al., 1967).

Chlorpromazine (CPZ)

Stein (1970) has demonstrated that CPZ administration blocks lateral hypothalamic ICSS, presumably by blocking NE neurons post-snyaptically. However, Hornykiewicz (1966) has argued that another major site of action of CPZ is in the striatum where high concentrations of dopamine and dopamine containing neurons are present.

There is evidence that CPZ may be involved with striatal function. For example, Nyback and Sedvall (1968) have presented data which shows that CPZ markedly accelerates the accumulation of labeled dopamine-C^{14} in the striatum while other brain regions are not affected. NE concentrations were not altered either in the striatum or other brain areas. The authors conclude that CPZ has a selective and major effect on the

nigro-neostriatal dopamine pathway (where synthesis of the transmitter occurs). O'Keeffe et al. (1970) have reported that CPZ (15 mg/kg) given daily for two weeks in cats produced a rise in the concentration of the acid metabolite of dopamine, homovanillic acid (HVA). Coyle and Snyder (1969) suggested that many of the phenothiazine drugs are potent inhibitors of dopamine uptake in the striatum. Thus, it appears that the regional action of CPZ probably involves DA as well as NE, and, perhaps, other transmitters as well.

Although Stein (1971) found that CPZ administration protects ICSS from 6-OHDA treatment, his data do not demonstrate an exclusive and specific action of 6-OHDA on NE or ICSS. For example, Antelman et al. (1971) observed that 6-OHDA reduces ICSS elicited from the MFB-lateral hypothalamus. By "priming" (i.e., experimenter instead of animal applies current for a brief period of time at start of test session), ICSS returned and after several days, priming was not necessary. Supplemental injections of phentolamine, an adrenergic blocker only reduced rates of ICSS by 10 to 30 per cent. Biochemical assays revealed that telencephalic NE levels were reduced by as much as 90 per cent. Since storage of NE was reduced and functional utilization of remaining NE was probably eliminated, it would appear that other transmitters besides NE may be involved with ICSS. In related work, Breese et al. (1971) have found that intracisternal injections of 6-OHDA decreased ICSS by 50 per cent and reduced both NE and DA by 83 per cent. A second injection of 6-OHDA reduced both amines to 7 per cent and eliminated ICSS. This evidence strongly suggests that 6-OHDA acts on both DA and NE containing neurons.

It has been suggested that DA, which is the immediate precursor

in the biosynthesis of NE, has a separate role in brain from NE (Brucke et al., 1969). Van Rossum (1970) postulated that NE is the major neurotransmitter that supports ICSS while other behaviors such as conditioned avoidance depend on both NE and DA and that sterotyped behavior depends mainly on DA. These hypotheses are based, however, on data accumulated from one species, the rat. Thus, the location of electrodes for the elicitation of ICSS has been within the MFB. Unfortunately, there is little information concerning drug action on other ICSS sites besides MFB, specifically those high in dopamine, such as the caudate.

It appears obvious, based on the previous literature review, that the psychoactive drugs influence broad regions of the brain and numerous proposed transmitters. The fact that a neuroanatomical site supports ICSS and contains mostly one type of amine does not indicate any exclusive specificity of action. The neural tissue at the tip of the electrode is probably not the end point of electrical activation. Instead, it is likely that several excitatory or inhibitory transmitters are released by stimulation, which then influence widespread sites in the limbic system and telencephalon.

Summary and Statement of Problem

The preceeding review of literature points out that stimulus parameters, brain lesions, and the psychoactive drugs have been investigated concerning their effects upon ICSS. The majority of this work, however, has been confined to one species, the rat. Nevertheless, extensive generalizations about motivation and mental disease have been made to human behavior.

In the early studies (i.e., Olds, 1968), "select" brain sites

that supported ICSS were found to be sensitive to certain psychoactive drugs such as amphetamine or CPZ. In the rat, as a matter of convenience, the brain region most commonly used was the MFB at the level of the lateral hypothalamus. NE containing neurons are located within this fiber tract which innervates much of the diencephalon and telencephalon. NE is also one of the amines that is released by and its re-uptake prevented by amphetamine so it is not surprising that the proposed site of action for amphetamine was the MFB. The fact that DA and 5-HT are also contained within the MFB and are released when it is stimulated, or the fact that there is good evidence that many psychoactive drugs also act on dopamine, 5-HT, and ACh has been largely ignored.

There are now data which imply that there are several different amine pathways in the brain that will support ICSS and that these pathways are differentially sensitive to the psychoactive drugs. There are also several studies which show that destruction of large portions of the MFB will not depress lateral hypothalamic ICSS. Thus, the data suggest that instead of a single positive reinforcement "system", there may be several brain regions and biochemical systems that mediate positive reinforcement. These notions are plausible since in many subjects, brain regions other than hypothalamus, such as putamen, caudate and central amygdala will support ICSS. These other sites have a different proportion and distribution of monoamines than hypothalamus as well as different neuro-anatomical connections and when stimulated produce, in addition to ICSS, other goal-directed behaviors.

The three major experiments described in this dissertation are not a direct test of the "noradrenergic hypothesis" of Stein. They are,

instead, intended to provide comparative data concerning self stimulation behavior in the squirrel monkey using brain sites where stimulation activates mostly NE pathways (lateral or anterior amygdala), a mixture of NE, DA, and 5-HT pathways (the lateral hypothalamus) and mostly DA pathways (central amygdala and head of caudate).

The experimental questions asked are:

1) Current parameters: What similarities and differences exist between brain sites that support self stimulation (caudate, hypothalamus, anterior, central and lateral amygdala) regarding rate of ICSS when current intensity and train duration are manipulated?

2) Amphetamine:

   a) Is rate of ICSS elicited from caudate, hypothalamus, anterior lateral and central amygdala changed as dose of amphetamine is increased from 0.25 mg/kg to 10.0 mg/kg?

   b) Do differences exist between brain sites regarding rate of ICSS following separate doses of amphetamine?

   c) Is there a difference in duration of amphetamine action in caudate, hypothalamus, anterior, central or lateral amygdala using ICSS as a behavioral measure?

3) Chloropromazine:

   a) Is rate of ICSS elicited from caudate, hypothalamus, anterior central and lateral amygdala changed as dose of CPZ is increased from 0.25 mg/kg to 2.0 mg/kg?

   b) Does a relationship exist between brain site stimulated and specific dose of CPZ regarding rate of ICSS?

   c) Is there a difference in duration of CPZ action in caudate, hypothalamus, anterior, central or lateral amygdala using the behavioral measure of ICSS?

4) Does administration of low doses of clonidine, a drug which acts mostly on NE neurons, block rate of ICSS from hypothalamus but not from caudate?

The working hypothesis that is developed from this review of literature is that there are several positive reinforcement pathways in the brain. Amphetamine and CPZ are proposed to act on NE, DA, 5-HT, and ACh neurons. The behavioral facilitating or inhibiting effects produced by these drugs probably involve numerous brain sites. Increasing the dose of these drugs is predicted to produce an inhibition in responding due to changes in availability of catecholamines as well as producing a change in "behavioral state" which influences rate of ICSS. The action of amphetamine on ICSS is predicted to be biphasic as a function of dose and its major sites of action are more widespread than Stein's data would imply.

CPZ, while affecting several amines in the brain is predicted to be most effective (low doses, 0.5 mg/kg) in the caudate. This hypothesis is based on previously reviewed literature which suggests a strong dopaminergic influence for this drug, e.g., it produces changes in DA levels and affects its re-uptake. As the dose of CPZ is increased, both NE and DA as well as ACh become involved and all ICSS sites would eventually be blocked from mediating ICSS. It is argued that Stein's suggestion that the major site of action of CPZ is in the hypothalamus is only partially correct.

More selective drugs such as clonidine should have their major effect on hypothalamic ICSS rather than caudate ICSS because clonidine acts on NE containing neurons. Activation of these neurons is thought to be the major factor in the elicitation of hypothalamic ICSS (Stein, 1971).

CHAPTER II

EXPERIMENT 1: STIMULUS INTENSITY AND TRAIN DURATION MEASURES FOR CAUDATE, AMYGDALA AND HYPOTHALAMIC SELF STIMULATION

Materials and Method

Subjects

The Ss in this experiment and in experiments 2, 3 and 4 were healthy adult male squirrel monkeys (Saimiri sciureus) that weighed between 600 and 800 grams. The animals were purchased from Primate Imports in Long Island, New York. Prior to shipment all animals were vaccinated against tuberculosis.

Housing Conditions

Each S was housed in an individual cage ($1\frac{1}{2}$ ft. x $1\frac{1}{2}$ ft. x 2 ft). The Ss were fed standard monkey chow or nutrient pellets and were watered and cleaned daily. The room in which the Ss were contained was well ventilated, and the temperature was constant at 72 degrees. Lights in the room were left on 24 hours a day.

Surgery

Each of the sixteen Ss was initially anesthetized with pentobarbital (25 mg/kg) and placed in a stereotaxic instrument. The cranium was exposed by making a longitudinal incision and retracting the temporalis muscle and skin. Stainless steel wire electrode pairs were

bilaterally implanted in the brain via small holes drilled in the skull. The electrode wire diameter was 0.25 mm. The tip separation was 10 mm and the active area was the cut end of the insulated wire. The brain sites selected and the respective coordinates utilized were as follows: Head of Caudate (AP 15.0, L 2.5, V 17.0 mm); Lateral Septum (AP 12.5, L 0.5, V 15.5 mm); Anterior Amygdala (12.5, L 7.5, V 7.0 mm); Central Amygdala (AP 9.5, L 7.0, V 9.0); Lateral Amygdala (AP 9.0, L 10.0, V 5.0 mm); Lateral Hypothalamus (AP 8.5-11.0, L 0.5-1.5, V 11.0 mm); Hippocampus (AP 2.0, L 8.5, V 10.0 mm); and Substantia Nigra (AP 6.5, L 2.0, V 11.0 mm) (Gergen and MacLean, 1962).

The leads from the electrodes and a ground wire were connected to an ELCO plug and the entire assembly was then embedded in dental repair acrylic cement which was fastened to the skull with machine screws. The temporalis muscles were then sutured and the longitudinal incision closed. As a precautionary measure against infection, bicillin (0.25 cc) was given before surgery and for several days following. A two-week recovery period followed surgery. Post-operative infections did not occur, but the recovery period was essential to permit recovery of the brain tissue from the trauma of electrode insertion.

Testing Apparatus

Two to three weeks prior to surgery each subject was adapted to sit in a restraint chair for up to two hours. Following recovery from surgery all Ss handled easily and quickly re-adapted to the restraint chair.

All experiments described in this dissertation were conducted in a ventilated sound-proof chamber (3½ ft. x 3 ft. x 4 ft., Lehigh

Valley). A light bulb was mounted in the ceiling and a one-way observation mirror (12 inches x 9 inches) for observation purposes was located on the door.

A steel plate was used to bolt the restraint chair inside the box. A lever (Lehigh Valley) was mounted in a vertical position in front of and two inches above the S so that the required response was to reach out and up to grasp the lever, a "natural" movement for the Ss.

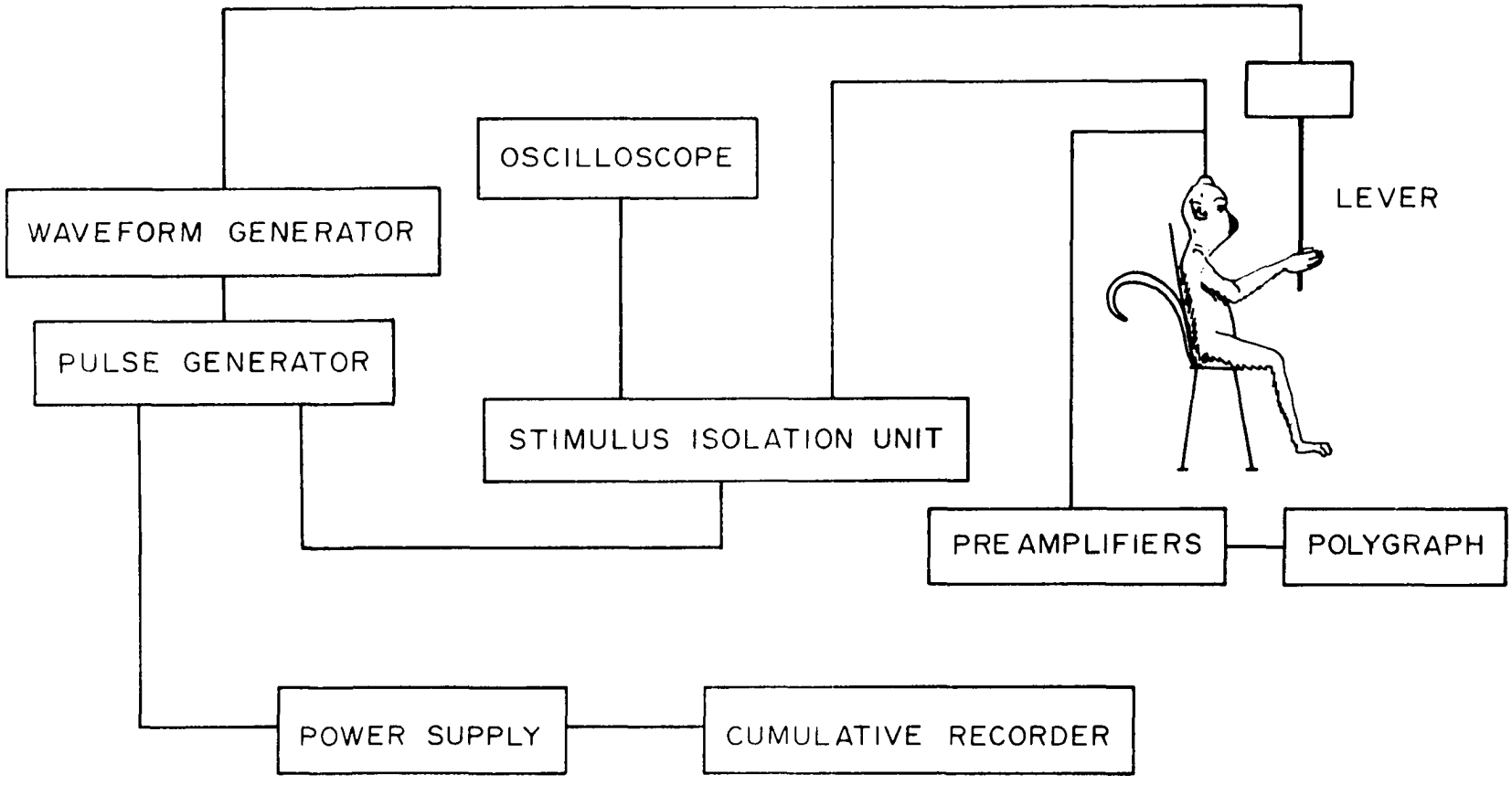
Each lever pull activated a microswitch which triggered a Tektronix waveform generator system. This system delivered, via a multiconductor cable attached to a male contact plug, rectangular trains of pulses (100 c/sec, pulse width 0.2 milliseconds). The current intensity, pulse width and waveform were monitored on a two-channel Tektronix Dual Beam Oscilloscope. A stepping device on a cumulative recorder (Model CR2D, Scientific Prototype) was also activated following each lever pull so as to provide a continuous record of the responses. Minimum interstimulus interval was 630 msec and the schedule of brain shock was continuous reinforcement (CRF).

A Grass 4 channel polygraph was used to record the electroencephalograph (EEG) of the S. The appropriate signal was initially sent through Grass pre-amplifiers mounted underneath the testing chamber. Figure 1 illustrates by block diagram the various components used in these experiments.

Testing Procedure

Mapping for the occurrence of ICSS. Each S was placed in the chamber for two consecutive days for periods up to two hours. This procedure was done to adapt the S to the experimental conditions. A lever

Figure 1 - Block diagram illustrating the components used in the intracranial self stimulation test in this dissertation.


WAVEFORM GENERATOR
OSCILLOSCOPE
LEVER
PULSE GENERATOR
STIMULUS ISOLATION UNIT
PRE AMPLIFIERS
POLYGRAPH
POWER SUPPLY
CUMULATIVE RECORDER

was placed in the chamber on the second day but its activation did not produce brain shock.

On the third day each S was then given brief current to one brain site (current and train duration varied from S to S). If the animal grimaced, vocalized or attempted to get out of the restraint chair, an inverted lever was placed in front of the S. By traditional shaping procedures, the S was operant trained to pull the bar to "escape" the brain shock which was manually applied by the experimenter. The site in this case was classified as having aversive properties.

If the S did not show the above signs, a lever was still placed in the chamber to determine whether the animal would activate the lever to receive brain shock. If the S did not activate the lever, current intensity and/or train duration were increased in slow ascending steps to 1000 microamperes (μamp) and 500 msecs, respectively. If the S did not activate the lever, the session was terminated. A second analysis was done at the same site on the following day. If the S still did not respond consistently, that particular site was classified as "neutral" and a new site was randomly selected for the next analysis. A site was classified as being "positive" when the following criteria were met: a) the S would initiate responding by activating the lever to receive current and the response rate would drop to zero when the current was turned off; b) as current intensity was decreased, the rate of lever pull showed a corresponding change, generally a decrease; c) minimum rate was 25 lever pulls per minute for 45 minutes; and d) all of the above were constant within one test session and between test days (five days maximum).

Results

Mapping for ICSS

Of the sixteen Ss that were initially implanted, four were not included in any analysis. Two Ss did not recover from surgery and two Ss died several weeks following surgery. The remaining data for the presence or absence of ICSS from the other twelve Ss are presented in Table 1. Twenty-four brain sites were classified as "positive" with respect to ICSS. Eight were in the head of the caudate; four were in the anterior amygdala; four were in the lateral amygdala; one was in the central amygdala; seven were in the hypothalamus; fifteen sites were neutral and three sites were aversive (two posterior hypothalamus and one substantia nigra).

Current Intensity and Train Duration Analysis

Caudate or hypothalamus. Stimulation of the caudate (n=6) or the hypothalamus (n=6) in separate groups of Ss produced increases in lever pulls as current was increased from 200 to 1000 μamps (Figures 2 and 3). In three Ss, current intensities that were higher than 800 amps produced some salivation and head shaking in the caudate as well as the MFB when train duration was 500 msecs.

The summary of a 2 x 4 x 5 analysis of variance with repeated measures on the last two factors (Winer, 1973) is presented in Table 2. A significant interaction occurred between brain site and current intensity ($F=5.13$, $1,10$ $p < .05$) and between brain site, current intensity and train duration ($F=5.78$, $1,10$ $p < .05$). The main effect of current intensity was significant ($F=16.87$, $1,10$ $p < .01$). To establish probability (p) levels, conservative degrees of freedom were used (Winer,

TABLE 1

SUMMARY OF BRAIN SITE MAPPING ANALYSIS FOR
SELF STIMULATION BEHAVIOR

Subject	Brain Site Tested	Positive	Neutral	Aversive	Used in Drug Analysis
Fred	Head of Caudate		*		No
	Ant. Amygdala		*		No
	MFB	*			Yes
Tippy	Head of Caudate		*		No
	Lat. Septum		*		No
	Ant. Amygdala	*			No
	MFB	*			Yes
	Hippocampus		*		No
Buttons	Head of Caudate	*			Yes
	Ant. Amygdala		*		No
	Lat. Amygdala		*		No
	Hippocampus		*		No
Chico	Head of Caudate		*		No
	MFB	*			Yes
	Lat. Amygdala		*		No
	Hippocampus		*		No
Torius	Head of Caudate	*			Yes
	Lat. Amygdala	*			Yes (partially)
	Cent. Amygdala	*			Yes (partially)
Joco	Head of Caudate	*			Yes
	Ant. Amygdala		*		No
	Lat. Amygdala	*			No
	MFB	*			Yes

TABLE 1--Continued

Subject	Brain Site Tested	Positive	Neutral	Aversive	Used in Drug Analysis
Alvin	Head of Caudate	*			Yes
	Lat. Septum		*		No
	Ant. Amygdala	*			No
	MFB	*			Yes
Paul	Head of Caudate	*			Yes
	Ant. Amygdala	*			Yes
Ringo	Head of Caudate	*			Yes
	MFB			*	No
	Sub. Nigra			*	No
George	Ant. Amygdala	*			Yes
	MFB	*			No
Larry	Head of Caudate	*			No
	Lat. Septum		*		No
	Lat. Amygdala	*			Yes
	MFB			*	No
	Hippocampus		*		No
Zip	Head of Caudate	*			No
	Lat. Amygdala	*			No
	MFB	*			Yes
	Hippocampus		*		No

Figure 2 - Graphic plot of mean self stimulation rate of six squirrel monkeys using five separate intensities of current and four train durations. Electrodes were implanted in the head of the caudate.

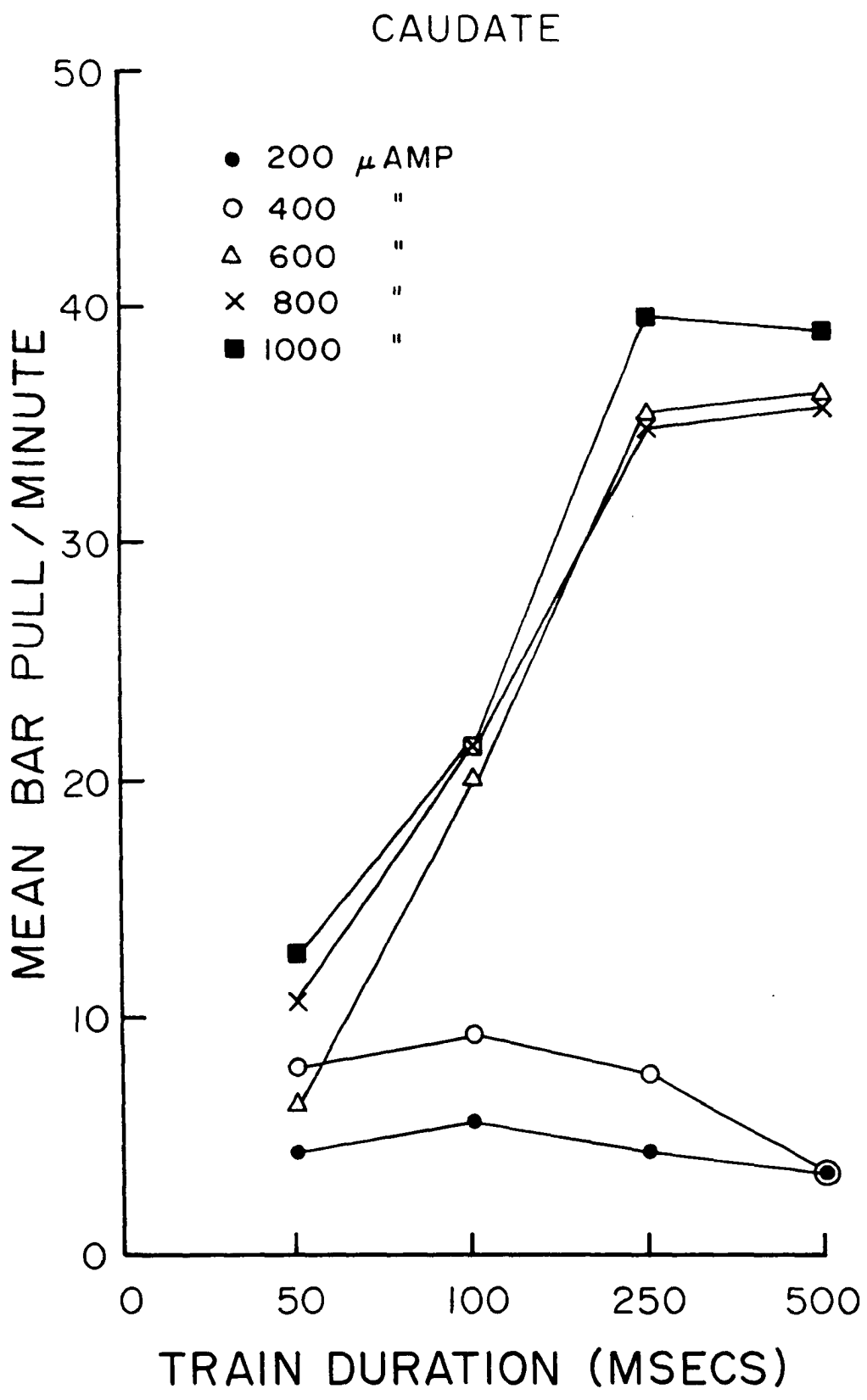

CAUDATE
50
40
30
20
10
0
MEAN BAR PULL / MINUTE
● 200 μAMP
○ 400 "
△ 600 "
× 800 "
■ 1000 "
0
50
100
250
500
TRAIN DURATION (MSECS)

Figure 3 - Graphic plot of mean self stimulation rate of six squirrel monkeys using five separate intensities of current and four train durations. Electrodes were implanted within the lateral hypothalamus-invading portions of the Medial Forebrain Bundle (MFB).

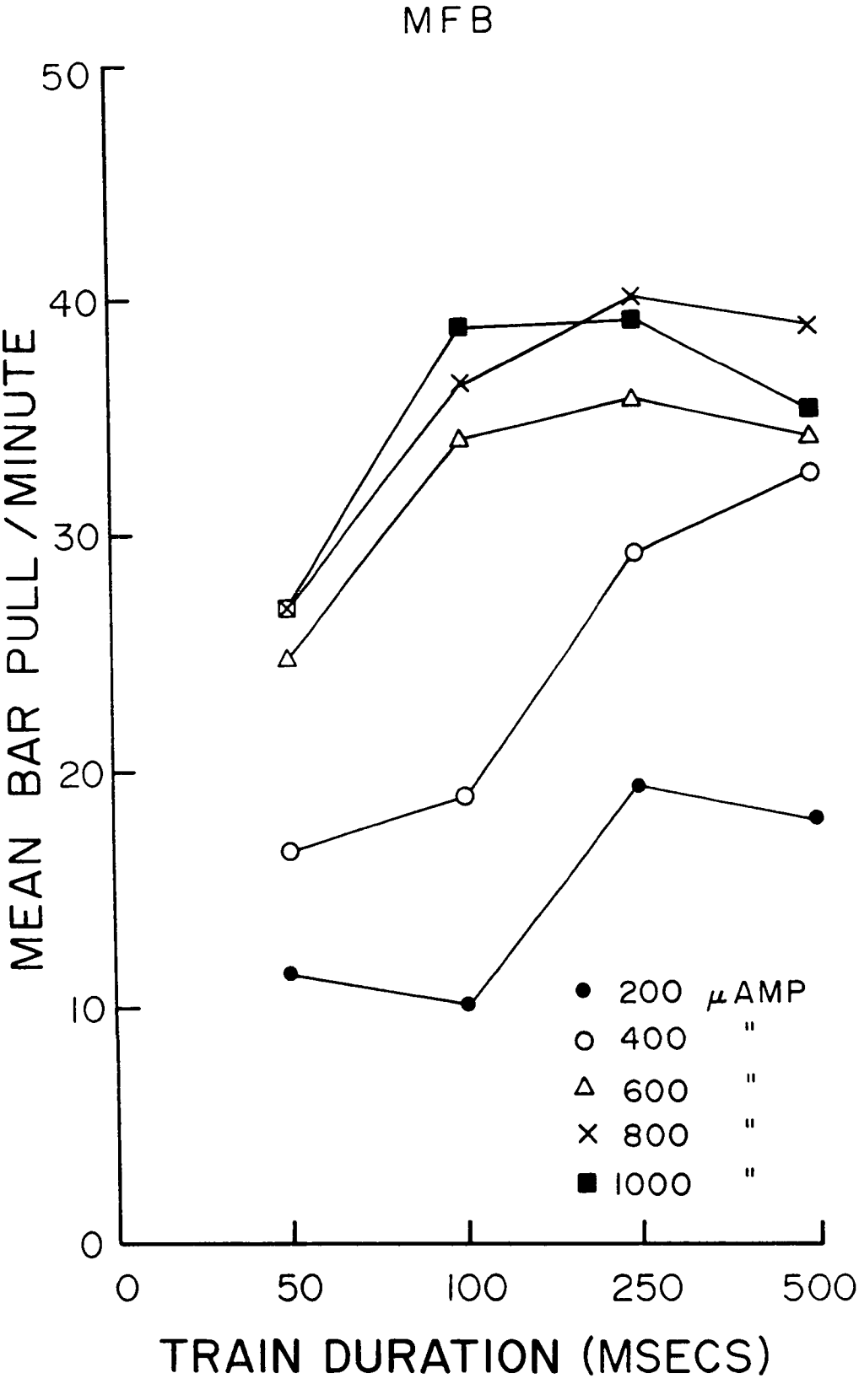

MFB
MEAN BAR PULL / MINUTE
50
40
30
20
10
0
0
50
100
250
500
TRAIN DURATION (MSECS)
200 μAMP
400 "
600 "
800 "
1000 "

TABLE 2

SUMMARY OF ANALYSIS OF VARIANCE COMPARING BRAIN SITE (CAUDATE OR MFB), CURRENT INTENSITY AND TRAIN DURATION

Source of Variation	SS	df	MS	F	
Between Subjects	20,242	11	1,840		
A (caudate or mfb)	5,005	1	5,005	3.28	n.s.
Subj w. groups	15,237	10	1,523		
Within Subjects	65,948	228			
B (train duration)	2,904	3 (1)	968	1.28	n.s.
AB	3,859	3 (1)	1,019	1.94	n.s.
B X subj w. groups	14,898	30 (10)	523		
C (current intensity)	12,355	4 (1)	3,083	16.87	**
AC	3,764	4 (1)	941	5.13	*
C X subj w. groups	7,327	40 (10)	183		
BC	1,659	15 (1)	110	1.06	n.s.
ABC	7,233	12 (1)	602	5.78	*
BC X subj w. groups	11,949	120 (10)	104		

n.s. Not Significant
* $p < .05$
** $p < .01$

1973). These values are in parentheses. For the interaction between current intensity and brain site, simple main effects were analyzed. Between group comparisons were made by the Scheffe Test (Winer, 1973).

The mean lever pulls for stimulation at each brain site at each current intensity is presented in Table 3. The values in parentheses are the standard deviations. Values underscored by the same line are not statistically different.

At caudate sites increases in current produced increases in lever pulls except at 800 and 1000 μamps where there was no difference between intensities. In the hypothalamus the lowest intensity produced significantly less lever activations than the other four current intensities which did not differ from each other.

Differences between brain sites were evident at all current intensities except 800 and 1000 μamp (Table 3). The hypothalamic group had higher response rates at current intensities between 200 to 600 μamps. In Table 3 any numerical value underscored by the same line does not differ.

Electroencephalographic (EEG) tracings taken from either hippocampus or septum during ICSS did not reveal the presence of seizure activity at any current level or train duration.

Anterior or lateral amygdala. Stimulation of the anterior amygdala (n=4) produced high rates of self stimulation provided that train duration was kept between 50 to 100 msec. When train duration was increased to 250 or 500 msec and current intensity was increased beyond 400 microamps, autonomic signs were evident. All animals demonstrated headshaking, eye blinking, copious salivation while lever pull activation was decreased (Figure 4). In contrast, the lateral amygdala group (n=4)

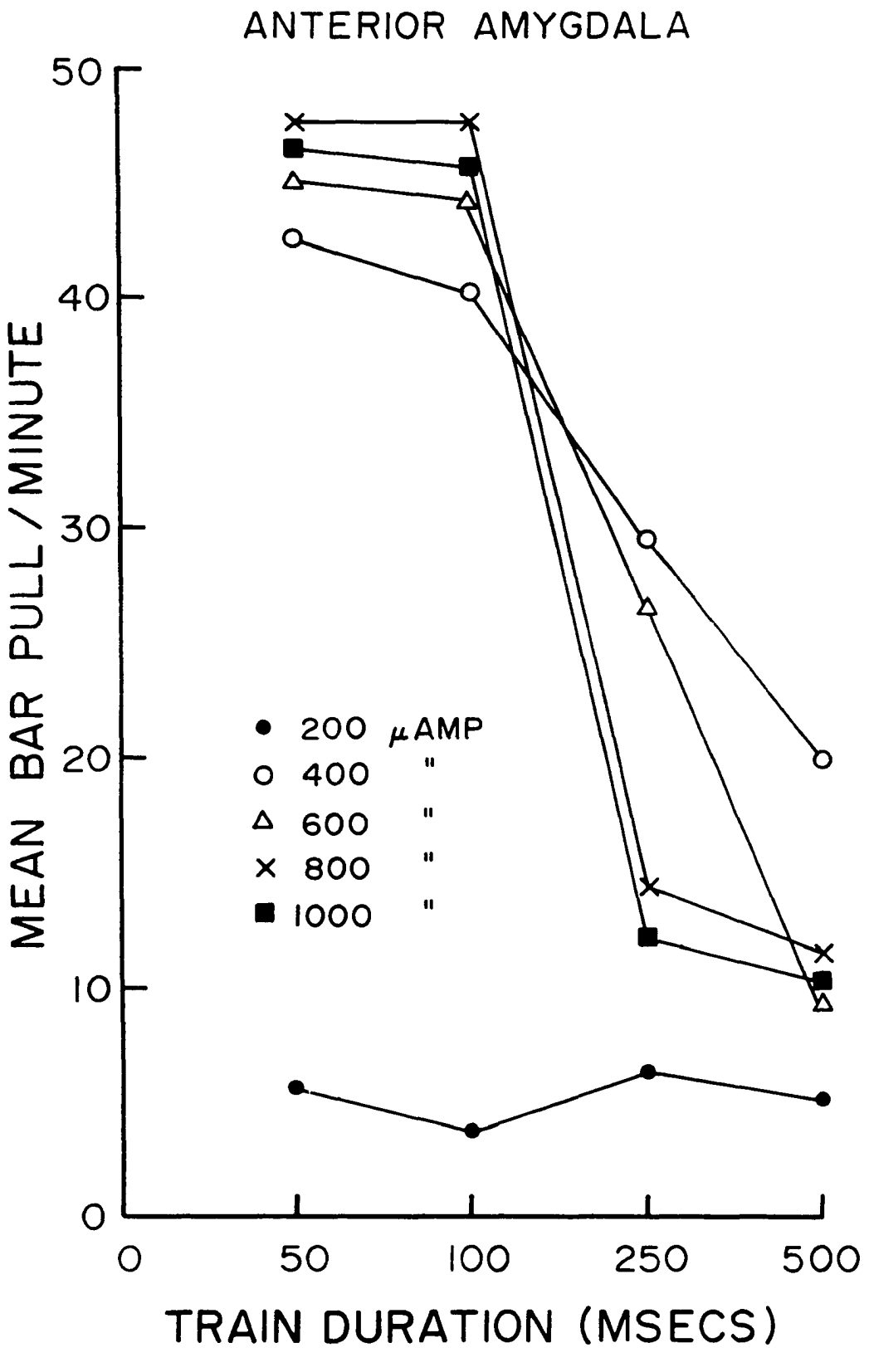
TABLE 3

MEAN LEVER PULLS PER MINUTE: BRAIN SITE AND INTENSITY COMPARISONS (IN MICROAMPERES)

	Microamperes				
Brain Site	200	400	600	800	1000
Caudate	4.25	10.00	25.12	27.00*	27.16
	(± 1.56)	(± 10.75)	(± 16.86)	(± 18.07)	(± 17.73)
MFB	18.79	30.00	32.29	30.33	30.95
	(± 17.50)	(± 16.17)	(± 19.58)	(± 20.87)	(± 20.63)

* Any value underscored by the same line is not statistically different.

Figure 4 - Graphic plot of mean self stimulation rate of four squirrel monkeys using five separate intensities of current and four train durations. Electrodes were implanted in the anterior amygdala.


ANTERIOR AMYGDALA
MEAN BAR PULL / MINUTE
50
40
30
20
10
0
0
50
100
250
500
TRAIN DURATION (MSECS)
200 μAMP
400 "
600 "
800 "
1000 "

demonstrated few lever pulls when train duration was at 50 or 100 msec, irrespective of current intensity. As train duration increased, lever pulls increased correspondingly (Figure 5).

Stimulation in the central amygdala generally produced results that were similar to those obtained from the lateral amygdala group.

Hippocampal or septal EEG tracings did not reveal the presence of seizure activity in either the lateral or anterior amygdala (Figures 6 and 7). In one animal, however, stimulation of the central amygdala, using high current intensities (600 to 1000 μamp., 250 or 500 msec train duration) did produce some initial seizures which were not consistently seen over the test session.

A summary of the analysis of variance comparing brain site (anterior or lateral amygdala, current intensity and train duration) is presented in Table 4. A significant interaction is reported between brain site and train duration ($F=31.47$, df 1,6 $p < .01$) and brain site, current intensity and train duration ($F=9.85$, df 1,6 $p < .01$). The main effect of current intensity was also significant ($F=24.35$, df 1,6 $p < .01$). To establish probability (P) levels, conservative degrees of freedom were used (Winer, 1973). These values are in parentheses in Table 4. For the interaction between train duration and brain site, simple main effects were analyzed. Between group comparisons were made by the Scheffe Test (Winer, 1973).

The mean lever pulls for stimulation at each brain site at each train duration is presented in Table 5. The values in parentheses are the standard deviations. Values underscored by the same line are not statistically different.

In the anterior amygdala no differences were obtained between

Figure 5 - Graphic plot of mean self stimulation of four squirrel monkeys using five separate intensities of current and four train durations. Electrodes were implanted in the lateral amygdala.

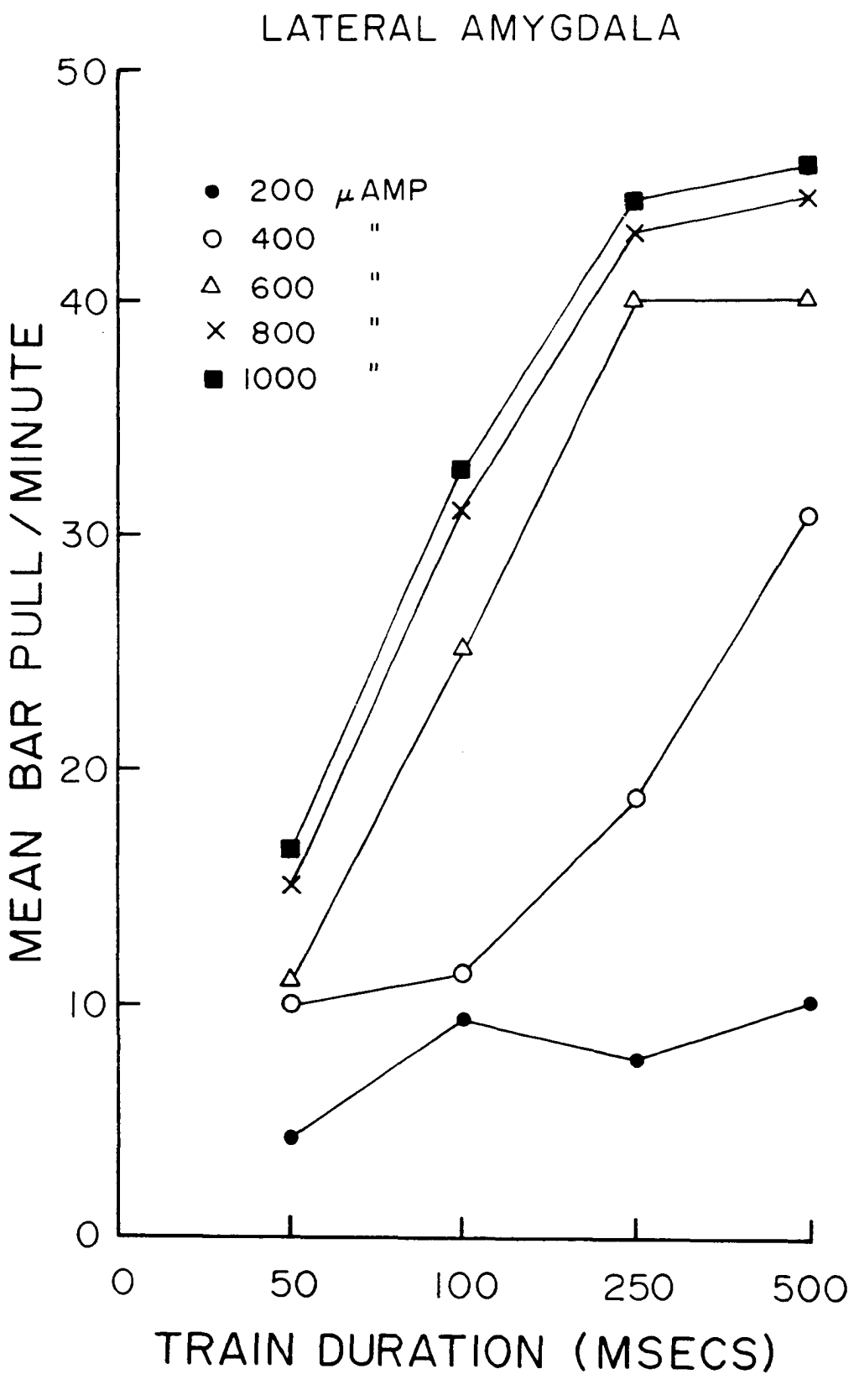

LATERAL AMYGDALA
50
40
30
20
10
0
MEAN BAR PULL / MINUTE
200 μAMP
400 "
600 "
800 "
1000 "
0
50
100
250
500
TRAIN DURATION (MSECS)

Figure 6 - Electroencephalographic recordings from one squirrel monkey self stimulating for current to the anterior amygdala. Recording electrodes in the hippocampus and medial septum; A=Free run with current turned off; B=Current intensity 500 μamps; train duration 50 msec producing high rate of response; C=Free run with current turned off; D=Current intensity 550 amp train duration 500 msec producing a reduced rate in responding and eliciting salivation, eyeblinking, head twitching; E=Continuation of D with current reduced to 475 μamp elicitation of autonomic components still evident and rate of response still reduced; F=Current turned off.

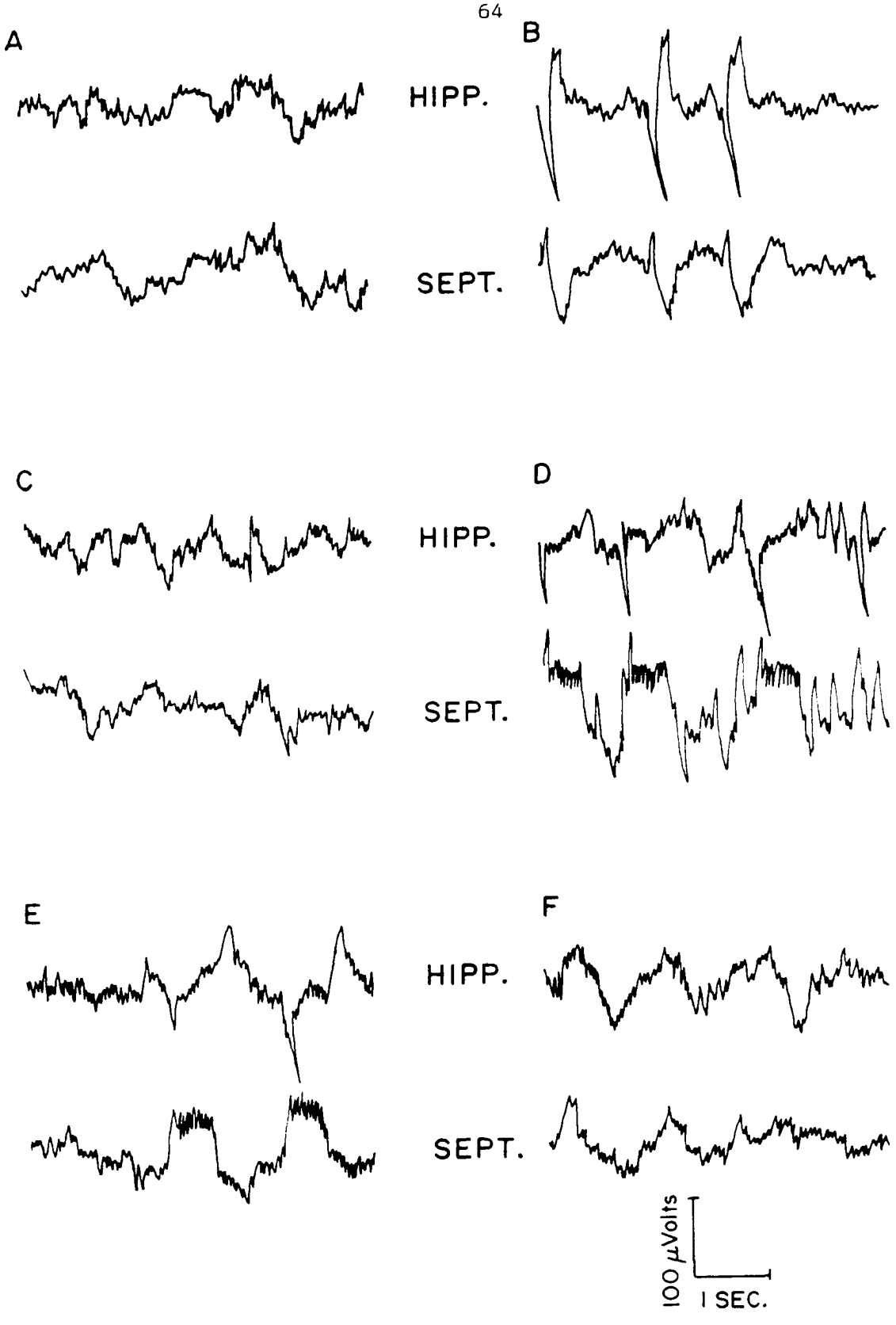

A
HIPP.
SEPT.
B
C
HIPP.
SEPT.
D
E
HIPP.
SEPT.
F
100 μVolts
1 SEC.

Figure 7 - Electroencephalographic recordings from one squirrel monkey self stimulating for current to the lateral amygdala. Recording electrodes in the hippocampus and medial septum; A=Free run with current turned off; B=Current intensity 550 μamp train duration 50 msec; little evidence of responding or the elicitation of autonomic components; C=Current intensity 550 μamp, train duration 500 msec. High rate of responding but little evidence of elicitation of autonomic components; D=Free run without current on.

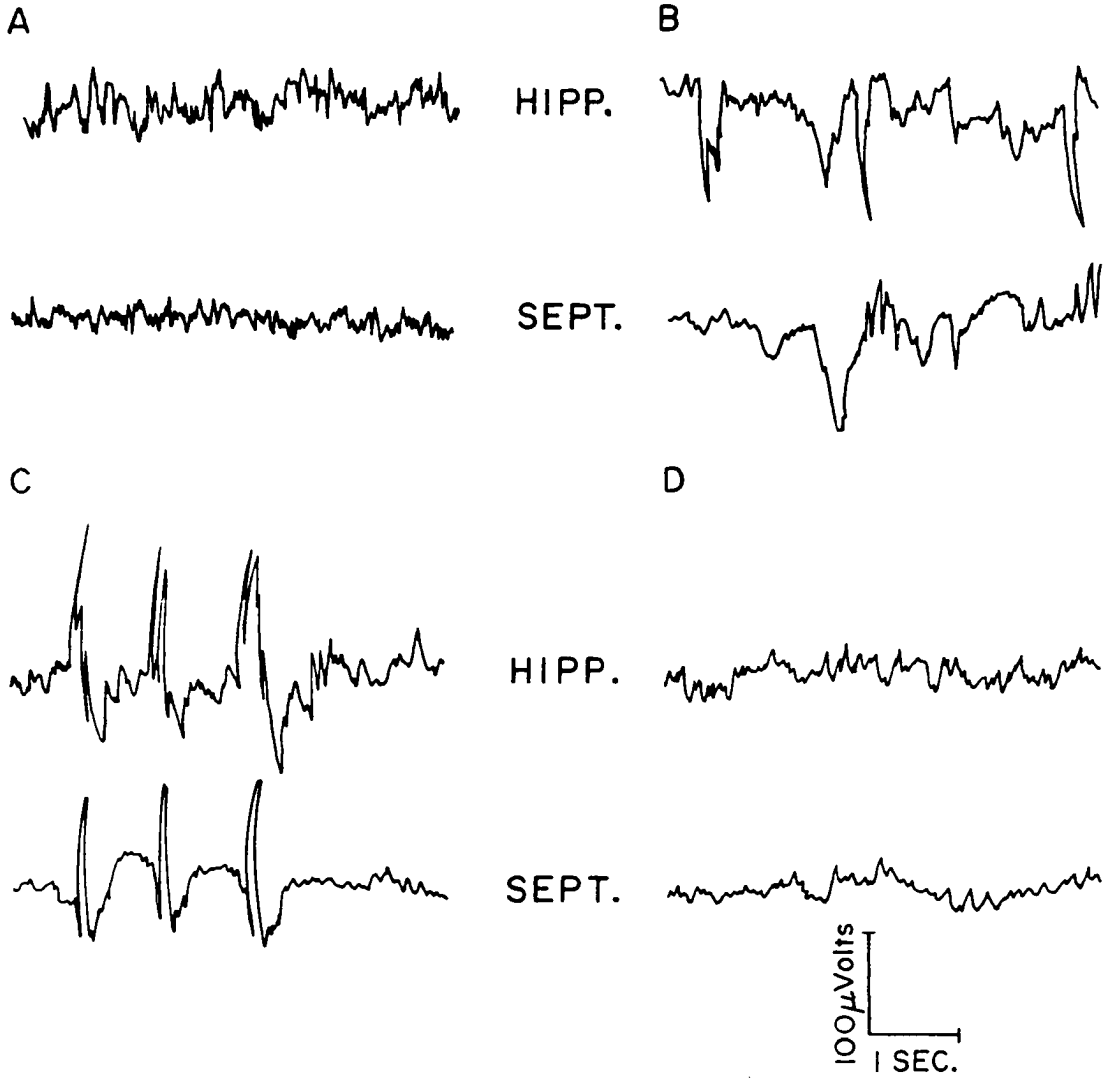

A
B
HIPP.
SEPT.
C
D
HIPP.
SEPT.
100μVolts
1 SEC.

TABLE 4

SUMMARY OF ANALYSIS OF VARIANCE COMPARING BRAIN SITE (ANTERIOR OR LATERAL AMYGDALA) CURRENT INTENSITY, AND TRAIN DURATION

Source of Variation	SS	df	MS	F	
Between Subjects	9,678	7			
A (anterior or lateral amygdala)	591	1	591	0.39	
Subj w. groups	9,087	6	1,514		
Within Subjects	55,689	152			
B (train duration)	364	3 (6)	121.33	0.70	n.s.
AB	16,354	3 (1)	5,451.21	31.47	**
B X subj w. groups	3,119	18 (6)	173.27		
C (current intensity)	18,141	4 (1)	4,535.25	24.35	**
AC	1,614	4 (1)	403.50	2.16	n.s.
C X subj w. groups	4,469	24 (6)	186.20		
BC	2,097	12 (1)	174.75	3.48	n.s.
ABC	5,924	12 (1)	493.66	9.85	*
BC X subj w. groups	3,607	72 (6)	50.09		

n.s. Not Significant
* $p < .05$
** $p < .01$

TABLE 5

MEAN LEVER PULLS PER MINUTE: BRAIN SITE AND TRAIN DURATION COMPARISON

	Train Duration (in milliseconds)			
Brain Site	50	100	250	500
Lateral Amygdala	12.40	19.00	27.00*	27.00
	(± 7.96)	(± 11.68)	(± 14.72)	(± 13.43)
Anterior Amygdala	37.30	36.85	18.85	14.25
	(± 20.84)	(± 20.87)	(± 13.35)	(± 8.43)

* Any value underscored by the same line is not statistically different.

50 and 100 msec, but rate decreased significantly at 250 and 500 msec. The anterior amygdala group self stimulated at higher rates than the lateral amygdala group at 50 and 100 msec while at 250 and 500 msec the lateral amygdala group showed the highest self stimulation rates.

Two Lever Preference Test

Since a 50 or 500 msec train duration was a relevant factor in determining if ICSS was to increase or decrease in amygdaloid implanted animals, a second test was used.

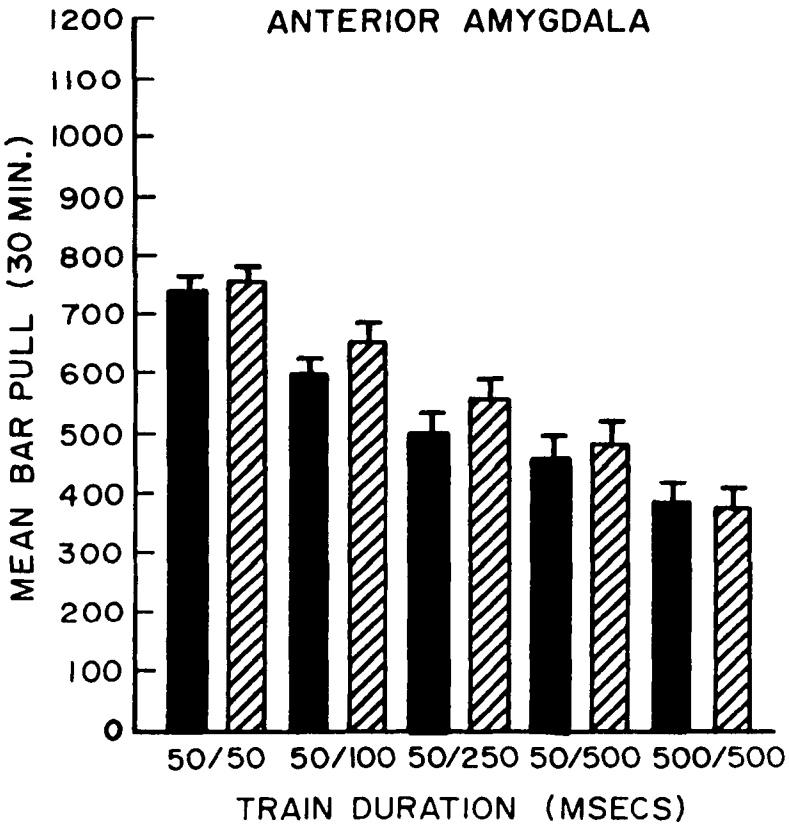
Two levers were placed side by side in front of and above the animal as previously described in the methods section. Activation on one lever produced a set current intensity (475 μamp) and a 50 msec train duration. Activation of the other lever produced the same current intensity as the first lever and either 50, 250, or 500 msec train duration. Thus, a preference analysis was made comparing 50 msec with one of the other train durations.

The preference test for any two combinations of current train duration was made in the A.M. with the session lasting for 45 minutes. In the afternoon the session was replicated with position of lever (left or right) for the respective train durations reversed (i.e., position counter balanced).

Figure 8 illustrates that in the anterior amygdala group absolute rate on both levers appears to decrease when one of the levers produces a longer train duration. In contrast, in the lateral amygdala the longer train durations produced an increase in mean bar pulls.

In Table 6 a summary of analysis of variance comparing brain site (anterior or lateral amygdala) and train duration is presented.

Figure 8 - Histogram comparing self stimulation preferences for different train durations in two separate groups of squirrel monkeys (anterior amygdala, n=4; lateral amygdala, n=4). 50/50 represents bar pulls in which right lever delivered 50 msec train duration and left lever delivered 50 msec train duration. 50/100 right lever delivered 50 msecs, left lever 100 msec. Current intensity was 475 microamperes for all comparisons.


ANTERIOR AMYGDALA
MEAN BAR PULL (30 MIN.)
1200
1100
1000
900
800
700
600
500
400
300
200
100
0
50/50
50/100
50/250
50/500
500/500
TRAIN DURATION (MSECS)

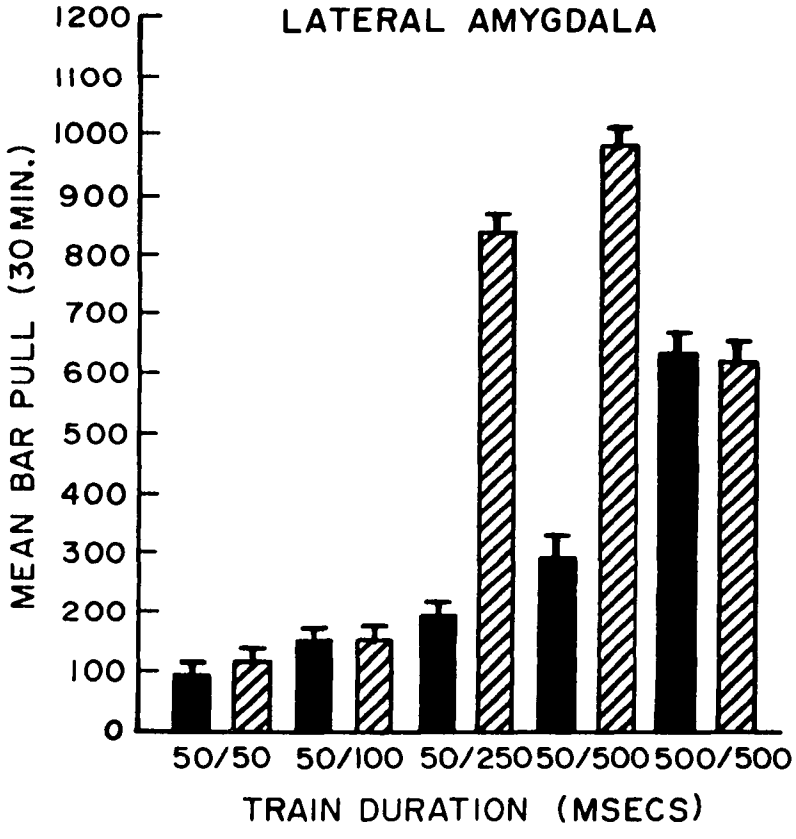

LATERAL AMYGDALA
MEAN BAR PULL (30 MIN.)
1200
1100
1000
900
800
700
600
500
400
300
200
100
0
50/50
50/100
50/250
50/500
500/500
TRAIN DURATION (MSECS)

TABLE 6

SUMMARY OF ANALYSIS OF VARIANCE COMPARING BRAIN SITE (ANTERIOR OR LATERAL AMYGDALA) AND TRAIN DURATION USING A TWO BAR PREFERENCE TEST (RATIO ANALYSIS)

Source of Variation	SS	df	MS	F
Between Subjects	167.56	7		
A (brain site)	90.14	1	90.14	6.98 *
Subj w. groups	77.42	6	12.90	
Within Subjects	271.99	24		
B (train duration)	93.65	3 (1)	31.21	7.15 *
AB	99.02	3 (1)	33.00	7.56 *
B X subj w. groups	78.52	18 (6)	4.36	

* $P < .05$

The dependent variable analyzed was the ratio of responding between the two levers at pre-selected train duration combinations.

Main effects for brain site (anterior or lateral amygdala) ($F=6.98$, df 1,6 $p < .05$) and for train duration ($F=7.15$, df 1,6 $p < .05$) were both significant. The interaction between these two variables was also significant ($F=7.56$, df 1,6 $p < .05$). Conservative degrees of freedom were used to establish P levels. For the analysis on the interaction, simple main effects were made by the method described by Winer (1973). Between group comparisons were made by the Scheffee Test (Winer, 1973).

In Table 7 means and standard deviations are presented for the ratio of responding between the two levers. In the anterior amygdala no differences were noted between any train duration combinations. In the lateral amygdala, however, the ratio of responding on the lever which delivered the longer train duration was significantly more than when both levers delivered either short or long train durations. When compared to the anterior amygdala the lateral amygdala group demonstrated a higher ratio for 250 or 500 msec train durations.

Discussion

The sites selected for the elicitation of ICSS in the present dissertation were chosen after a careful review of the literature regarding electrode placements in self stimulation experiments in primates. Thus, when certain electrode coordinates were used and stimulation of a particular electrode yielded the appropriate behavior, all remaining subjects were implanted using the same coordinates. The high percentage of electrodes producing ICSS (55 per cent) is probably a result of this

TABLE 7

MEAN LEVER PULL PER MINUTE (RATIO ANALYSIS): COMPARISON BETWEEN BRAIN SITE AND TRAIN DURATION (IN MSEC)

	Train Duration (in msec)			
Brain Site	50/50	50/250	50/500	500/500
Anterior Amygdala	1.34*	1.61	1.00	1.42
Lateral Amygdala	1.00	6.87	8.75	1.00

* Any value underscored by the same line is not statistically significant.

"selective" implant procedure. Had a systematic mapping study been undertaken, the percentage and distribution of "positive", "neutral", and "aversive" sites might have been quite different from that reported.

Mapping Results

Lateral hypothalamus. The general findings reported in this dissertation regarding brain sites that support ICSS are in agreement with other studies for a wide range of species (Olds, 1962; Doty, 1967; Plotnik, 1972). For example, the lateral hypothalamus has been described as consistently supporting high rates of ICSS in most species although many hypothalamic sites can also produce aversive behavior if the stimulation parameters are increased (Olds, 1962).

In the only other study concerned with ICSS in squirrel monkeys, Renfrew (1968) has mapped brain sites with regard to the presence or absence of self stimulation. Most sites within the lateral hypothalamus were aversive; however, in examining Renfrew's results several procedural differences can be found. The type of stimulation used was "monopolar" rather than "bipolar" so it is possible that the volume of brain tissue stimulated in Renfrew's study was larger and less well defined than reported here. Stimulation may have activated aversive pathways due to the large current spread. Also, the anterior-posterior coordinates used by Renfrew were generally in the posterior portions of the hypothalamus (AP 7.5-9.5). The MFB at this AP level is quite compact with a low probability that an electrode will actually penetrate it. The surrounding regions which include dorsal and ventral hypothalamus and zona incerta were described by Olds (1962) as containing mixed effects (e.g., both positive and aversive sites). Thus, it seems likely that

stimulation in the more posterior MFB may produce aversive as well as positive self stimulation.

In the results reported in this experiment, the brain sites that did contain aversive elements were located in the more posterior parts of the hypothalamus (See Appendix, Figure 45). At more anterior points (AP 10.0 to 11.5) the MFB becomes larger, or more diffuse, and the probability of penetrating the MFB with an electrode is, correspondingly, higher. All of these MFB sites produced high and consistent self stimulation with no evidence that any site was aversive. Since Renfrew did not fully investigate the more anterior portions of the lateral hypothalamus as well as amygdaloid and caudate structures, his data are not fully comparable to that reported here, and his mapping data for ICSS are incomplete.

Caudate. The high occurrence of self stimulation elicited from the head of the caudate found in these experiments corresponds to recent work reported in the rhesus monkey. For example, Plotnik et al. (1972) found that of 42 electrodes implanted in the head of caudate, 14 were positive with respect to ICSS. No other region of the brain ranging from caudate-septum laterally to globus pallidus through hypothalamus to the ventromedial tegmentum yielded even a third of the number of positive sites found in the head of the caudate. One other region in Plotnik's study that produced ICSS was the tip of the internal capsule. This is consistent with Routtenberg's et al. (1971) results in rhesus monkeys.

In other species such as the cat (Wilkinson and Peels, 1963; O'Donohue and Hagamen, 1967) and rabbit (Bruner, 1967), as well as the rat (Olds, 1962), caudate self-stimulation is either intermittent or

nonexistent. Earlier claims of positive caudate self stimulation in cats (Sidman, Brady, Boren and Conrad, 1955) has been challenged by later evidence (Justesen et al., 1963) which suggested that caudate ICSS was the result of hyperkinesis induced by the stimulation. If motor excitation were a consequence of caudate stimulation, depending on the location of the lever or pedal, it is possible that motor involvement could obscure interpretation of any "rewarding" effect.

Another factor that has been proposed to explain caudate self stimulation is that possible deep penetration of the electrode might activate other structures (Bruner, 1967). The caudate is anatomically close to septal areas. If current intensity was high enough, ascending or descending MFB fibers passing through the septum might be activated accordingly.

However, in the experiments reported here, a maximum amount of care was taken when electrodes were implanted in the head of the caudate to avoid both septum and internal capsule. The anterior (AP 15.0) and ventral coordinates of 17.0 mm were chosen so as to place the electrode at least 2 to 3 mm away from these regions. Although final electrode penetration in several animals did approach within 1 mm of the septum (See Appendix, Figure 33), no discernable differences were noted between these sites and more lateral placements for response rate, current threshold or overt behavior. None of these sites when stimulated produce overt signs of motor involvement or seizures except in two animals (Jo and Fr) at higher current intensities (800-1000 μamps, 500 msec). EEG data failed to show the presence of either septal or hippocampal seizures. Evidence that this area of the telencephalon is fairly select with regard to ICSS is that at AP 13.0 electrodes around the putamen, claustrum or

tip of the internal capsule (See Appendix, Figure 34), produced obvious signs of motor involvement, e.g., head shaking, arm and hand tremors, and salivation. Response rate was variable and the sites were classified as neutral.

Amygdala. Previous mapping studies in the cat for amygdala ICSS (Wilkinson and Peele, 1963) reported only negative effects. However, Brady (1961) and Plotnik (1972) demonstrated positive sites within amygdala of the rhesus monkeys. Generally, anterior and lateral sites were neutral whereas the central amygdala sites were positive. In a thorough mapping study of the rat amygdala, Wurtz and Olds (1963) found that the most consistent ICSS was in the corticomedial group, especially the central and medial nuclei. The basolateral division was aversive.

The amygdala sites chosen in the present research represented the major divisions of the amygdala. All divisions of the amygdala in the squirrel monkey were positive with respect to ICSS although current parameters (see next section) selectively affected response rate.

The apparent disparity between these findings and others for ICSS in the lateral amygdala in primates may have occurred because of a lack of previous systematic mapping within this division. It is of interest that Kaada (1972) reports that stimulation of the lateral amygdala in cats produces a searching investigatory response. This same type of response is seen in rats prior to the establishment of ICSS.

Current Intensity and Train Duration Relationships

Lateral hypothalamus and caudate. The finding that in the squirrel monkey both caudate and MFB ICSS sites (Figures 2 and 3) are responsive to increases in current intensity and train duration increases

up to a point, is consistent with reports by Olds (1962). When electrodes are implanted in the posterior hypothalamus of rats, increases in current cause a monotonic increase in response rate. When asymptote is reached, further increase in current produces motor seizures that can interfere with the response sequence and thus produce a decrease in rate.

One interpretation placed on the finding that MFB stimulation may eventually become aversive is that increases in stimulation intensity increases activation of aversive sites (Olds, 1961). The MFB fiber tract contains many neurons which synapse with dendrites from other brain nuclei (Millhouse, 1969). Stimulation, especially in the more posterior portions of the hypothalamus, could activate via the MFB surrounding aversive sites by "recruiting" additional neuron paths. In the more anterior regions of the hypothalamus (AP 10.0-11.5), there was no evidence of aversive effects. The larger size and diffuseness of the anterior bundle and lack of any co-joining region containing aversive path neurons might explain why no elicitation of negative reinforcement was observed at these coordinates.

Olds (1962) found that telencephalic sites require larger current intensities and train durations to elicit ICSS than hypothalamic sites. Reports contained in this dissertation are consistent with this proposal. For example, until current intensity was increased to 500 μamp, MFB ICSS was significantly higher (Table 3) than caudate ICSS.

There are two possible interpretations for these findings. One would be that there are several major positive reinforcement pathways which differ in current intensity characteristics. One pathway could be the dorsal MFB originating in part from the locus coeruleus and projecting through the hypothalamus to the cortex. Clavier (1973) has

evidence that this proposed pathway is a positive site in the rat. Another pathway (caudonigral) would go from head of caudate projecting in part to the rostral part of the substantia nigra, anterior to the oculomotor roots. This pathway has been implicated in ICSS in the rhesus monkey (Routtenberg, 1971). The substantia nigra could be one important relay for receiving and sending efferents to the cortex via the ventral and medial thalamus, ascending reticular system as well as through the caudate. It is relevant to note that previous studies (Valenstein and Campbell, 1966) have revealed the apparent redundancies in pathways supporting ICSS. For example, diencephalic lesions of the rat MFB do not eliminate subcortical telencephalic ICSS.

Alternatively, Olds (1962) contends that the major reinforcement pathway (MFB) is bidirectional connecting cortex, forebrain limbic areas, and brainstem structures. Areas such as caudate nucleus would support ICSS by the indirect activation of the MFB, probably via a variety of thalamic and midbrain projections.

The substantia nigra, when stimulated, was always aversive (Table 7). Other investigators (Brady, 1961; Olds, 1961) have reported that regions such as the ventral and dorso medial nucleus of the thalamus when stimulated also produce fear-like symptoms and avoidance responses in cats and monkeys. All of these findings, however, may not be inconsistant with Routtenberg (1971) who proposed that these structures sometimes support ICSS. Stimulation within the midbrain and brainstem probably activates a complex of both positive and negative reinforcing systems. The final outcome of any experiment series would depend on the area and pathway(s) activated, and this may be a matter of chance. Thus, wide variations regarding the presence of ICSS would be expected.

Amygdala sites. A discussion of ICSS elicited from amygdalar nuclei now follows.

Anterior amygdala. When current train duration was increased beyond 250 msec, stimulation in the anterior amygdala of the squirrel monkey produced large amounts of salivation, facial movements (e.g., smiling) some micturitation and defecation. Similar findings have been reported in cats (Kaada, 1972). Concomitant with the elicitation of these components, lever pull rate decreased (Figure 4). However, the decrease in response rate did not indicate that the stimulation was necessarily aversive since in a preference test, while rate decreased, there was no difference between responding for long or short train durations (Figure 8). This finding points out the validity of Valenstein's argument (1964) that rate measures can be a misleading index of the quality of positive reinforcement.

Hippocampal or septal EEG recordings did not establish the presence of seizures following caudate, hypothalamic or amygdalar stimulation. This finding is consistant with other reports (Brady, 1961). If seizures do exist, especially during amygdalar stimulation, they may be more localized specifically to amygdalar nuclei and are not propagated to either the hippocampus or septum even though other aspects of neural responses (evoked potentials) were (Figures 6 and 7).

That an extreme range of behavioral responses can be elicited from the anterior amygdala is predictable because of its close anatomical interconnection with the MFB and hypothalamus. In many hypothalamic and limbic regions of the rhesus monkey, Robinson (1964) has reported that stimulation caused more than thirty different reactions from simple motor and autonomic reflexes to complex alimentary and aggressive

behaviors. Many distinct behaviors could be elicited by moving the probing electrode 1/2 to 1 mm. These results indicate that an intermixture of neural elements exists to subserve various behavioral patterns.

The anterior amygdala is considered to be the bed nucleus of the lateral extension of the MFB (Cowan, 1965). This portion of the amygdala is an intermediary for reciprocal relationships between the amygdalopiriform complex, the preoptic area and hypothalamus (via the MFB); the dorsomedial and rostral midline nuclei of the thalamus (via the inferior thalamic peduncle) and the habenula via the stria medullaris. It is significant that all of these pathways have been implicated with ICSS in primates (Routtenberg, 1971). The stria terminalis, a major efferent pathway interconnects the anterior with the cortico-medial division of the amygdala, another area which appears to be a major site for eliciting ICSS in rats (Wurtz and Olds, 1963; Grossman, 1967).

The reciprocal relationship between anterior amygdala and the afore-mentioned pathways might explain its low threshold for ICSS as well as the high probability of eliciting numerous other responses. Stimulation within this region is also likely to activate one or more pathways that support ICSS because of existing multiple interconnections. The longer the current is left on, the more likely other regions which receive projections from the anterior amygdala are also activated or inhibited. Two of these regions, the claustrum and olfactory tract nucleus, when stimulated, elicit both motor and autonomic components (Kaada, 1972). Competing motor or autonomic responses could interfere with the ICSS response rate if either nucleus were activated. Duration, rather than current intensity, appears to be the critical parameter for uncovering numerous underlying stimulus elicited responses from the anterior amygdala.

Central amygdala. Stimulation of the central amygdala in one animal produced the highest rates of self-stimulation at the longer train durations. Some salivation was noted; however, this was generally observed at the higher train durations (250-500 msec). Stimulation of the central nucleus in rats is also positively reinforcing (Wurtz and Olds, 1963).

The central nucleus is generally thought to be a continuum of the bed nucleus of the stria terminalis, one of the major efferent pathways of the amygdala (Lammer, 1972). Its close proximity to the anterior amygdala area (Hall and Jensen, 1971) may explain the elicitation of some of the observed autonomic components.

Stimulation within the central amygdala activates its major efferent pathway, the stria terminalis going to the anterior amygdala, septum and hypothalamus (Kaada, 1971). Further, it is likely that DA neurons are activated when the central amygdala is stimulated. Fuxe et al. (1970) have described a mesolimbic DA pathway whose cell bodies are located in the interpeduncularis nucleus and project in part to the central amygdala as well as the substantia nigra.

Lateral amygdala. In most species, when the lateral amygdala is stimulated, a type of bewildered, searching occurs and with higher current intensity signs of fright, anger, or hissing are seen. None of these behavior sequelae were observed in the present experiments nor were any sites aversive. Although there may be a species difference regarding some stimulus elicited behaviors, much of the later amygdala in the squirrel monkey needs to be more fully mapped.

Stimulation of the lateral amygdala probably activates its major efferent pathway, the ventral amygdalofugal which projects into the pyri-

form cortex thalamic nuclei, septum, head of caudate and anterior hypothalamus (Kaada, 1972). One important projection of the ventrofugal pathway in mediating both electrophysiological and behavioral responses is to the lateral hypothalamus. Gloor (1955) has reported short latency evoked responses in the hypothalamus following basolateral stimulation.

ICSS from the lateral hypothalamus is also modified by concurrent ipsilateral lateral or cortico-medial amygdaloid stimulation (Szabo, 1971). When both amygdala and hypothalamus were stimulated, an increase in press rate and a decrease in threshold were observed. When the stria terminalis was lesioned the effect was still observed thus suggesting that ICSS might be mediated by the ventrofugal pathway.

To summarize this discussion, electrical stimulation within amygdalar nuclei produces a high and predictable ICSS response rate. One critical parameter that appears to influence rate of response is train duration. It is postulated that as the period of time that the current is left on increases, the more likely (within certain amygdalar nuclei such as anterior) other elicited responses may appear and jointly act to influence ICSS response rate. Many of the elicited autonomic responses may block or prevent the animal from maintaining high response rates.

Stimulation within the lateral and central amygdalar nuclei do not produce elicitation of autonomic components. The lack of other interfering response components may decrease the likelihood that rate of ICSS will be affected by increasing stimulus intensities or durations.

Several pathways connecting amygdalar nuclei, the stria terminalis and the ventral amygdalofugal are involved with the elicitation of ICSS. However, since these pathways project to other regions of the brain that also support ICSS, it remains to be clarified where final response integration occurs.

CHAPTER III

EXPERIMENT 2: DOSE RESPONSE AND DURATION OF ACTION OF AMPHETAMINE ON CAUDATE, AMYGDALOID AND HYPOTHALAMIC SELF STIMULATION

Materials and Methods

Subjects

The Ss used in this experiment were the same Ss as were used in Experiment 1.

Testing Procedure

From the results of Experiment 1, six animals were selected that had stable response rates in the head of the caudate; six other Ss were chosen that had stable response rates in the hypothalamus. Two Ss that also had positive electrodes in the anterior amygdala, two that had positive sites in the lateral amygdala and one that had a positive site within the central amygdala were also used.

Dose response analysis. Five separate doses of amphetamine were given to all Ss in the following manner. The first two days were used to establish a saline baseline. The animals were given 20 minutes in the chamber in which every lever pull produced a brain shock. Intensity of current was reduced to that level which produced a low rate of responding, e.g., 15-20 responses per minute. The animal was then

removed from the chamber, given a marshmallow with saline dissolved inside. Fifteen minutes later the animal was placed back in the chamber and the session was continued for 45 minutes to an hour. The same procedure was followed on the second day and generally the between baseline variance was minimal. On the third day, amphetamine dissolved in saline (either 0.25, 0.50, 1.0, 2.0, or 10.0 mg/kg) was given in a randomized order in place of the saline.

The 10.0 mg/kg solution of amphetamine was given intraperitoneally (I.P.) because many of the Ss would not take this dose orally.

Duration analysis. Analysis of duration of drug effects was done in the following manner. The initial testing session began from 15 minutes following drug ingestion for the next one hour. Three hours following drug administration, the animal was placed back in the experimental chamber and re-tested for 45 minutes. The same procedure was used at six hours and at 24 hours. Then every 24 hours until the animal's rate had returned to its original baseline level. Each time the animal was placed in the chamber, a marshmallow was given. All animals were food deprived for twelve hours prior to the test session. The interval between drug administration was ten days.

Threshold analysis. The analysis for current thresholds was done in the following way. A drug dose of either 0.25, 0.50, or 1.0 mg/kg was given and a current intensity that produced a low rate of response was used. Following drug administration, when the animal's rate increased to its maximum point (generally twice baseline), the current intensity was decreased in steps of 50 μamp until the animal stopped responding.

Results

Statistical analysis was performed on the absolute response rate per minute (drug to saline ratio x 100). A 2 x 5 (brain site and drug dose) analysis of variance (see Table 8) with repeated measures on the last factor (Winer, 1972) revealed a significant main effect for brain site ($F=8.90$, df 1,10, $p < .05$) and drug dose ($F=47.31$, df 1,10, $p < .01$). The interaction between brain sites and drug dose was not significant ($F=4.81$, df 1, 10, $p > .05$). Conservative degrees of freedom (Winer, 1973) were used to establish all P levels. Between group comparisons were analyzed using the Scheffe Test (Winer, 1973).

Table 9 demonstrates that increasing dose from 0.25 mg/kg to 0.50 mg/kg significantly increased the ratio of responding (low response rate) at both brain sites. At 1.0 mg/kg the MFB group still demonstrated response facilitation while the ratio between drug/saline in the caudate group was decreased. At 2.0 mg/kg a significant inhibition in response rate was noted at both sites. Values in parentheses represent standard deviations. The biphasic effect of amphetamine on ICSS as a function of dose is presented in Figure 9.

The mean per cent change in ICSS threshold is presented in Figure 10. The analysis for this data was a 2 x 2 (brain site and drug dose) design with repeated measures on the last factor. A significant main effect for drug dose ($F=40.00$, df 1,20, $p < .01$) was revealed. Both caudate and MFB sites demonstrated a reduction in ICSS thresholds at 0.5 mg/kg of amphetamine. This was the only dose analyzed because fewer than four animals were used at the other doses and this particular dose was the one reported to reduce current thresholds in rats (Stein, 1964).

TABLE 8

SUMMARY OF ANALYSIS OF VARIANCE COMPARING BRAIN SITE (CAUDATE OR MFB) AND DRUG DOSE (AMPHETAMINE)

Source of Variation	SS	df	MS	F	
Between Subjects	24,548	11			
A (brain site)	11,565	1	11,565	8.90	*
Subj w. groups	12,983	10	1,298.30		
Within Subjects	359,965	48			
B (drug dose)	274,640	4 (1)	68,660.00	47.31	**
AB	27,267	4 (1)	6,816	4.81	n.s.
B X subj w. groups	58,058	40 (10)	1,451		

n.s. Not Significant
* $P < .05$
** $P < .01$

TABLE 9

MEAN PER CENT CHANGE (DRUG SALINE RATIO) IN RATE OF SELF STIMULATION: BRAIN SITE AND AMPHETAMINE DOSE COMPARISONS

	Drug Dose (mg/kg)				
Brain Site	.25	.50	1.0	2.0	10.0
Caudate	105.83	188.50	125.25	19.66*	11.00
	± 13.04	± 17.56	± 29.65	± 5.24	± 5.60
MFB	109.50	194.83	173.80	16.16	10.06
	± 9.55	± 9.13	± 10.30	± 8.87	± 5.80

* Any value underscored by the same line is not statistically different.

Figure 9 - Graphic plot of changes in self stimulation rate in squirrel monkeys (expressed as mean per cent of previous control run) following separate doses of amphetamine. Vertical bar represents standard error of the mean. Caudate, n=6; MFB, n=6; Lateral amygdala, n=2; Anterior amygdala, n=1.

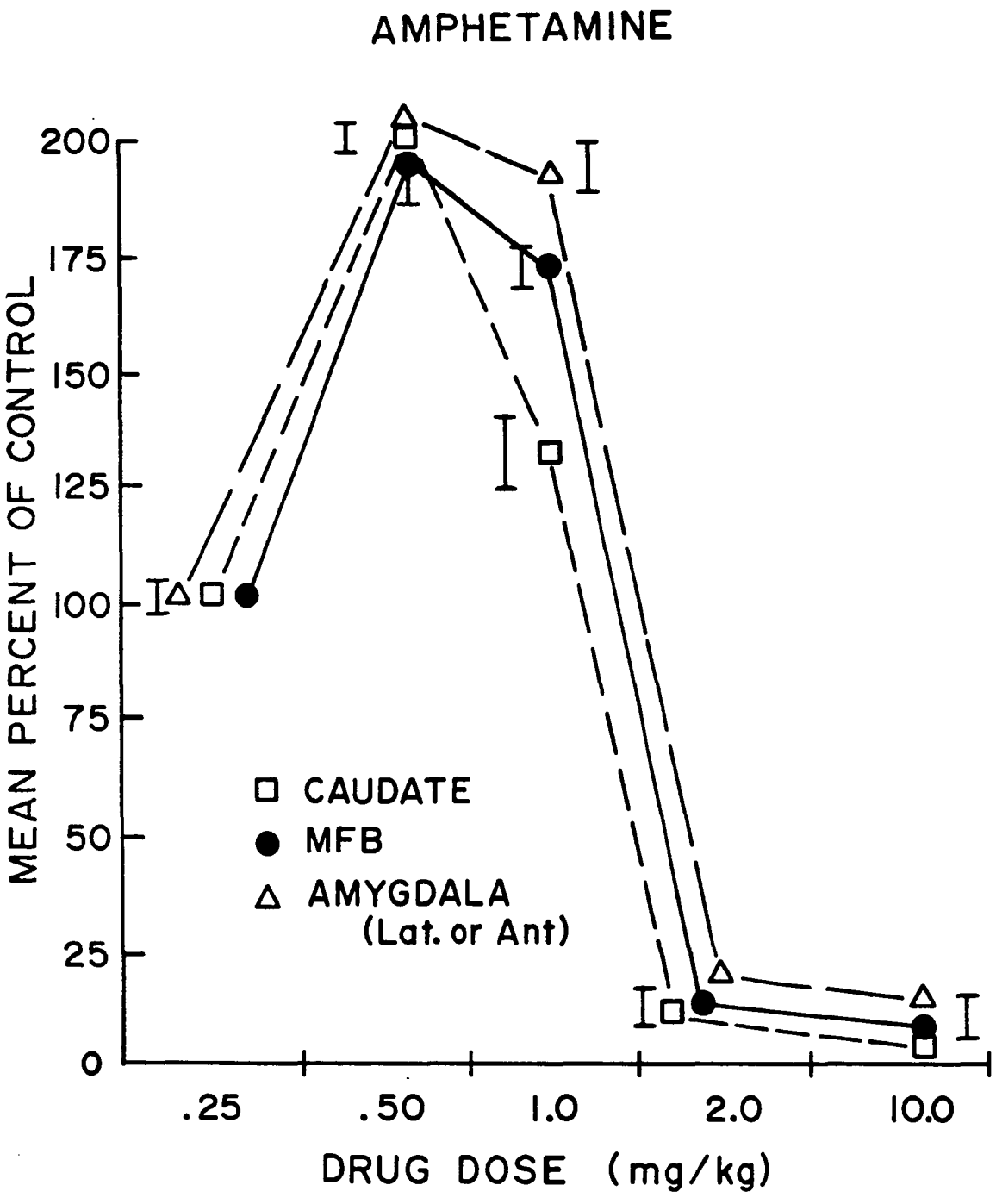
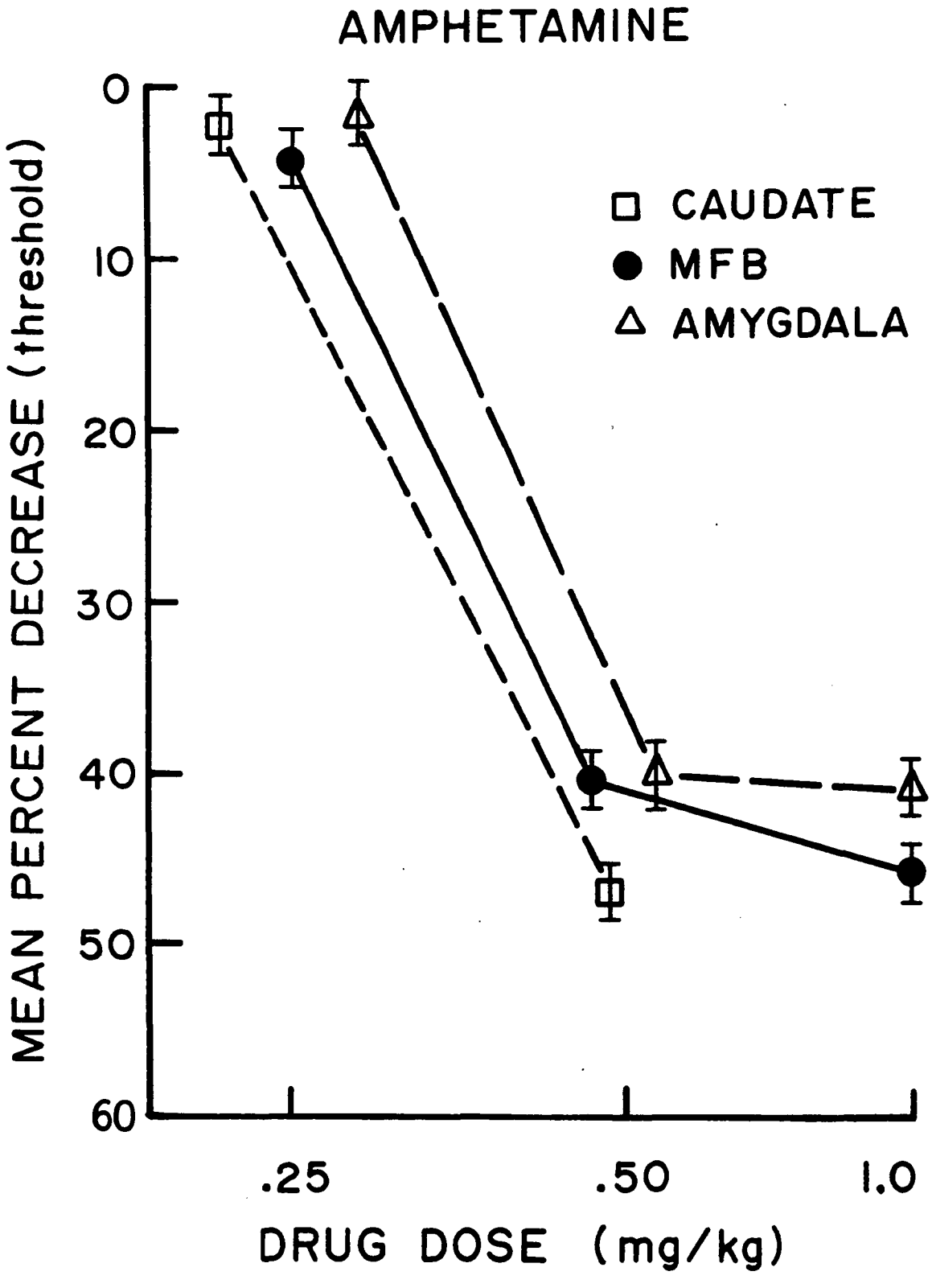

AMPHETAMINE
MEAN PERCENT OF CONTROL
200
175
150
125
100
75
50
25
0
CAUDATE
MFB
AMYGDALA
(Lat. or Ant)
.25
.50
1.0
2.0
10.0
DRUG DOSE (mg/kg)

Figure 10 - Graphic plot of mean per cent change in self stimulation threshold in squirrel monkeys following separate doses of amphetamine. Vertical bar represents standard error of the mean. Caudate, n=6; MFB, n=6; Lateral amygdala, n=2; Anterior amygdala, n=1.


AMPHETAMINE
MEAN PERCENT DECREASE (threshold)
0
10
20
30
40
50
60
CAUDATE
MFB
AMYGDALA
.25
.50
1.0
DRUG DOSE (mg/kg)

In Figure 11, histograms compare the duration of action of amphetamine at various doses. Since the unit of measure (hours to return to baseline) was arbitrarily set by the experimenter, e.g., animals were re-tested every 12-24 hours, some doubt existed as to using this data in a parametric analysis. Instead, a planned comparison using non-parametric ranking procedures (Segal, 1962) was used for the two highest doses. At 2.0 mg/kg and 10.0 mg/kg the caudate group showed a significantly longer return to baseline for ICSS ($U=0$, $p < .001$; $U=0$, $p < .001$) when compared to the MFB group.

Figures 12-21 are cumulative records demonstrating the potentiation or inhibition of ICSS rate following various doses of amphetamine. The mixed effects of amphetamine (both excitation and inhibition) are seen in Figure 18. This effect was only observed in the caudate group at this drug dose (1.0 mg/kg).

Amphetamine at high doses (10.0 mg/kg) produced several forms of bizarre behavioral manifestations in squirrel monkeys similar to those reported in both cats and monkeys by Ellinwood (1970). These include loss of motor initiative, dysjunctive posture, cataleptic, and stereotypic activity.

Discussion

The finding reported here that certain doses of amphetamine in squirrel monkeys potentiate low rates of operant behavior for positive reinforcement and decrease current thresholds for eliciting ICSS replicates earlier studies (Dews and Morse, 1961; Horovitz et al., 1962; Stein, 1964, 1970). Increasing doses of amphetamine produced a

Figure 11 - Histogram of the return of self stimulation rate to pre-drug baseline condition in squirrel monkeys following separate doses of amphetamine. Vertical bar represents standard error of the mean. Caudate, n=6; MFB, n=6; Lateral amygdala, n=2; Anterior amygdala, n=1.

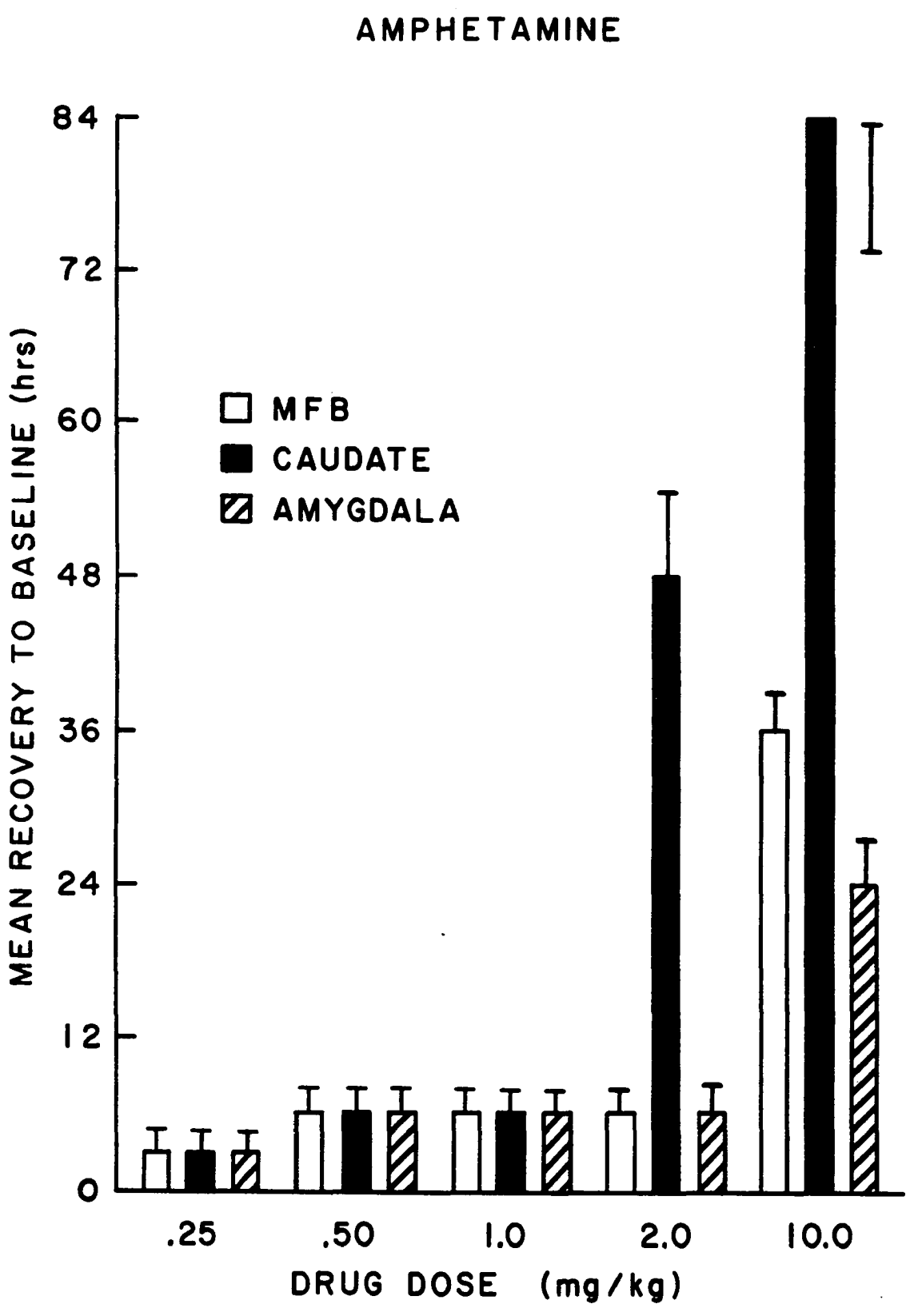

AMPHETAMINE
84
72
60
48
36
24
12
0
MEAN RECOVERY TO BASELINE (hrs)
MFB
CAUDATE
AMYGDALA
.25
.50
1.0
2.0
10.0
DRUG DOSE (mg/kg)

Figure 12 - Cumulative record of one squirrel monkey self stimulating for brain current. Comparison is between saline condition and amphetamine (0.25 mg/kg). Electrodes implanted in head of caudate. Maximum excursion of pen set at 500 responses. Schedule is continuous reinforcement (CRF).

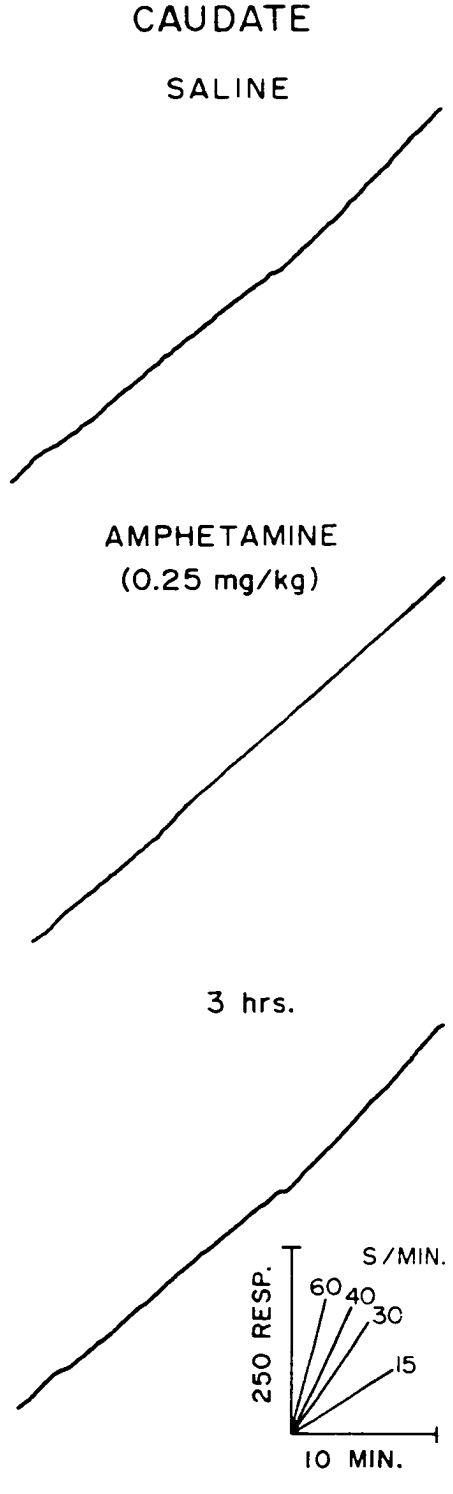

CAUDATE
SALINE
AMPHETAMINE
(0.25 mg/kg)
3 hrs.
S/MIN.
60
40
30
15
250 RESP.
10 MIN.

Figure 13 - Cumulative record of one squirrel monkey self stimulating for brain current. Comparison is between saline condition and amphetamine (0.5 mg/kg). Electrodes implanted in the head of caudate. Maximum excursion of pen set at 500 responses. Schedule is CRF.

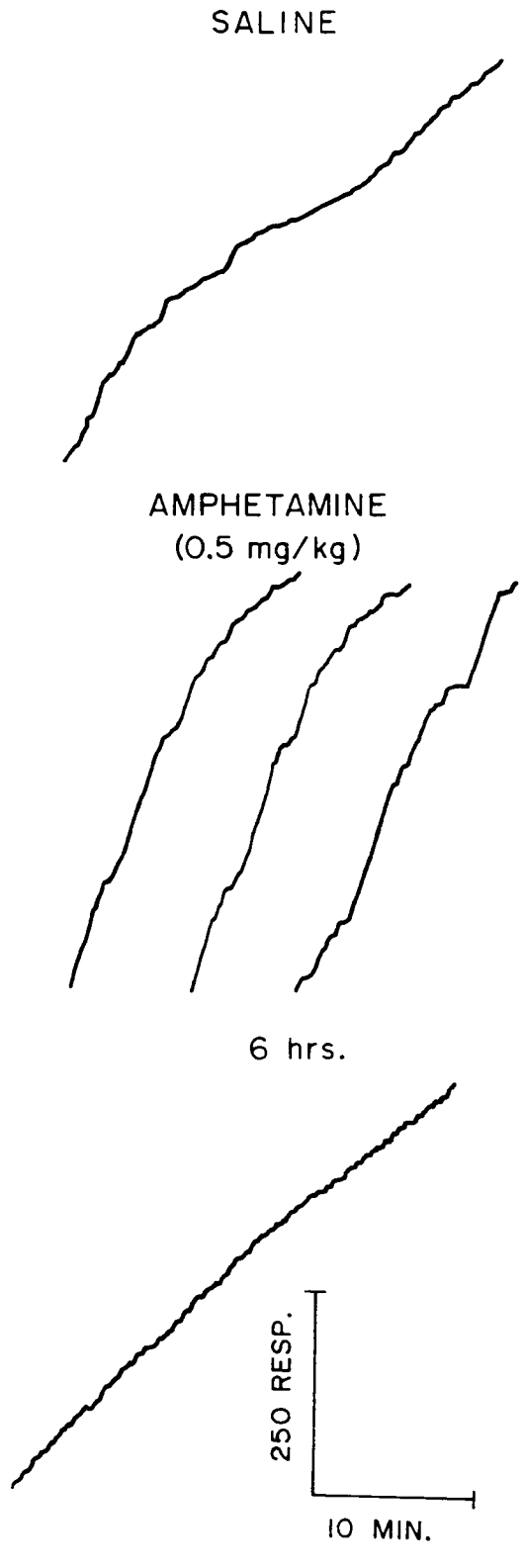

CAUDATE
SALINE
AMPHETAMINE
(0.5 mg/kg)
6 hrs.
250 RESP.
10 MIN.

Figure 14 - Cumulative record of one squirrel monkey self stimulating for brain current. Comparison is between saline condition and amphetamine (0.50 mg/kg). Electrodes implanted in the MFB. Maximum excursion of pen set at 500 responses. Schedule is CRF.

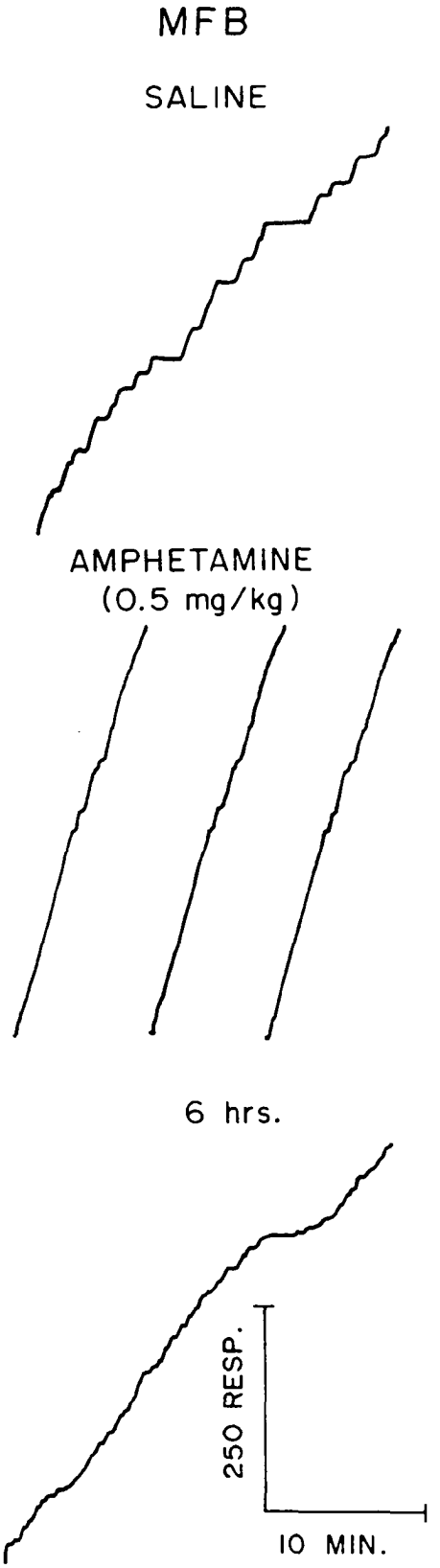

MFB
SALINE
AMPHETAMINE
(0.5 mg/kg)
6 hrs.
250 RESP.
10 MIN.

Figure 15 - Cumulative record of one squirrel monkey self stimulating for brain current. Comparison is between saline condition and amphetamine (0.5 mg/kg). Electrodes implanted in the anterior amygdala. Maximum excursion of pen set at 500 responses. Schedule is CRF.

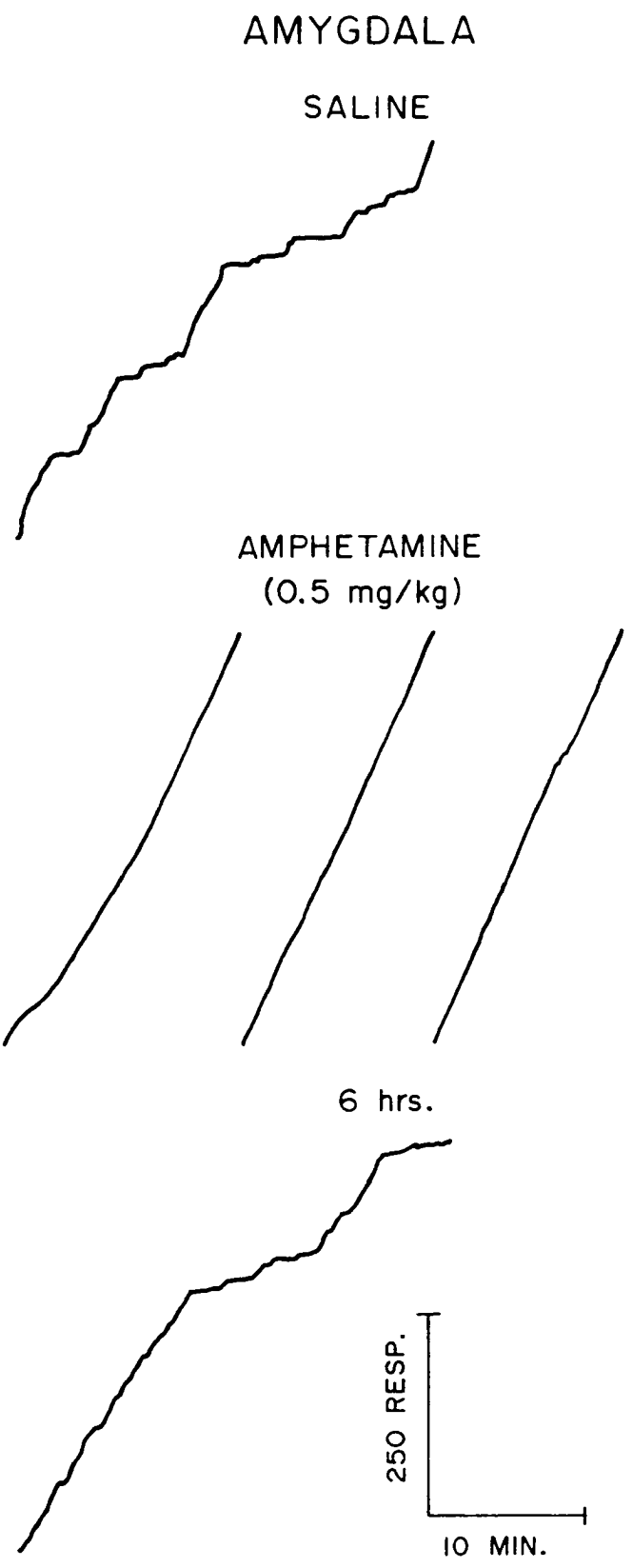

AMYGDALA
SALINE
AMPHETAMINE
(0.5 mg/kg)
6 hrs.
250 RESP.
10 MIN.

Figure 16 - Cumulative record of one squirrel monkey self stimulating for brain current. Comparison is between saline condition and amphetamine (0.50 mg/kg). Current was turned off for fifteen minutes and the animal stopped responding. High rate reappeared when current was turned on again. Electrodes implanted in Lateral Amygdala. Maximum excursion of pen set at 500 responses. Schedule is CRF.

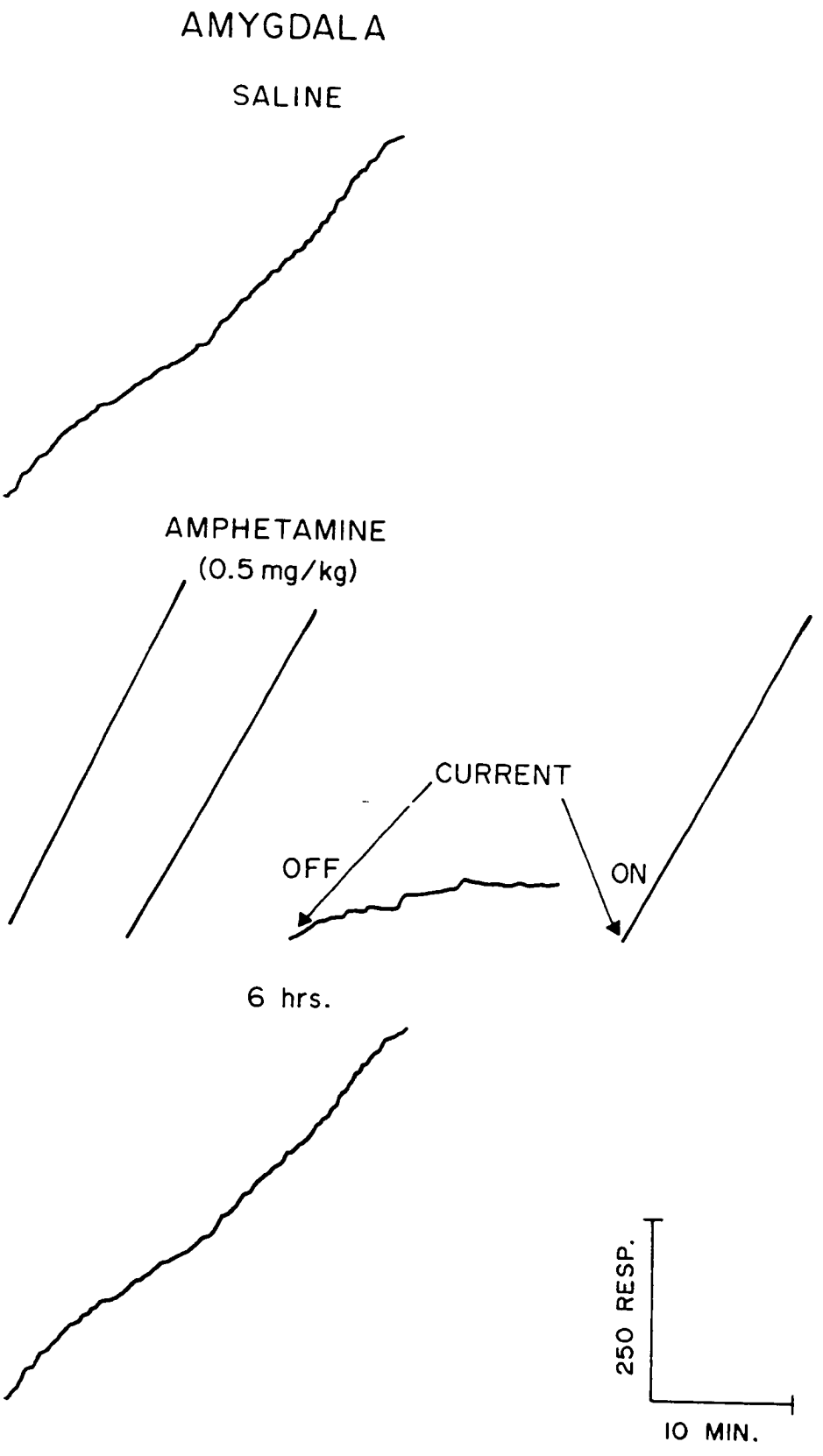

AMYGDALA
SALINE
AMPHETAMINE
(0.5 mg/kg)
CURRENT
OFF
ON
6 hrs.
250 RESP.
10 MIN.

Figure 17 - Cumulative record of one squirrel monkey self stimulating for brain current. Comparison is between saline condition in which rate exceeded 35 responses per minute and amphetamine (0.50 mg/kg). Electrodes implanted in Lateral Amygdala. Maximum excursion of pen set at 500 responses. Schedule is CRF.

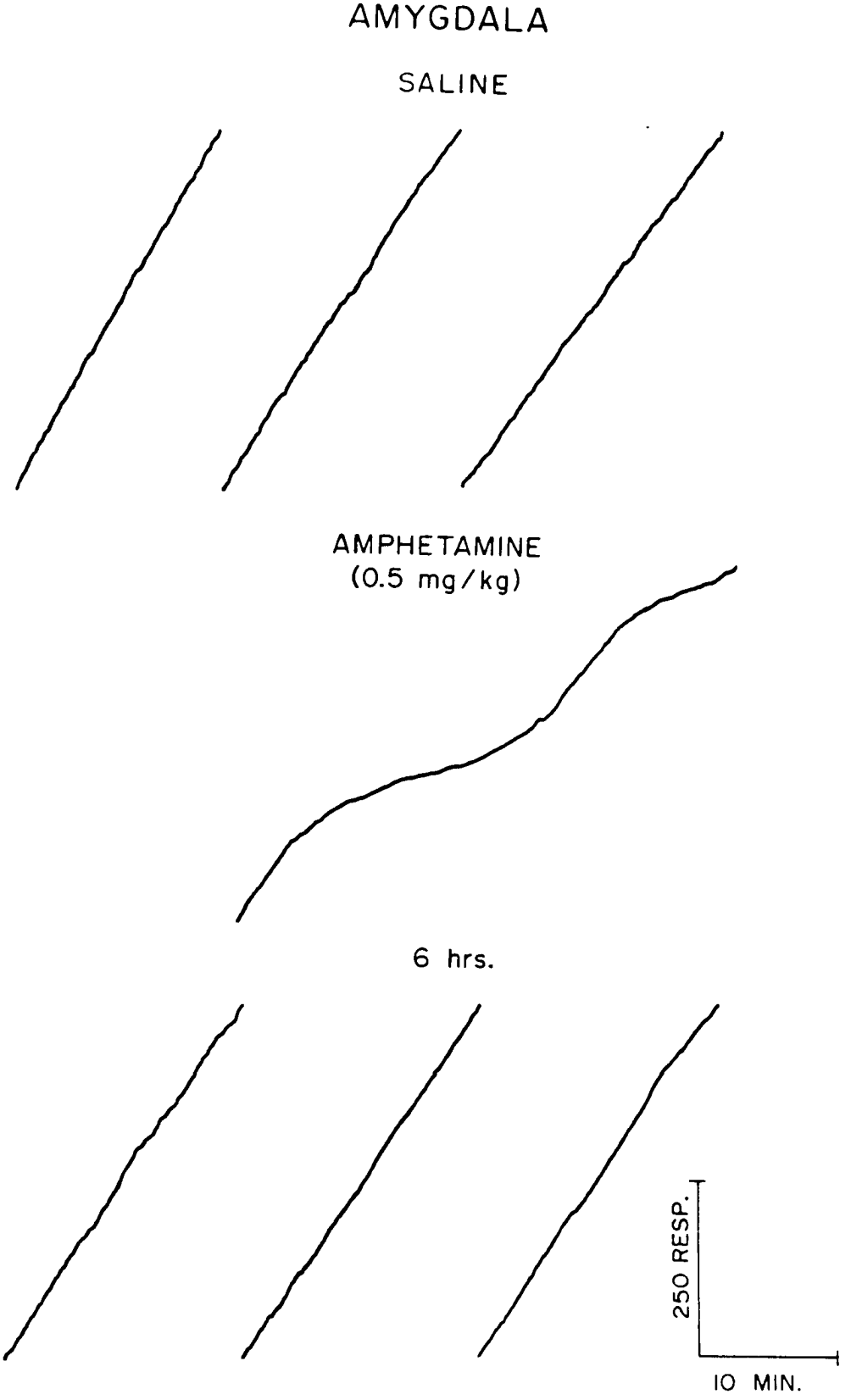

AMYGDALA
SALINE
AMPHETAMINE
(0.5 mg/kg)
6 hrs.
250 RESP.
10 MIN.

Figure 18 - Cumulative record of one squirrel monkey self stimulating for brain current. Comparison is between saline condition and amphetamine (1.0 mg/kg). Electrodes implanted in head of caudate. Maximum excursion of pen set at 500 responses. Schedule is CRF.

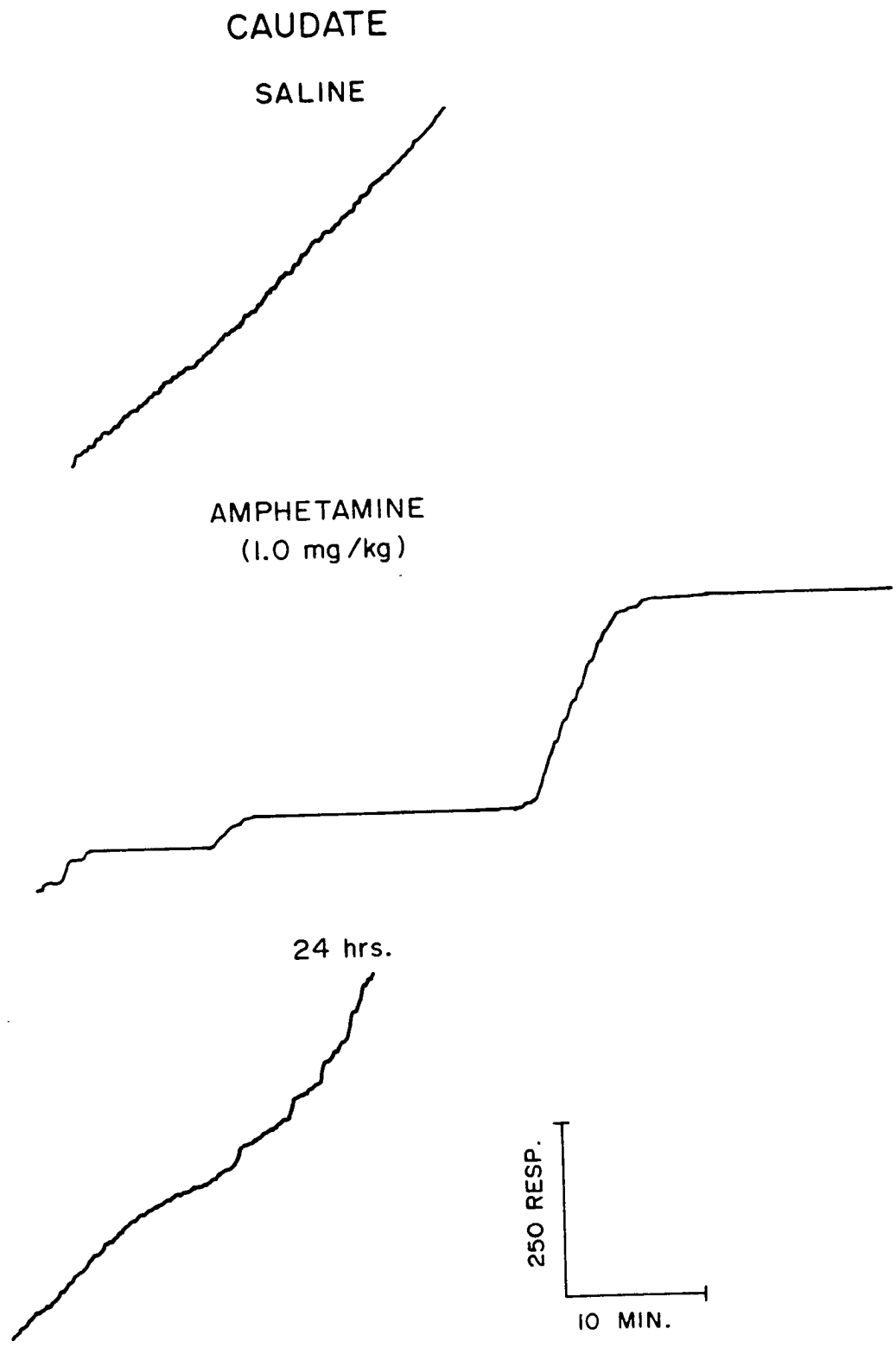

CAUDATE
SALINE
AMPHETAMINE
(1.0 mg/kg)
24 hrs.
250 RESP.
10 MIN.

Figure 19 - Cumulative record of one squirrel monkey self stimulating for brain current. Comparison is between saline condition and amphetamine (1.0 mg/kg). Electrodes are implanted in the Anterior Amygdala. Maximum excursion of pen set at 500 responses. Schedule is CRF.

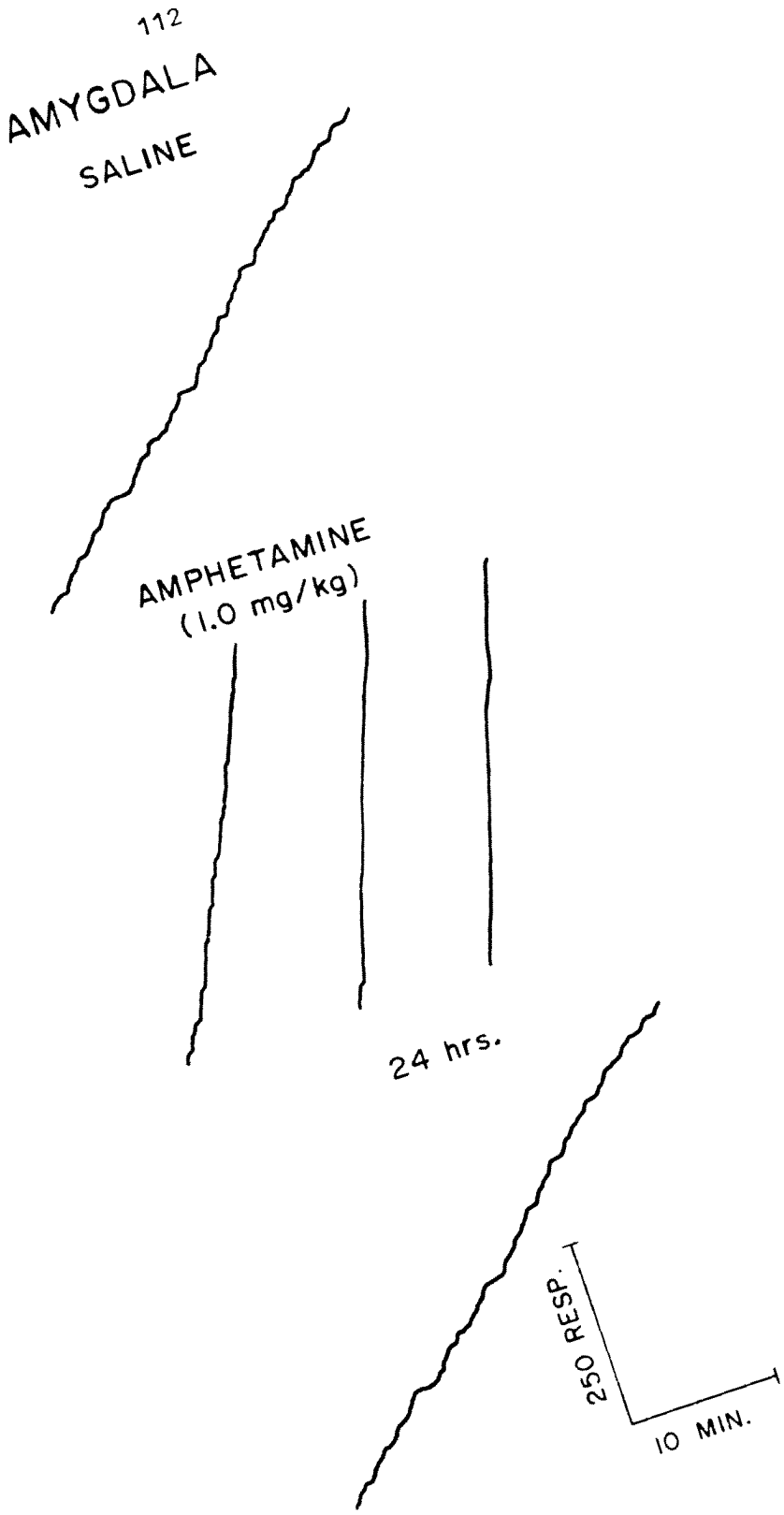
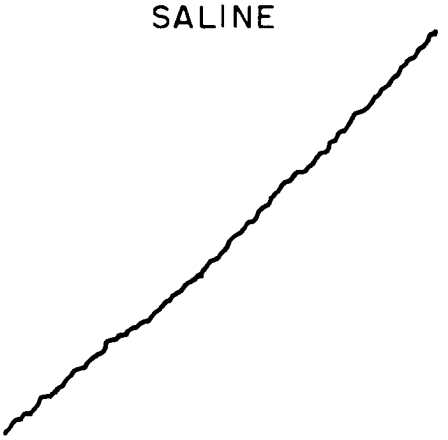

AMYGDALA
SALINE
AMPHETAMINE
(1.0 mg/kg)
24 hrs.
250 RESP.
10 MIN.

Figure 20 - Cumulative record of one squirrel monkey self stimulating for brain current. Comparison is between saline condition and amphetamine (2.0 mg/kg). Electrodes implanted in the Head of Caudate. Maximum excursion of pen set at 500 responses. Schedule is CRF.

CAUDATE

SALINE



AMPHETAMINE
(2.0 mg/kg)

48 hrs.

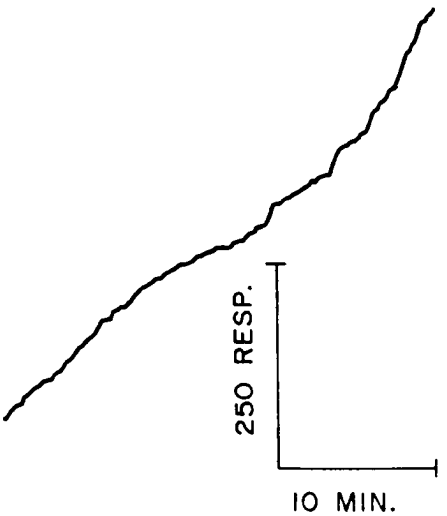
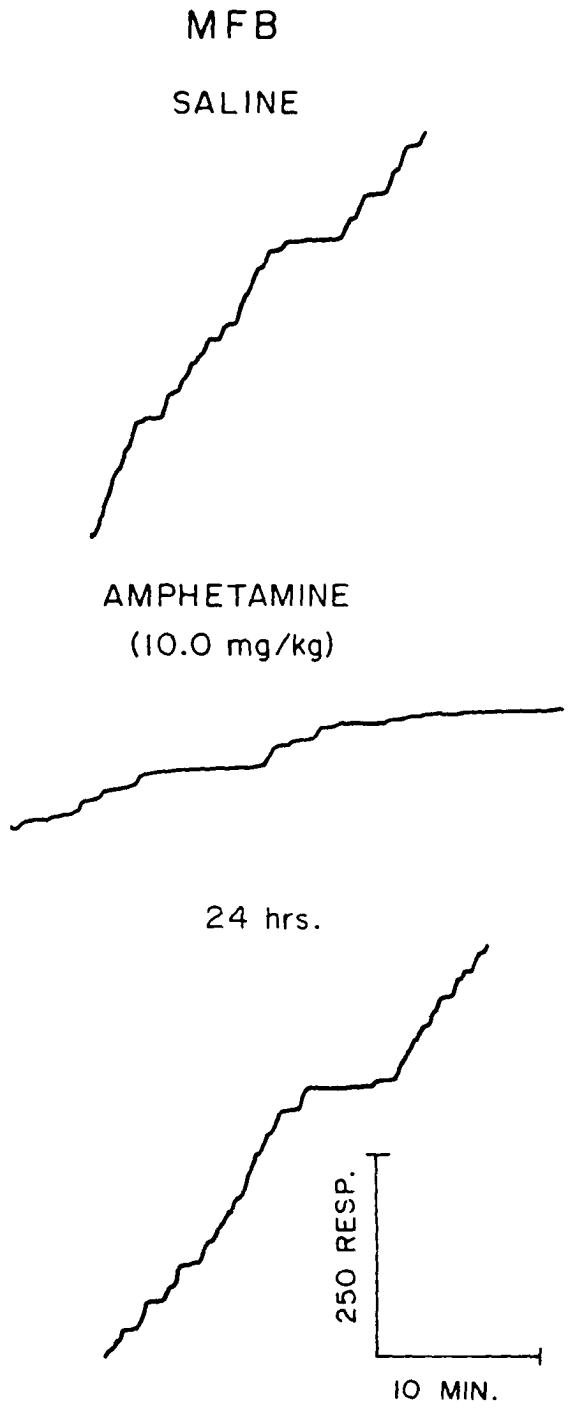


Figure 21 - Cumulative record of one squirrel monkey self stimulating for brain current. Comparison is between saline condition and amphetamine (10 mg/kg). Electrodes implanted in the MFB. Maximum excursion of pen set at 500 responses. Schedule is CRF.


MFB
SALINE
AMPHETAMINE
(10.0 mg/kg)
24 hrs.
250 RESP.
10 MIN.

depressant effect on ICSS (Figure 9) and this finding corresponds to work done in cats (Umemoto and Kido, 1967). Thus, amphetamine influences ICSS in squirrel monkeys as well as rats, cats and the rhesus monkey (Malis et al., 1960).

All stimulated brain sites were equally responsive to the effects of amphetamine suggesting a widespread drug action. The finding that the behavioral facilitating effect stops when the current is turned off (Figure 16) demonstrates that the potentiation of operant rate is not due to a non-specific augmentation of motor activity. Stein (1964) reports a similar finding in rats.

Although amphetamine's effects are complex, there is good evidence that at the biochemical level amphetamine acts on the catecholamines in brain probably by blocking their re-uptake into the nerve ending (Axelrod, 1971). Also, if amphetamine is administered after the intraventricular injection of labeled NE, the drug is able to displace NE from storage sites in the brain, e.g., cortex and hypothalamus. Subcellular distribution studies have also revealed that amphetamine releases labeled NE from nerve endings in the hypothalamus (Glowinski and Axelrod, 1966). The regional location of the catecholamines shows that high amounts of NE are located within the hypothalamus, a region which will support ICSS (Olds, 1962).

DA is also responsive to amphetamine administration. If the caudate nucleus (which contains high amounts of dopamine) is labeled with 3-H dopamine and the ventricular system is perfused with d-amphetamine, an increase in efflux of 3-H dopamine is observed in the ventricles (Voigtlander et al., 1972). The efflux of 3-H dopamine evoked by amphetamine is dependent upon impulse activity of neurons in the nigro-

striatal pathway since chronic lesions within these fibers reduces the output of dopamine. This finding is significant since the nigro-striatal pathway has been implicated as supporting ICSS in primates and may be a separate pathway for positive reinforcement apart from the MFB (Routtenberg, 1971).

In addition to amphetamine-induced release of the catecholamines, electrical stimulation of caudate, substantia nigra or the hypothalamus will either release DA or NE, respectively (Voigtlander et al., 1972). Thus, both drugs and electrical stimulation will cause a release of catecholamines from regions of the brain that also support ICSS.

When "behavior" is then superimposed onto drug action and/or neuro-chemistry, the interpretation of mechanisms involved becomes more difficult. For example, Stein and Wise (1969) have demonstrated that hypothalamic ICSS causes an increase in labeled NE in both the amygdala and the hypothalamus. When amphetamine was injected, NE was released from the amygdala but not from the hypothalamus. Stein suggests that the NE acts as an inhibitory transmitter which depresses the activity of behaviorally suppressant cell groups in the forebrain. That is, amphetamine facilitates behavior by the release of NE which has an disinhibitory action. However, since release of DA 5-HT and ACh have all been correlated with the occurrence of ICSS (Arbuthnott et al., 1970, 1971; Holloway, 1972; Olds and Yuwiler, 1972), Stein's hypothesis may not fully explain ICSS. One particular difficulty in attempting to relate drug action and brain neurochemistry with hypothalamic ICSS is conclusively demonstrating that any amine or any brain region is functionally separate.

The results contained in this experiment cannot be used to analyze this problem. Rather, they suggest that the site of action for amphetamine, regardless of which specific amine(s) are more important to supporting "behavior", probably involves at least noradrenergic and dopaminergic sites. Both diencephalic and telencephalic regions of the brain were found to be sensitive to amphetamine-induced potentiation or inhibition of ICSS.

The neurophysiological action of amphetamine is to hyperpolarize the nerve membrane (Himwich, 1970). Low doses might act on receptors to first cause facilitation of behavior through the disinhibition of suppressant cells throughout the forebrain. Higher doses might act on more receptors to produce inhibition in behavior. When d-amphetamine is injected I.P. in anesthetized rats, single cells in the caudate and reticular formation are first facilitated and then depressed (Groves et al., 1974). These effects are most obvious at higher doses (2.0 mg/kg and 4.0 mg/kg). The authors suggest that amphetamine-induced depression in caudate can be related to the facilitation of DA transmission. For example, the effect of amphetamine could be reversed by haloperidol (a DA receptor blocker) which when administered alone produced significant increases in activity. Since the dopaminergic nigrostriatal pathway has been implicated in ICSS studies (Routtenberg, 1971), reported differences in drug effects on caudate neurophysiology may have functional significance to certain underlying behaviors.

Administration of amphetamine also influences general "behavioral state" (e.g., in humans it can produce hallucinogenic or stereotypic symptoms). Further, amphetamine injections can act as a positive reinforcement. For example, rats will emit operant responses to receive

injections of the drug (Picken et al., 1970). When given in low doses animals may seek additional stimulation (ICSS) to maximize the positive reinforcing effects of the drug. With higher doses the behavioral state could change to a hallucinogenic one and the animal may not require additional self stimulation (via ICSS) since the positive effects of the drug are sufficient. If this latter hypothesis is correct, the biphasic action of amphetamine on operant behavior, as a function of dose, is explicable.

Single low doses of amphetamine (0.25-1.0 mg/kg) had short durations of effect and no differences were recorded between brain sites. When larger doses were given (2.0 mg and 10.0 mg/kg) rate of self stimulation of caudate was inhibited for a longer period of time than hypothalamus or amygdala (Figure 11). The explanation that the effect may have been "state" dependent does not appear to be feasible since these differences were observed within the same animal as well as between groups. That is, animals would self-stimulate their hypothalamus but not caudate.

The findings previously reported that several amines and brain sites are sensitive to amphetamine administration challenges Stein's hypothesis of exclusive hypothalamic action by amphetamine. The finding reported in this dissertation that caudate ICSS is more sensitive to high doses of amphetamine administration than hypothalamic ICSS (using duration of action as a measure) begins to challenge Stein's hypothesis at another level of analysis.

The duration of action of drugs that affect CNS function is regulated in part by general metabolism, by enzymes and by binding phenomena at the cell and synapse (Ungerstedt, 1973). The exact

mechanisms responsible for affecting duration of action of a drug are complex. Separate neural and metabolic mechanisms are probably involved both for individual drugs and for varying doses of any given drug.

To summarize this discussion, any drug's effect on behavior (dose response or duration of action) is secondary to its action within the brain. Amphetamine interacts with many enzymes, altering their function, or with important substrates for enzyme activity. These alterations of the biochemistry of the cell produce changes in cellular function which in turn affects tissue and organ function. Amphetamine acts to release, block re-uptake, and to affect the receptor binding characteristics for DA, NE, and 5-HT. The threshold dose for any type of action will vary as a function of particular transmitter and the type of neurons involved. The drug affects neurone interaction to varying extents throughout various brain areas. The contribution of each brain area to various "motivated behaviors" is complex but may be unique for each site. Amphetamine's effects then, on behavior, are a complex sum of the effect on brain areas which is in turn a function of dose.

Many "behavioral states" of the organism (e.g., arousal, wakefulness, motor activity, affective patterns, memory, sensory abilities) are co-jointly modified by the action of amphetamine. Any final response output probably does not adequately reflect amphetamine's sole action because it has changed so many other behaviors that interact with the one selected behavior under study.

CHAPTER IV

EXPERIMENT 3: DOSE RESPONSE AND DURATION OF ACTION OF CHLORPROMAZINE ON CAUDATE, AMYGDALOID AND HYPOTHALAMIC SELF STIMULATION

Materials and Methods

Subjects

The Ss used in this experiment were the same Ss used in Experiments 1 and 2.

Testing Procedure

From the results of Experiment 1, six animals were selected that had stable response rates (head of caudate); six other Ss were chosen that had stable response rates in the hypothalamus. Two Ss that also had positive electrodes in the anterior amygdala, two that had a positive site in the lateral amygdala and one that had a positive site in the central amygdala were used.

Dose response analysis. Four separate doses of CPZ were randomly given to all Ss in the following manner. The first two days were used to establish a saline ICSS baseline. The Ss were given fifteen minutes in the chamber in which every lever pull produced a brain shock. Intensity of current was increased to a level which produced high and consistent rates of responding, e.g., 35-50 responses per minute. The S was then removed from the chamber and given a marshmallow with saline

dissolved inside. Fifteen minutes later the animal was placed back in the chamber and the session was continued for 45 minutes to an hour. On the second day the same procedure was followed and generally the between baseline variance was minimal. On the third day CPZ (either 0.25, 0.50, 1.0, or 2.0 mg/kg) was given in a randomized order in place of the saline.

The interaction of amphetamine with chlorpromazine was examined by first giving amphetamine (0.5 mg/kg). Following potentiation of ICSS rate, CPZ 1.0 mg/kg was given. This analysis of interaction effects was done in three animals that had positive sites in either caudate, hypothalamus or amygdala.

In three animals that had positive sites in both caudate and MFB, second and third CPZ replications were performed at 0.5, 1.0, and 2.0 mg/kg. First, one site was tested for 30-45 minutes and then the other site was analyzed (order counterbalanced).

The duration analysis was done in the following manner. The initial testing session lasted from fifteen minutes to one hour and fifteen minutes following drug ingestion. Three hours after drug injection the animal was placed back in the chamber and re-tested for 45 minutes. The same procedure was used at 6, 12, and 24 hours, and then every 12 hours until the animal's rate had returned to its original baseline level (plus or minus ten per cent). Every time the animal was placed in the chamber a marshmallow was given. The interval between drug administrations was ten days.

Results

Statistical analysis was performed on the absolute response rate

per minute (drug to saline ratio x 100). A 2 x 4 analysis of variance (brain site and drug dose) with repeated measures on the last factor (Winer, 1973) revealed a significant interaction between drug dose and brain site ($F=9.68$, 1, 10 $p < .05$) as shown in Table 10. Since this interaction described a relationship between a specific brain site and dose of CPZ during ICSS, main effects were ignored although they were significant ($F=34.34$, 1, 10 $p < .01$) and ($F=74.46$, 1, 10 $p < .01$). Simple effects analysis was performed as well as analyzing between treatment totals using the Scheffe Test (Winer, 1973). Conservative degrees of freedom were used (Winer, 1973).

Table 11 and Figure 22 show that increasing doses of CPZ from 0.50 and 1.0 mg/kg produced a significant decrease in responding in the caudate group but less of a change in the MFB group. As dose was increased to 2.0 mg/kg, a reduction in response rate was observed in both groups. The animals, however, still responded to a tail or leg pinch at this dose. Dose response analysis for the one animal that had electrodes in the central amygdala found effects similar to those in the caudate group.

In Figure 23 histograms compare the duration of action of CPZ at various doses. Since the unit of measure (hours to return to baseline) was arbitrarily set by the experimenter, a non-parametric analysis (Segal, 1962) based on signed ranks was performed on the two highest doses. At 1.0 and 2.0 mg/kg the caudate group demonstrated a significantly longer return to baseline responding ($U=0$ $p < .001$; $U=1$ $p < .001$) when compared to the MFB group.

Figures 24 to 31 are cumulative records demonstrating that as CPZ dose is increased, ICSS is reduced at all brain sites. Differences

TABLE 10

SUMMARY OF ANALYSIS OF VARIANCE COMPARING
BRAIN SITE (CAUDATE OR MFB) AND
DRUG DOSE (CHLORPROMAZINE)

Source of Variation	SS	df	MS	F	
Between Subjects	11,260	11			
A (brain site)	8,721	1	8,721	34.34	**
Subj w. groups	2,539	10	253.90		
Within Subjects	58,497	36			
B (drug dose)	46,247	3 (1)	15,415.66	74.46	**
AB	6,029	3 (1)	2,009	9.68	*
B X subj w. groups	6,221	30 (10)	207		

* $P < .05$
** $P < .01$

TABLE 11

MEAN PER CENT CHANGE IN RATE OF SELF STIMULATION:
BRAIN SITE AND CPZ DOSE COMPARISONS

	Drug Dose (mg/kg)			
Brain Site	.25	.50	1.0	2.0
Caudate	89.33	32.83*	28.00	11.83
	(± 15.18)	(± 19.61)	(± 12.17)	(± 7.01)
MFB	100.00	93.33	65.33	12.04
	(± 7.45)	(± 9.42)	(± 18.48)	(± 7.28)

* Any value underscored by the same line is not statistically different.

Figure 22 - Graphic plot of changes in self stimulation rate in squirrel monkeys (expressed as mean per cent of previous control run) following separate doses of chlorpromazine. Vertical bar represents standard error of the mean. Caudate, n=6; MFB, n=6; Lateral Amygdala, n=2; Anterior Amygdala, n=1.

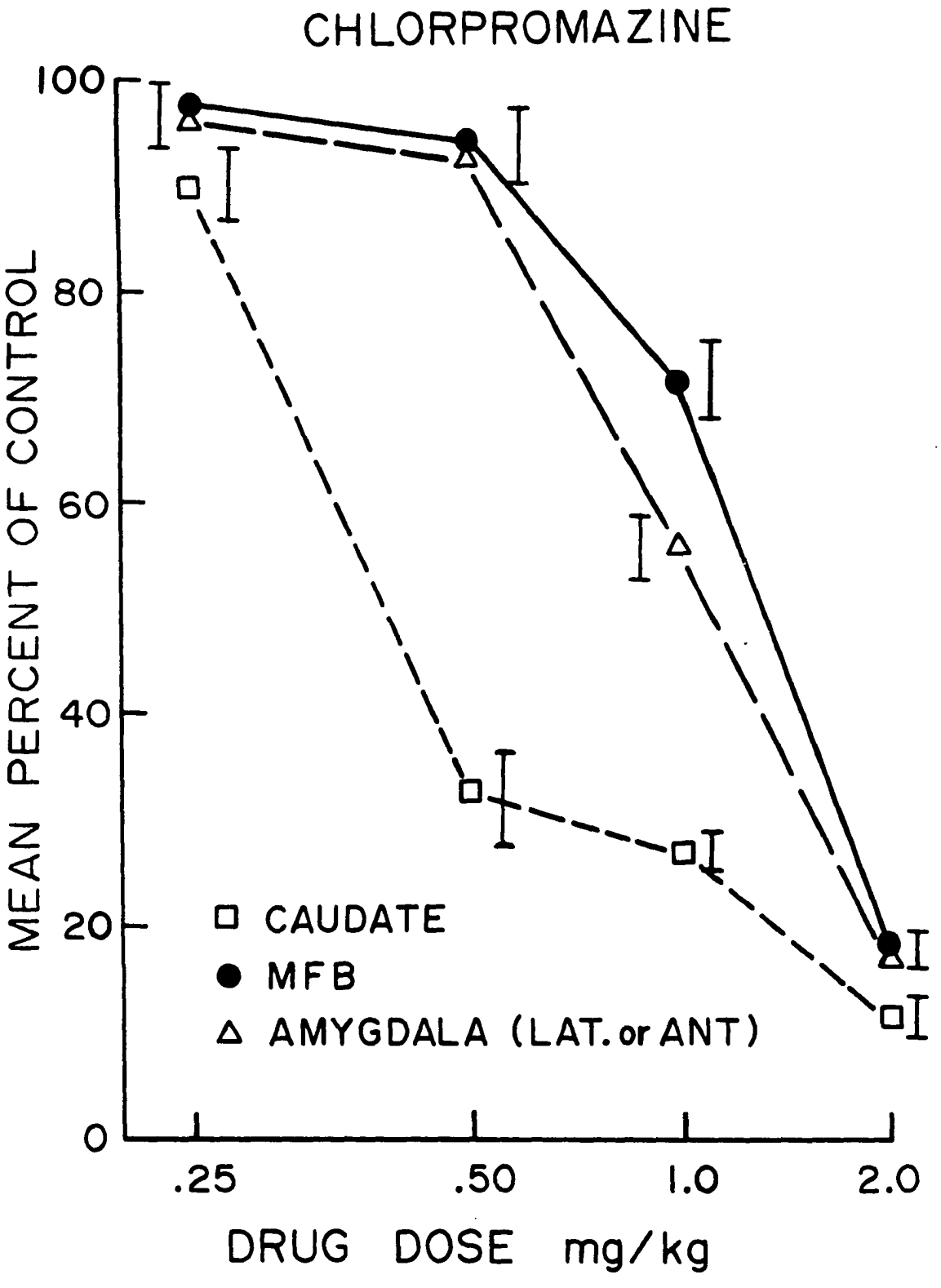

CHLORPROMAZINE
100
80
60
40
20
0
MEAN PERCENT OF CONTROL
CAUDATE
MFB
AMYGDALA (LAT. or ANT)
.25
.50
1.0
2.0
DRUG DOSE mg/kg

Figure 23 - Histogram of the return of self stimulation rate to pre-drug baseline condition in squirrel monkeys following separate doses of chlorpromazine. Vertical bar represents the standard error of the mean. Caudate, n=6, MFB, n=6; Lateral Amygdala, n=2; Anterior Amygdala, n=1.

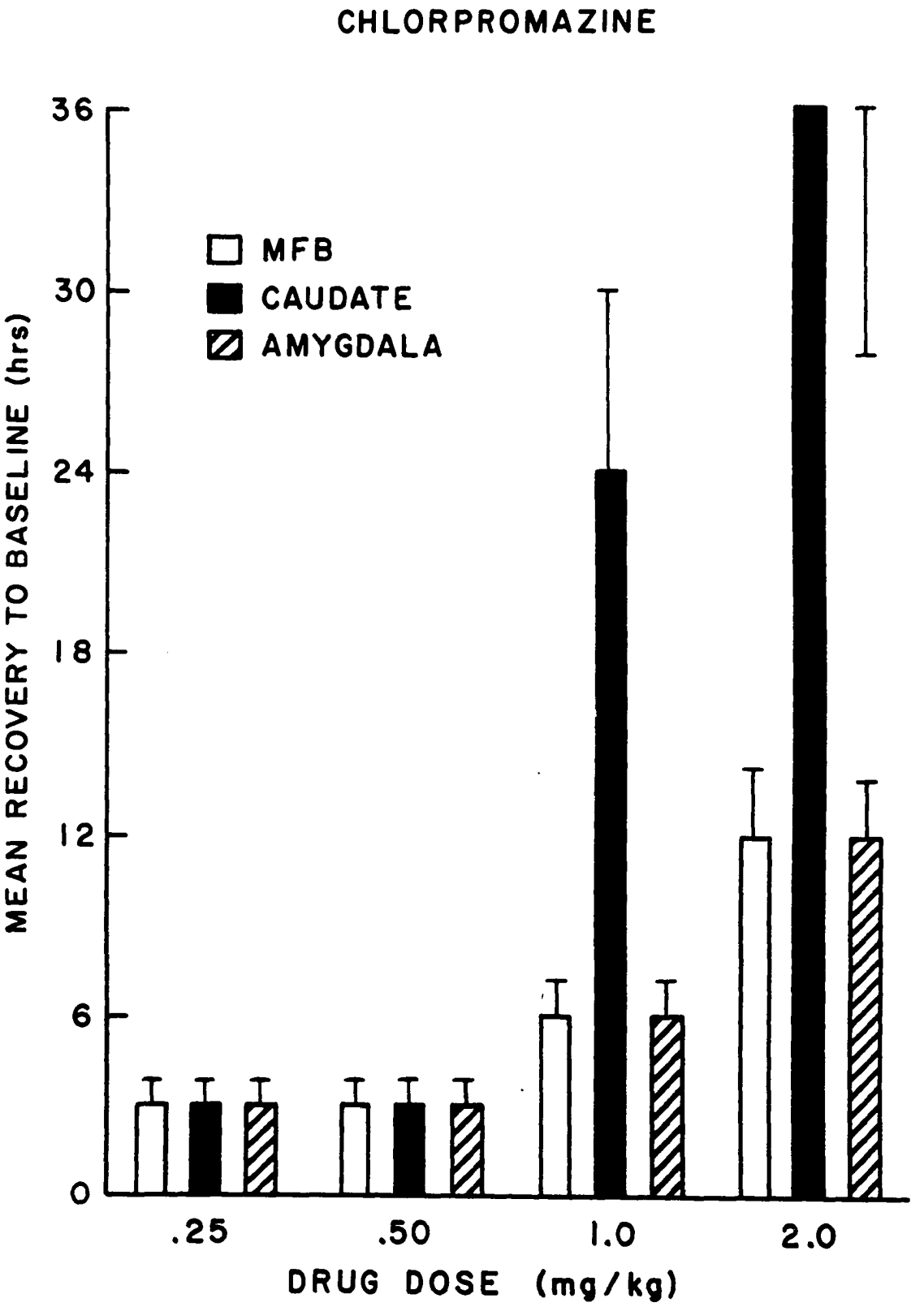

CHLORPROMAZINE
MFB
CAUDATE
AMYGDALA
MEAN RECOVERY TO BASELINE (hrs)
36
30
24
18
12
6
0
.25
.50
1.0
2.0
DRUG DOSE (mg/kg)

Figure 24 - Cumulative record of one squirrel monkey self stimulating for brain current. Comparison is between saline condition and chlorpromazine (0.5 mg/kg). Electrodes are implanted in the Head of Caudate. Maximum excursion of pen set at 500 responses. Schedule is CRF.

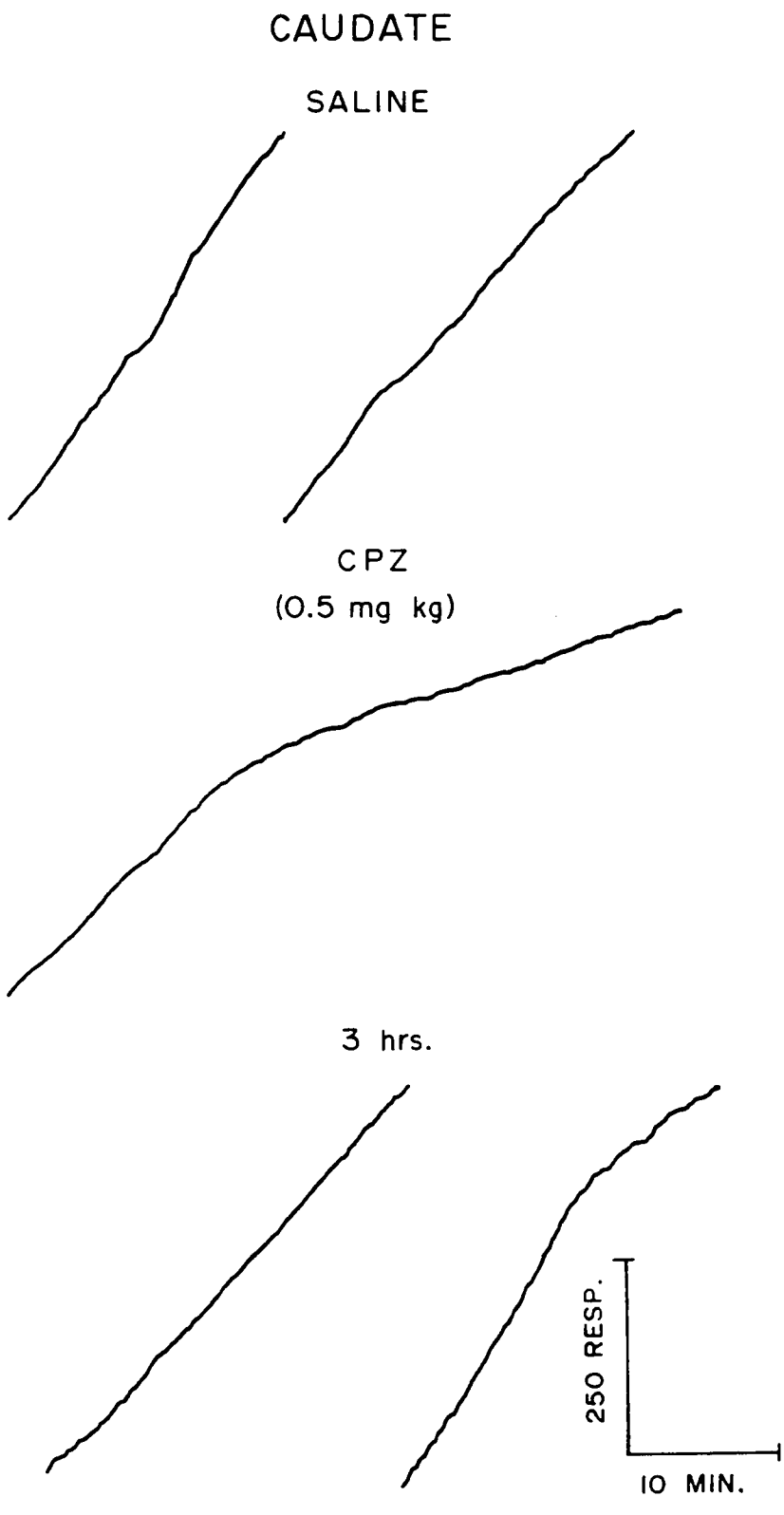

CAUDATE
SALINE
CPZ
(0.5 mg kg)
3 hrs.
250 RESP.
10 MIN.

Figure 25 - Cumulative record of one squirrel monkey self stimulating for brain current. Comparison is between saline condition and chlorpromazine (0.50 mg/kg). Electrodes are implanted in the MFB. Maximum excursion of pen set at 500 responses. Schedule is CRF.

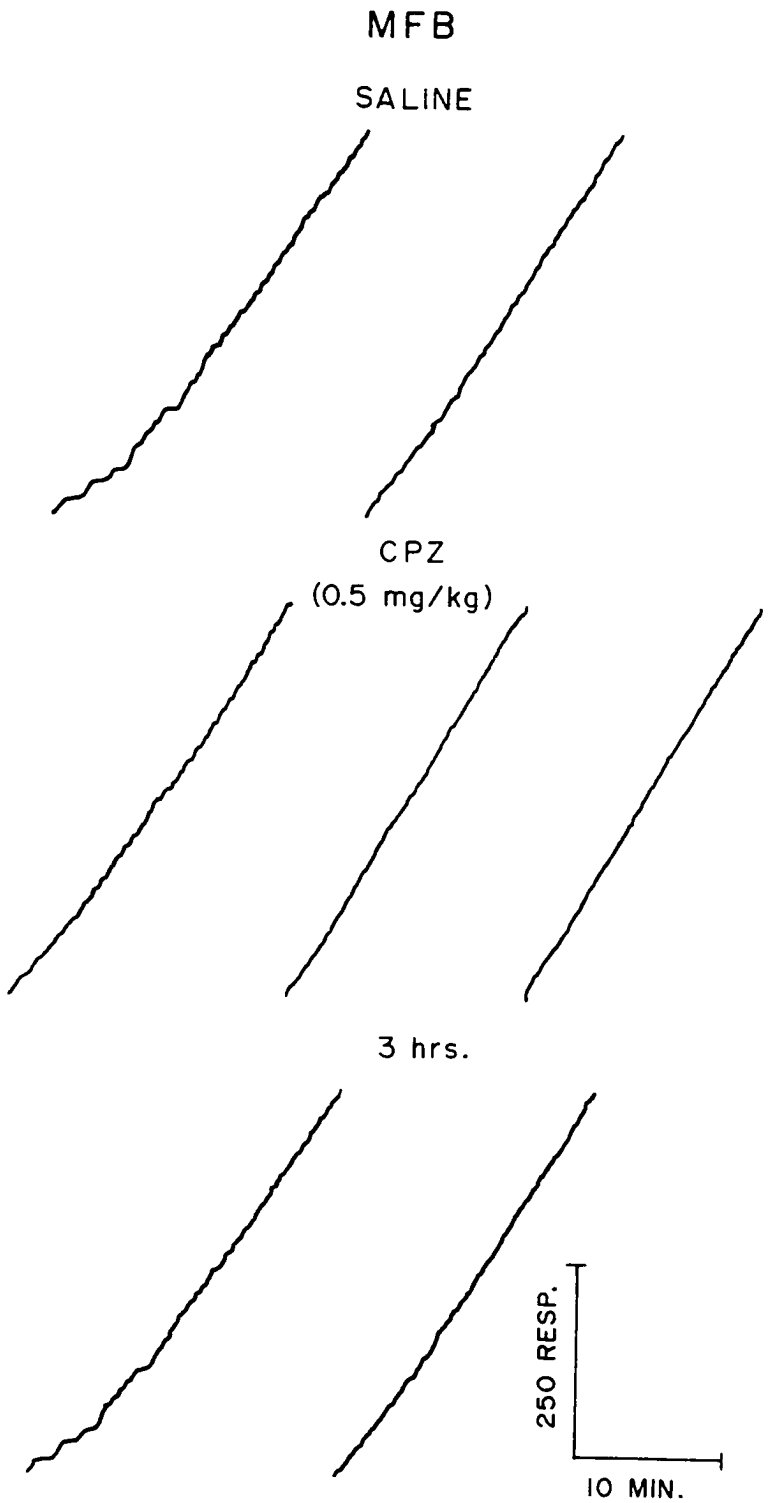

MFB
SALINE
CPZ
(0.5 mg/kg)
3 hrs.
250 RESP.
10 MIN.

Figure 26 - Cumulative record of one squirrel monkey self stimulating for brain current. Comparison is between saline condition and chlorpromazine (1.0 mg/kg). Electrodes implanted in Head of Caudate. Maximum excursion of pen set at 500 responses. Schedule is CRF.

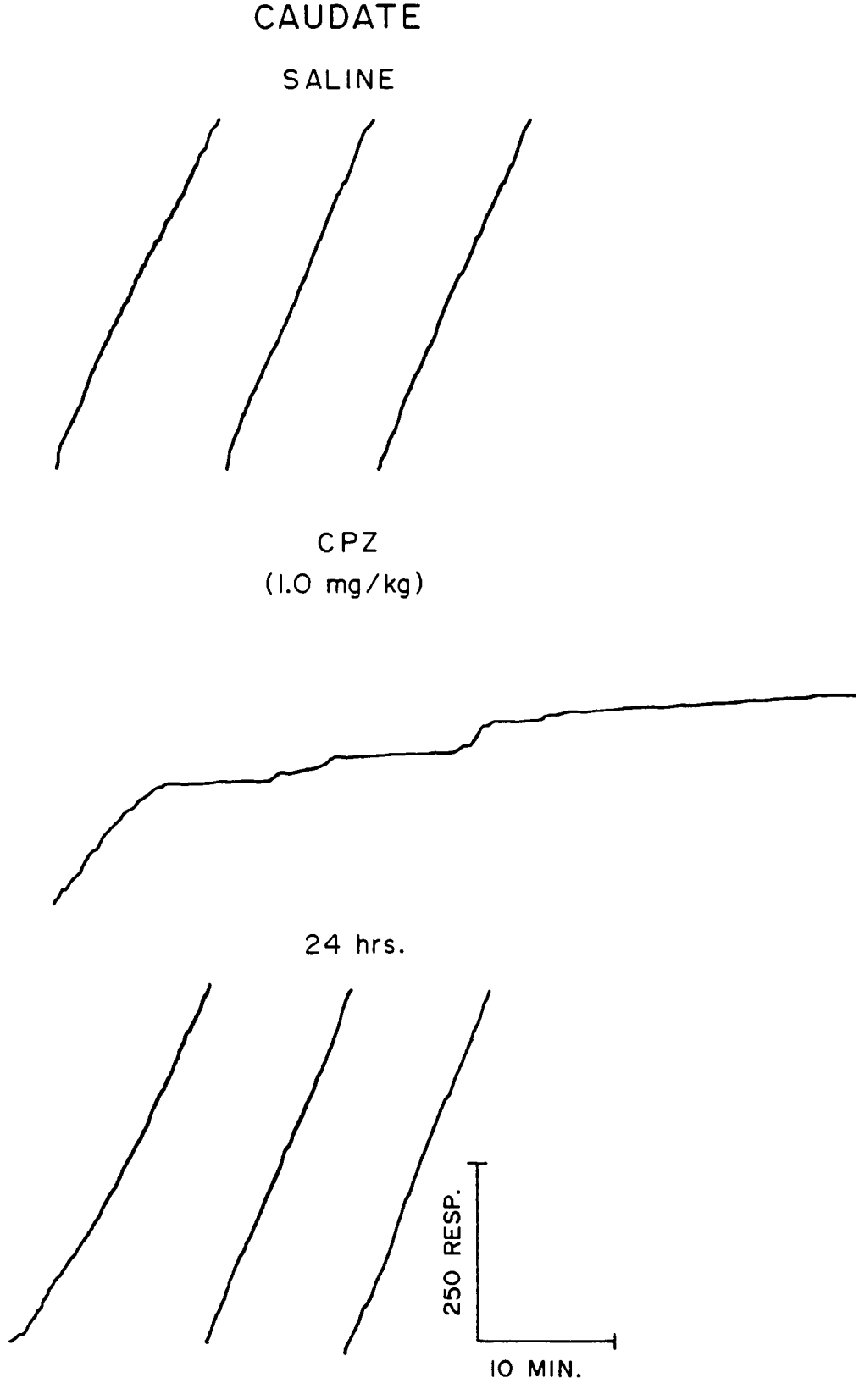

CAUDATE
SALINE
CPZ
(1.0 mg/kg)
24 hrs.
250 RESP.
10 MIN.

Figure 27 - Cumulative record of one squirrel monkey self stimulating for brain current. Comparison is between saline condition and chlorpromazine (2.0 mg/kg). Electrodes are implanted in the MFB. Maximum excursion of pen set at 500 responses. Schedule is CRF.

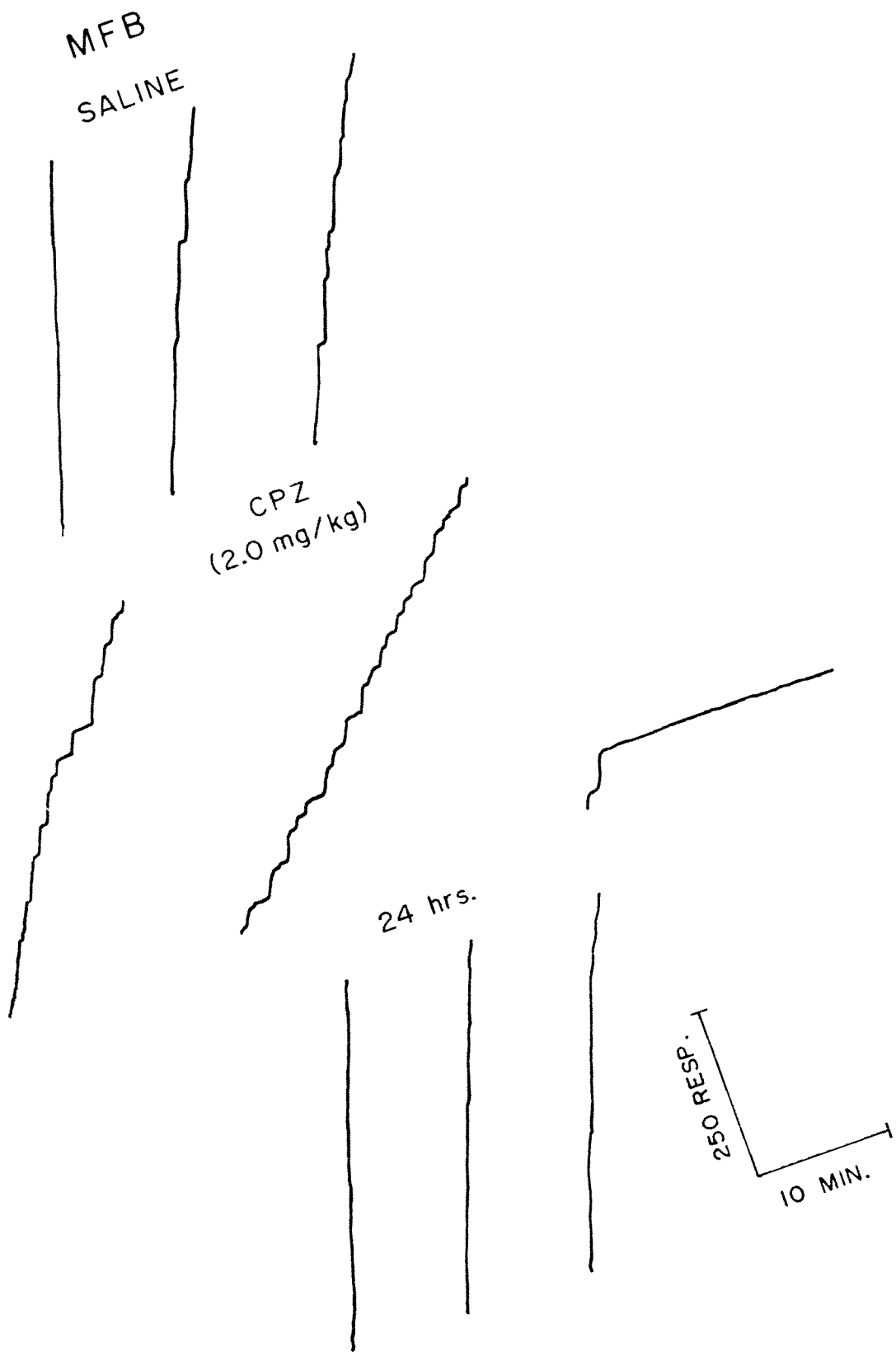

MFB
SALINE
CPZ
(2.0 mg/kg)
24 hrs.
250 RESP.
10 MIN.

Figure 28 - Cumulative record of one squirrel monkey self stimulating for electric current to brain. Comparison is between saline condition and chlorpromazine (0.5 mg/kg) at two separate brain sites, Head of Caudate or MFB. Drug appears to produce inhibition in responding at caudate but MFB is not affected. Maximum excursion of pen set at 500 responses. Schedule is CRF.

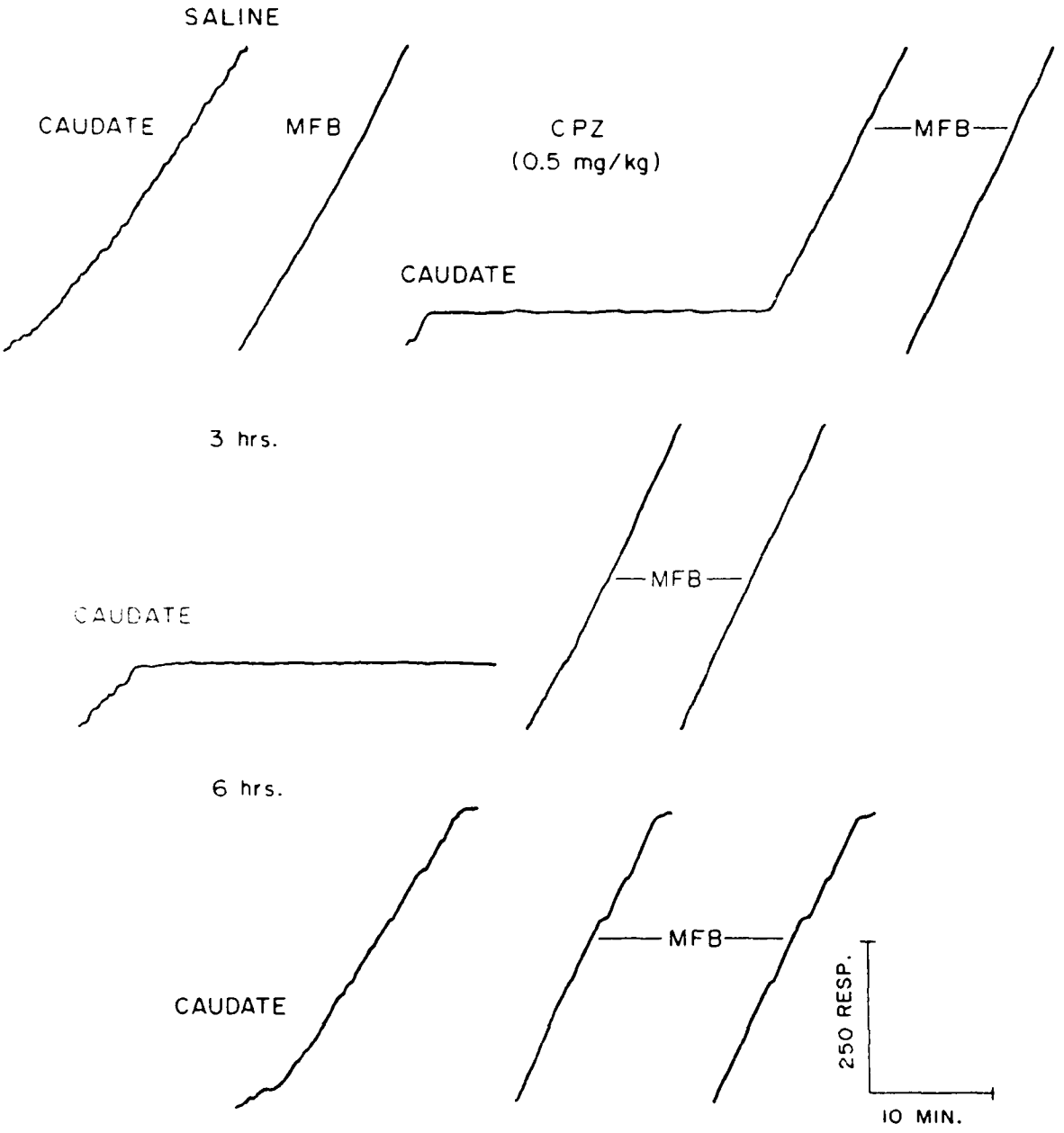

SALINE
CAUDATE
MFB
CPZ
(0.5 mg/kg)
MFB
CAUDATE
3 hrs.
MFB
CAUDATE
6 hrs.
CAUDATE
MFB
250 RESP.
10 MIN.

Figure 29 - Cumulative record of one squirrel monkey self stimulating for electric current to brain. Comparison is between saline condition and chlorpromazine (1.0 mg/kg) at two separate brain sites, Head of Caudate or MFB. Drug appears to produce inhibition in responding at caudate but MFB is not affected. Maximum excursion of pen set at 500 responses. Schedule is CRF.

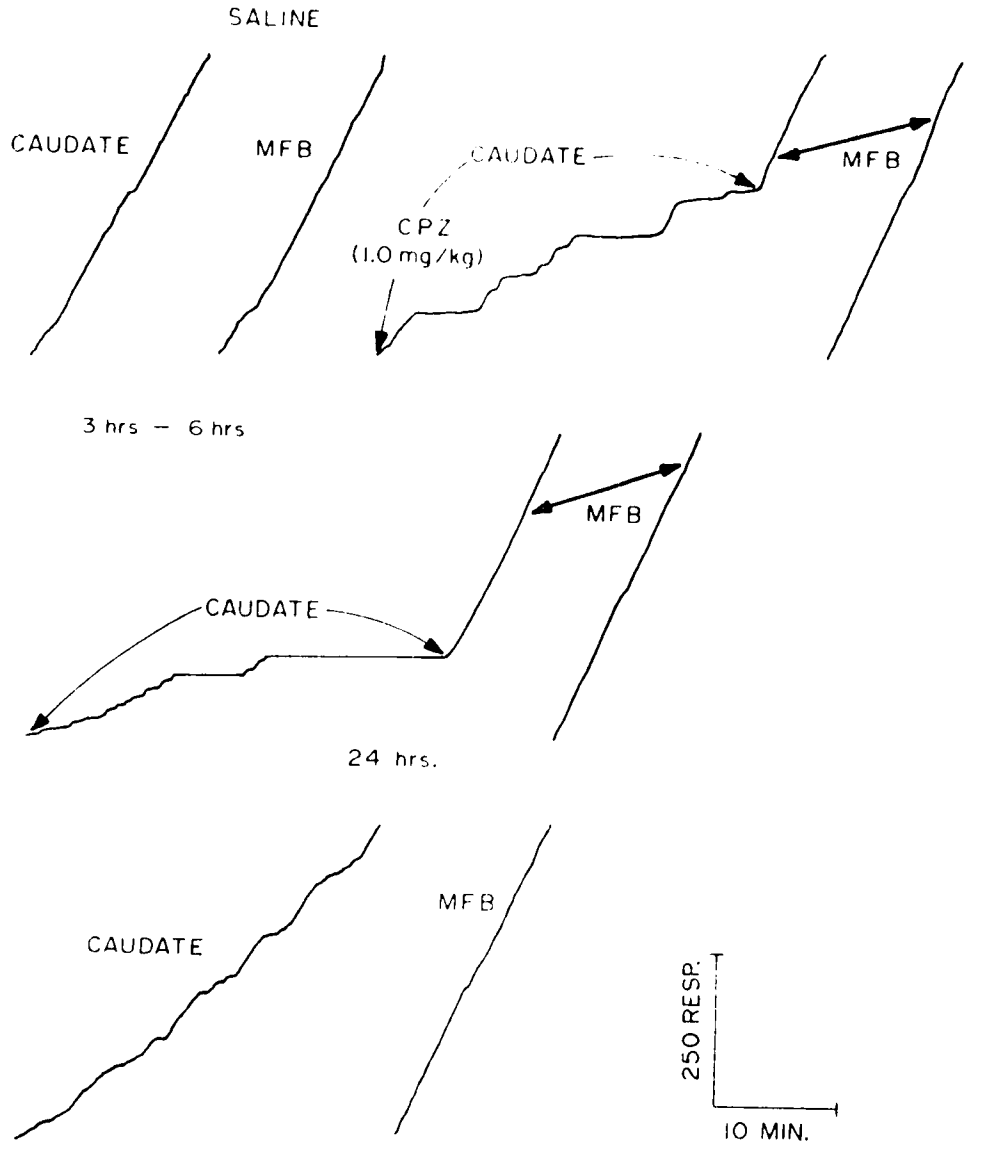
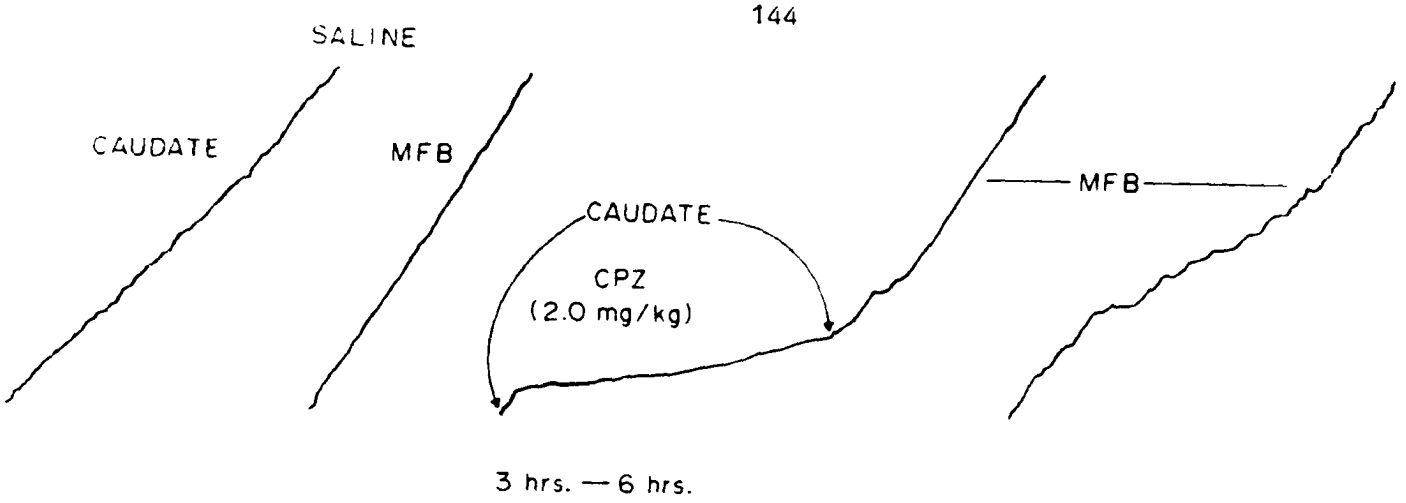
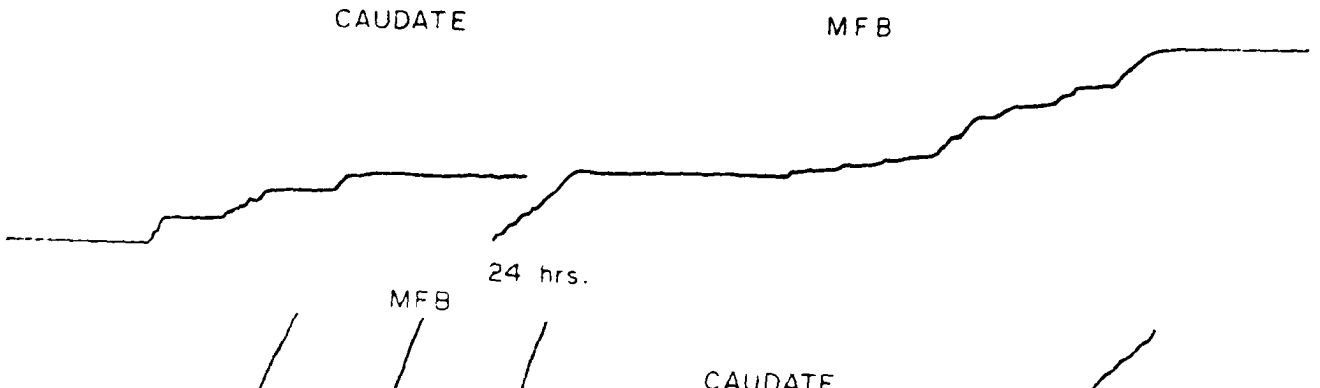
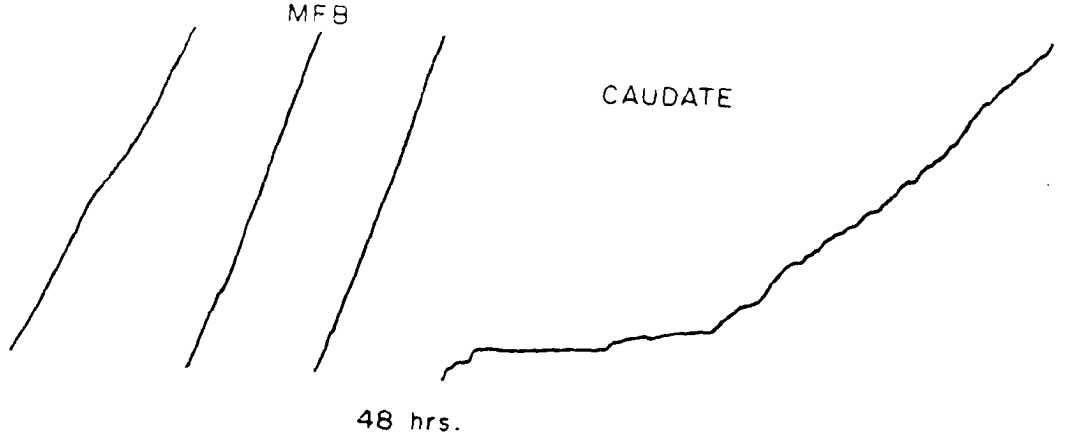

SALINE
CAUDATE
MFB
CAUDATE
MFB
CPZ
(1.0 mg/kg)
3 hrs – 6 hrs
MFB
CAUDATE
24 hrs.
CAUDATE
MFB
250 RESP.
10 MIN.

Figure 30 - Cumulative record of one squirrel monkey self stimulating for electric current to brain. Comparison is between saline condition and chlorpromazine (2.0 mg/kg) at two separate brain sites, Head of Caudate or MFB. Drug produces reduction and then inhibition in responding at both sites. The MFB self stimulation site returns to baseline by 24 hours while the caudate returns at 48 hours. Maximum excursion of pen set at 500 responses. Schedule is CRF.


SALINE
CAUDATE
MFB
CAUDATE
CPZ
(2.0 mg/kg)
MFB
3 hrs. — 6 hrs.


CAUDATE
MFB
24 hrs.


MFB
CAUDATE
48 hrs.

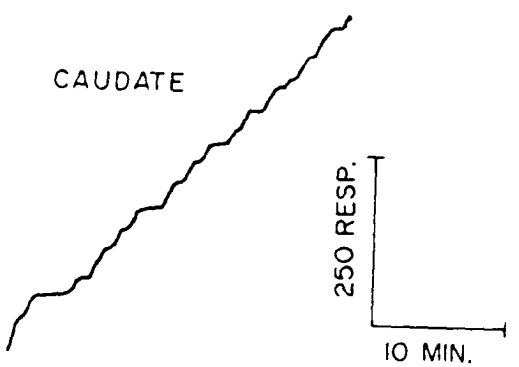
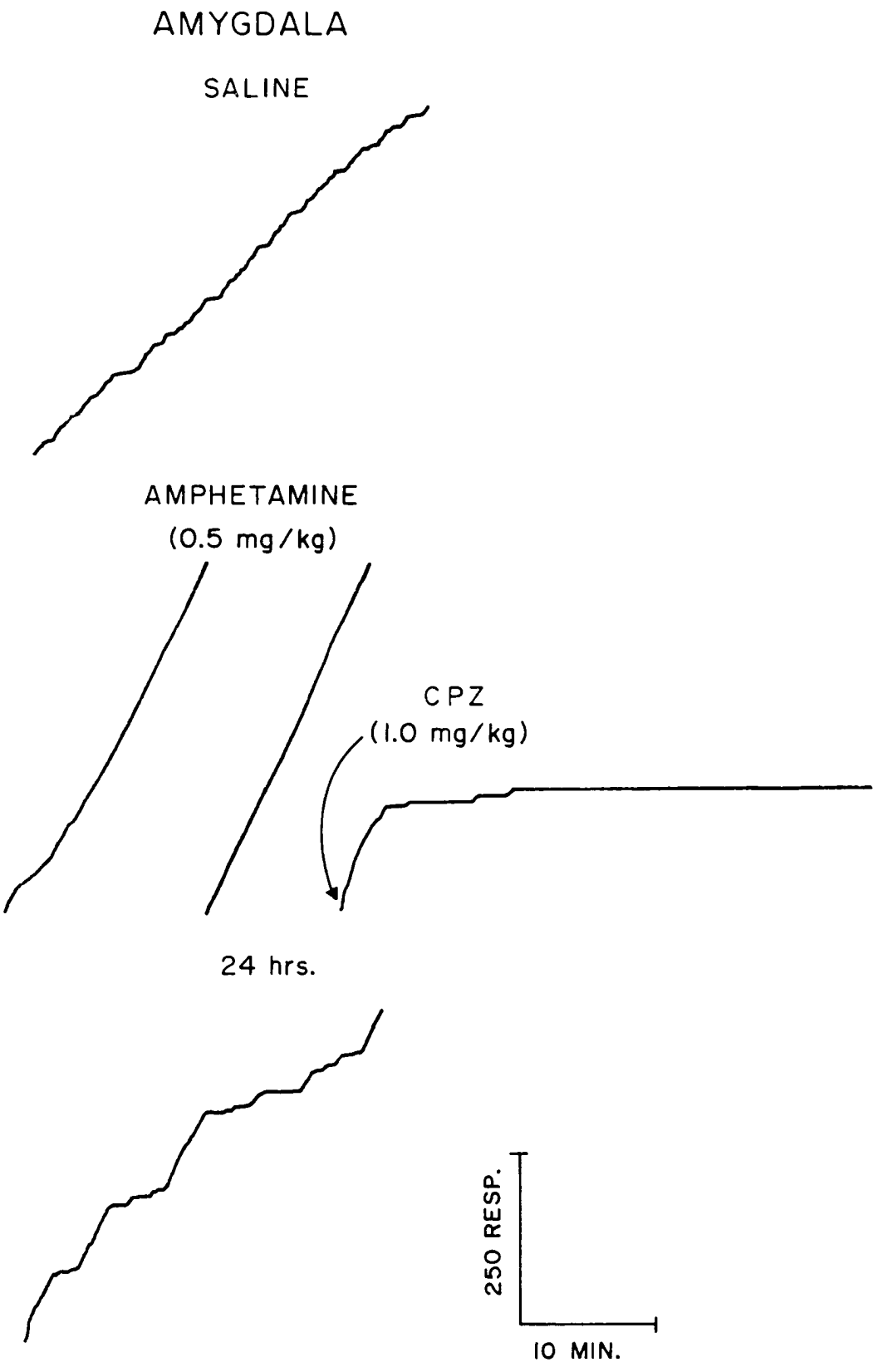

CAUDATE
250 RESP.
10 MIN.

Figure 31 - Cumulative record of one squirrel monkey self stimulating for brain current. Comparison is between saline condition and amphetamine (0.50 mg/kg). Following response facilitation, chlorpromazine (1.0 mg/kg) is then given. Note inhibition of previous amphetamine induced facilitation. Electrodes implanted in Lateral Amygdala. Maximum excursion of pen set at 500 responses. Schedule is CRF.


AMYGDALA
SALINE
AMPHETAMINE
(0.5 mg/kg)
CPZ
(1.0 mg/kg)
24 hrs.
250 RESP.
10 MIN.

were observed not only between groups but within three animals who had positive sites in both caudate and MFB (Figures 29-31). Again, the caudate appeared to be more sensitive to various doses of CPZ than hypothalamus. The last Figure (31) shows that previously administered low doses of amphetamine which produce increases in ICSS can be blocked by subsequent administration of 1.0 mg/kg of CPZ. This effect was observed at all brain sites in three animals.

Discussion

The finding that CPZ inhibits ICSS from various positive reinforcing sites is consistent with other reports (Olds, 1958; Olds and Olds, 1964; Stein, 1971). It is interesting that caudate sites were more sensitive to lower doses of CPZ than either hypothalamic or amygdalar ones (Figure 22). The only other report that CPZ blocks ICSS from mostly dopaminergic neurons is that of van Rossum (1970). In his study, ICSS from the pars compacta of the substantia nigra was reduced after administration of CPZ. However, since no dose response curve was presented or any comparison made between DA and NE sites, regional differences in brain for CPZ's action on behavior were not established.

As this research shows, it is important to examine dose-response functions in fairly complete fashion. The finding that DA regions of the brain are more responsive to CPZ than noradrenergic ones at low drug doses would not have been established had just one or two doses been used.

As drug dose was increased, CPZ completely blocked all sites from eliciting ICSS. It is likely that the blocking effects of CPZ on all amine transport mechanisms becomes more apparent at higher doses.

Neurophysiological studies (e.g., Olds, 1973) report similar findings. At 4.0 mg/kg, CPZ suppressed ICSS from the posterior hypothalamus of the rat while also completely abolishing single excitatory unit responses in the anterior hypothalamus. Firing patterns in those units were correlated 100 per cent with ICSS and thus were thought to be related to a "reinforcement pathway" in the hypothalamus. Unfortunately, since other brain regions or the effects of lower drug doses were not examined, the complete action of CPZ was not established.

Since both anatomical and pharmacological data point to an important role for dopamine in many forms of behavior (Randrup, 1967), it seems likely that ICSS elicited from regions high in DA would also be sensitive to drugs such as CPZ or apormorphine which act on DA containing neurons. Brokkamp and van Rossum (1974) found that apomorphine, a proposed direct stimulant of DA receptors, will influence ICSS from DA fibers in the rat. Apomorphine (0.2 mg/kg) facilitated ICSS from dopaminergic cell bodies in the A9-A10 cell group (Crow, 1972). In other animals, however, ICSS elicited from the lateral hypothalamus and locus coeruleus was also modified by the drug. These results would appear to indicate that both DA and NA can influence ICSS. Keats (1973) also reports that dopaminergic regions such as the caudate are responsive to CPZ and may interact with the hypothalamus to influence ICSS. Thus, when CPZ was applied directly to the head of the caudate, ICSS from the lateral hypothalamus was suppressed. The action appeared to be fairly select because when CPZ was applied directly to the cortex, hypothalamic ICSS was unchanged.

There is also pharmacological evidence that points to a strong

dopaminergic-link in CPZ action. In a detailed study of the regional effect of CPZ on catecholamine synthesis and turnover, Nyback and Sedvall (1969) reported that CPZ does act on DA metabolism in select regions of the brain. When 15 mg/kg of CPZ was given, labeled DA in the striatum was accelerated. This effect was not observed in other regions of the brain. Moreover, NE in the striatum or hypothalamus was not affected. Possibly, CPZ may act within the nigrostriatal dopamine pathway where synthesis of transmitter is increased by the blockade of DA receptors. This pathway has also been implicated in ICSS in primates (Routtenberg, 1971).

The duration of action of CPZ effects on ICSS revealed that higher doses (2.0 mg/kg) were longer lasting in caudate or central sites than in hypothalamus or anterior or lateral amygdala. In the rat, Olds (1973) has reported that the duration of action of CPZ on hypothalamic ICSS using similar doses was 24 hours. Old's data are not complete since other brain sites were not sampled and a more complete analysis was not made on duration (e.g., every 12 hours).

The reported differences between brain sites regarding duration of CPZ action have to be related to various receptor binding characteristics or differences of enzyme-receptor reactivation. Both of these actions are related to particular brain sites and transmitter involved.

To summarize this discussion, CPZ appears to have a widespread action of various brain sites that support ICSS. However, dose is an important variable in establishing differences between brain sites. Low doses appear to first block caudate sites while higher doses block all sites from eliciting ICSS.

Both amphetamine and CPZ have wide sites of action and it is unlikely that any one transmitter is solely involved with the effects of these drugs on behavior. Data are needed comparing brain sites that contain mostly NE or DA pathways with drugs that act specifically on these amines. If differences are reported between sites, the relative importance of one or more pathways in the support of ICSS can begin to be established.

Some pilot work to be described in the next chapter attempts to further look at the effect of clonidine on ICSS elicited from caudate or hypothalamus.

CHAPTER V

EXPERIMENT 4: A PILOT STUDY OF THE EFFECT OF CLONIDINE ON CAUDATE OR HYPOTHALAMIC SELF STIMULATION

Material and Methods

Subjects

The Ss used in this experiment were the same animals that had been used in Experiments 1, 2, and 3.

Procedure

A total of six animals was used in this pilot study. Three had positive sites within the caudate and three were within the hypothalamus. Two of these animals had positive sites within both caudate and hypothalamus.

Dose response analysis. Three separate doses of clonidine (0.05, 0.10, and 0.25 mg/kg) were used. The drug was administered orally and order of presentation was random. The remaining analysis was like that described for Experiments 2 and 3.

Histological analysis. Following completion of this final experiment, all animals were sacrificed with an overdose of nembutal. Fifty cc of saline and 50 cc of a ten per cent formalin solution was perfused through the heart. The brain was removed and kept in a ten per

cent solution of formalin for 48 hours. Electrode track verification was made by cutting frozen cross sections (100 micron) of brain tissue. The sections were mounted on slides and stained with cresyl violet acetate. Electrode verification data is presented in the Appendix.

Results

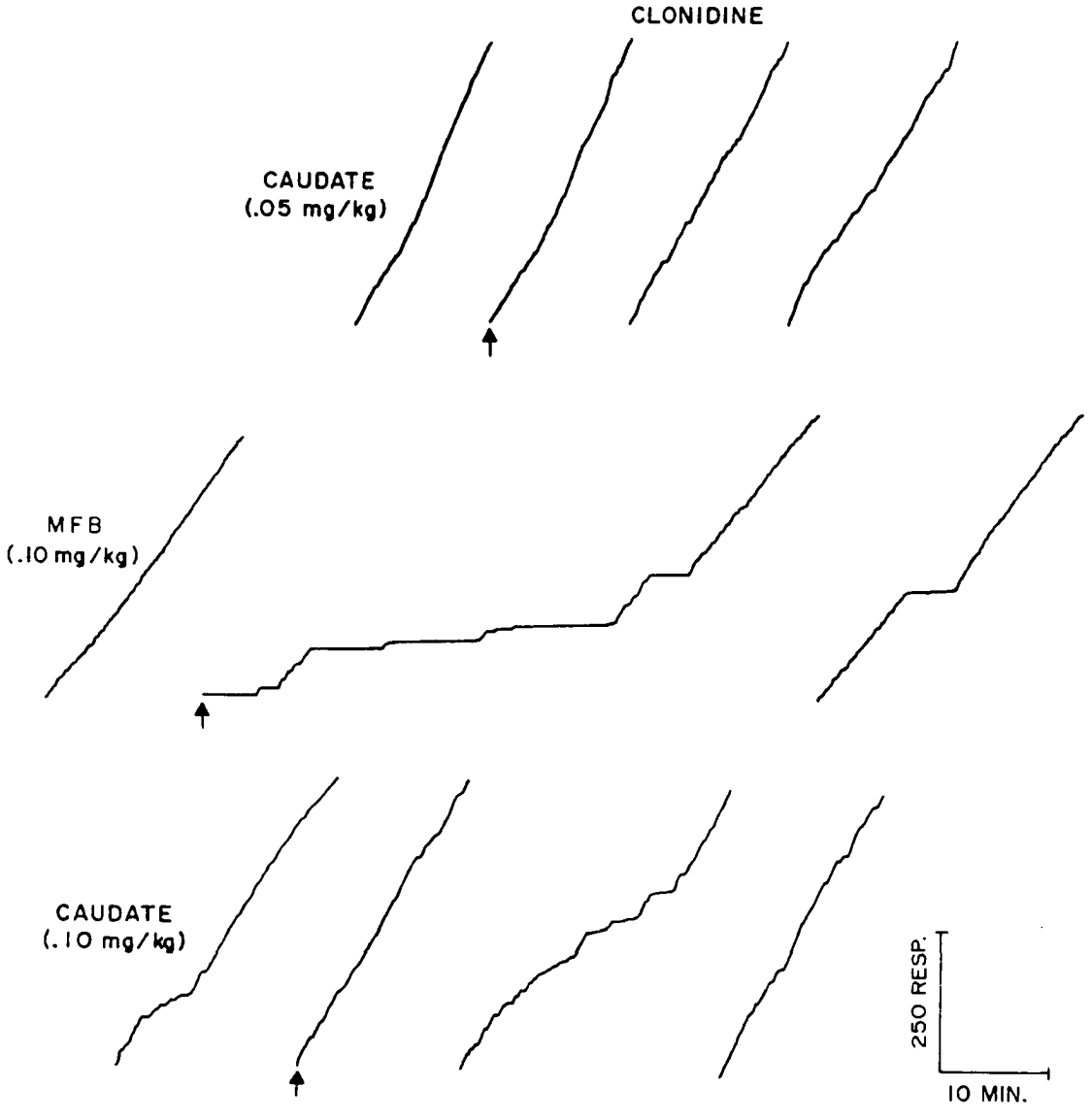
Low oral doses of clonidine (0.05 mg/kg) had no effect on ICSS elicited from either caudate or MFB. Doses of 0.10 mg/kg reduced ICSS from the hypothalamus for about 30 minutes but had much smaller effects on caudate sites (Figure 32). These results were consistent between groups of three animals as well as within two animals when replicated (order counterbalanced). Higher doses (0.25 mg/kg) produced a sedative action usually lasting for 45 minutes. At this dose level both sites were equally effected.

Discussion

Clonidine has been postulated to be much more of a specific acting sedative than the traditional psychoactive drugs, e.g., CPZ. For example, Laverty and Taylor (1969) reported that in rats clonidine was five to seven times more potent than CPZ in prolonging chloral hydrate sleeping time and inhibiting exploratory activity or conditioned avoidance. NE is probably the major transmitter acted on by clonidine since an increase in stored NE, but not DA or 5-HT was observed.

When clonidine is placed directly into the hypothalamus a large reduction in heart rate and lowering of blood pressure occurs (Boudier and van Rossum, 1972). The strongest cardiovascular effects were seen near the MFB which contains a large proportion of NE neurons (Dahlstrom

Figure 32 - Cumulative record of one squirrel monkey self stimulating for electric current to either head of caudate or MFB. Clonidine (0.05 mg/kg) produces no effect on caudate self stimulation. Clonidine (0.10 mg/kg) produces a small reduction on caudate self stimulation but much more of a response inhibition to MFB stimulation. Tip of arrow indicates when drug was given. Maximum excursion of pen is set at 500 responses. Schedule is CRF.


CLONIDINE
CAUDATE
(.05 mg/kg)
MFB
(.10 mg/kg)
CAUDATE
(.10 mg/kg)
250 RESP.
10 MIN.

and Fuxe, 1964). In later work, Broekkam and van Rossum (1972) reported the effect of intrahypothalamically applied clonidine on behavior. The drug induced greater amounts of eating behavior than was observed when NE was applied. Although these authors did not investigate other brain sites in the rat, neither DA nor 5-HT sensitive neurons within the snail brain were activated when clonidine was applied. In general, the literature indicates that the hypothalamus is a major target of clonidine.

The results of this pilot study confirm the hypothesis that the hypothalamus and, possibly NE activation, are more sensitive to lower doses of clonidine than caudate where ICSS is the behavioral measure.

The finding that clonidine has a select dose response effect in hypothalamus while low doses of CPZ have selective effects in the caudate may implicate several amines with ICSS. If more than one major positive reinforcement pathway exists within the brain, it is likely that a great deal of neuroanatomical and neuropharmacological overlap exists too. Stimulation within the hypothalamus may activate severel transmitters but mostly NE. Stimulation in the caudate will produce primarily a release of dopamine and activate a striatal-nigro pathway. Both pathways project to the cortex where they have their own respective synapses with NE or DA containing neurons (Thierry et al., 1973).

It is proposed that low doses of clonidine act first on NE synapses while low doses of CPZ first act on DA synapses to influence ICSS. Increasing doses of either drug completely block ICSS, probably through mutual interaction of several pathways and brain sites.

CHAPTER VI

SUMMARY

A series of experiments was conducted on twelve squirrel monkeys investigating similarities and differences between brain sites that support intracranial self stimulation (ICSS) but differ in their neuroanatomical connections and neurochemistry. Electrodes were implanted at: a) head of caudate nucleus; b) medial forebrain bundle (MFB) lateral hypothalamus; and c) anterior, lateral, and central amygdaloid nuclei.

High and consistant rates of ICSS were obtained from both the head of caudate and MFB.

Rates of ICSS from anterior and lateral amygdala were most sensitive to manipulation of train durations and stimulus intensities. In the anterior amygdala, increases in train duration from 50 to 500 msec elicited certain autonomic components (salivation, eye blinking, and head twitching) and produced either a decrease or complete inhibition in lever pulls (response). In contrast to the anterior amygdala, within the lateral amygdala, rate of ICSS increased monotonically with increases in train duration and there were no signs of autonomic involvement. In a two-lever choice test, Ss with electrodes in the lateral amygdala preferred longer train durations whereas Ss with electrodes in the anterior amygdala demonstrated no preference. However, as train duration

increased, rate decreased. Hippocampal or septal electroencephalographic tracings did not indicate seizure activity during stimulation of either amygdala site.

In the next phase of the dissertation, two major psychoactive drugs, amphetamine and chlorpromazine (CPZ) were each orally administered on different occasions to examine their effects on ICSS elicited from caudate, hypothalamus or amygdalar sites.

In separate groups of animals, a low dose of amphetamine (0.5 mg/kg) increased ICSS 180 to 200 per cent and decreased threshold for responding by 50 per cent at all sites. A moderate dose (1.0 mg/kg) produced excitation and inhibition in response rate from the caudate and central amygdala but just excitation from the MFB, anterior or lateral amygdala. Higher doses of amphetamine (2.0 mg/kg) produced complete response inhibition at all sites. Analysis of duration of action revealed that caudate ICSS did not return to baseline for up to 84 hours following the 10 mg/kg dose. Rate of ICSS from other sites returned to baseline by 24 hours at this dose.

CPZ (0.5 mg/kg) blocked caudate ICSS (-54 per cent) whereas lateral hypothalamic sites were unaffected (-8 per cent). Doses of 1.0 and 2.0 mg/kg increasingly blocked all sites from eliciting ICSS. Analysis of duration of action again showed that with a higher dose of CPZ (2.0 mg/kg), caudate ICSS did not return to baseline until 36 hours, whereas hypothalamic ICSS returned by 24 hours. These differences were observed both between groups and within three Ss.

The effects of clonidine, a drug which stimulates central norepinephrine (NE) receptors without influencing dopamine (DA) or

serotonin (5-HT) receptors, were then investigated. A dose of 0.10 mg/kg reduced MFB ICSS (-55 per cent) while caudate ICSS was not changed (-0.10 per cent). Higher doses (0.25 mg/kg) blocked both sites (-85 per cent).

These findings suggest: 1) consistent rates of ICSS can be obtained from broad regions of the squirrel monkey brain, and rate of ICSS from certain amygdalar nuclei may be influenced by the elicitation of other underlying responses; 2) the dose effects of amphetamine and CPZ on ICSS are not only observed within the hypothalamus but are extensive throughout the brain involving telencephalic as well as diencephalic structures; and 3) since hypothalamus but not caudate ICSS can be blocked by drugs which have select sites of action, other pathways besides the MFB may also support ICSS.

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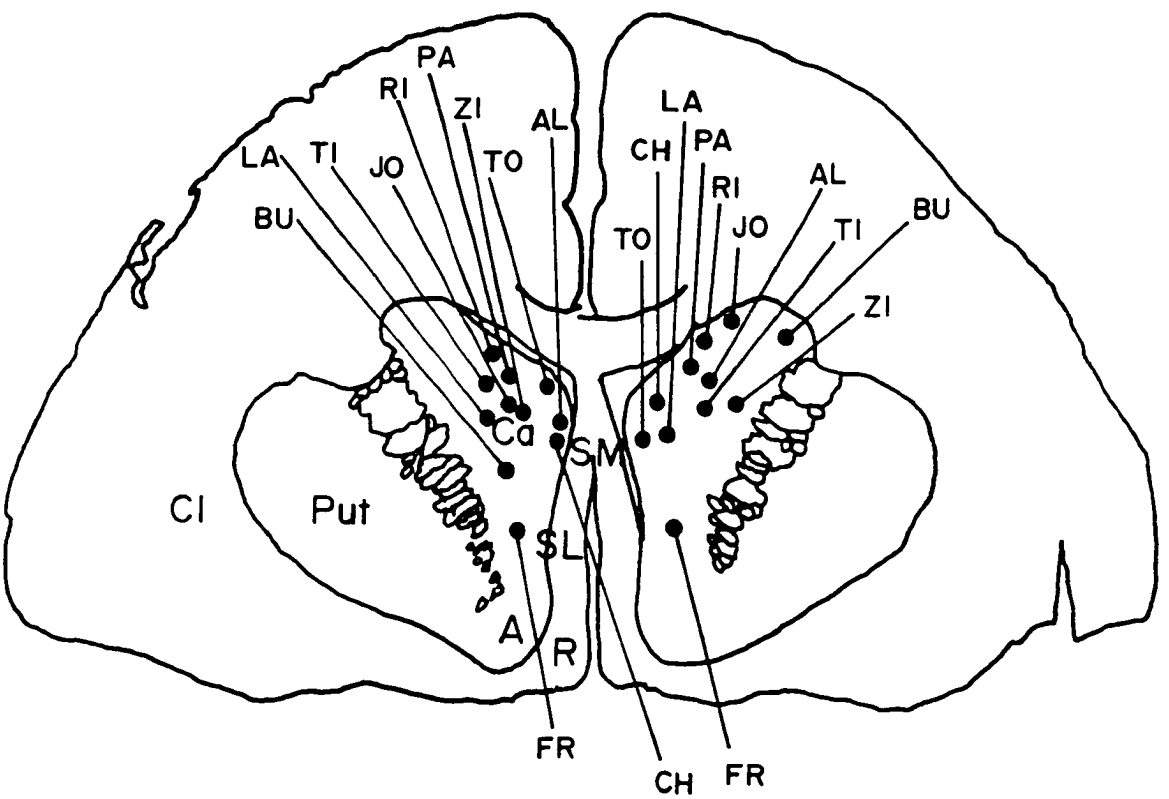
APPENDIX

Histology Plates

Figure 33 - Final placement of electrodes in head of Caudate for eleven squirrel monkeys (AP=15.0). Abbreviations: (+) positive with respect to ICSS; (0) neutral; (-) aversive. Zi-Zip (+); Bu-Buttons (+); Ti-Tippy (+); Al-Alvin (+); Jo-Joco (+); Ri-Ringo (+); Pa-Paul (+); La-Larry (+); Ch-Chico (+); To-Torius (+); Fr-Fred (+); Ca-caudate nucleus; Put-putamen; CI-internal capsule; ci-cingulate gyrus; SM-medial septum; SL-lateral septum; A-accumbens nuclei; R-gyrus rectus.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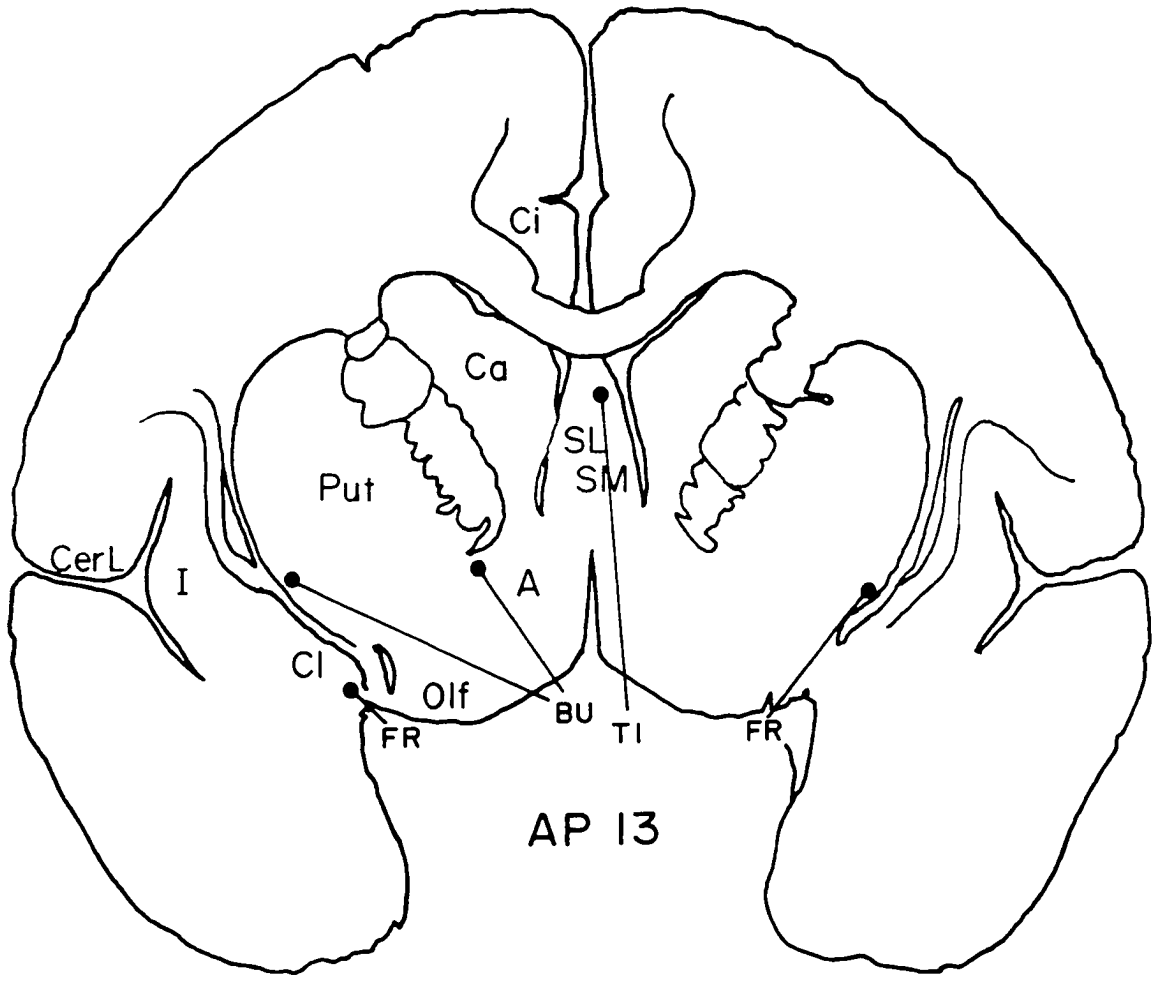
AP 15

Figure 34 - Final placement of electrodes for three squirrel monkeys (AP=13.0). Abbreviations: (+) positive with respect to ICSS; (0) neutral; (-) aversive. Fr-Fred (0); Bu-Buttons (0); Ti-Tippy (0); Ca-caudate nucleus; Put-putamen; CI-internal capsule; ci-cingulate gyrus; SM-medial septum; SL-lateral septum; Olf-area olfactoria; CerL-lateral fissure; I-insula.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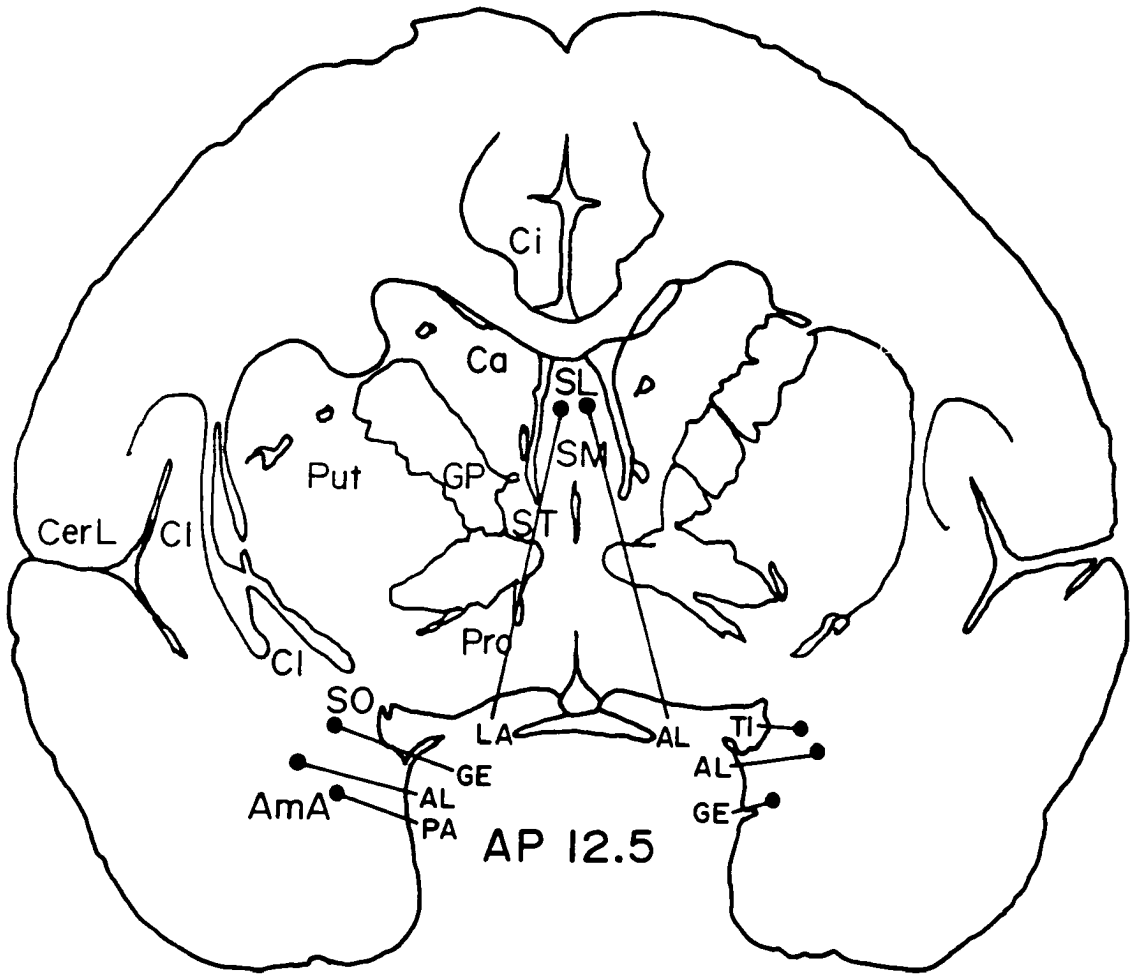
AP 13

Figure 35 - Final placement of electrodes for five squirrel monkeys (AP=12.5). Abbreviations: (+) positive with respect to ICSS; (0) neutral; (-) aversive. Pa-Paul (+); Al-Alvin (+); Ge-George (+); La-Larry (0); Ti-Tippy (+); Ca-caudate nucleus; Put-putamen; GP-globius pallidus; ST-Striae terminalis; SL-lateral septum; SM-medial septum; Pro-preoptic area; SO-striae olfactory nucleus; AmA-anterior amygdaloid area; CI-internal capsule; CerL-lateral fissure; Ci-cingulate gyrus.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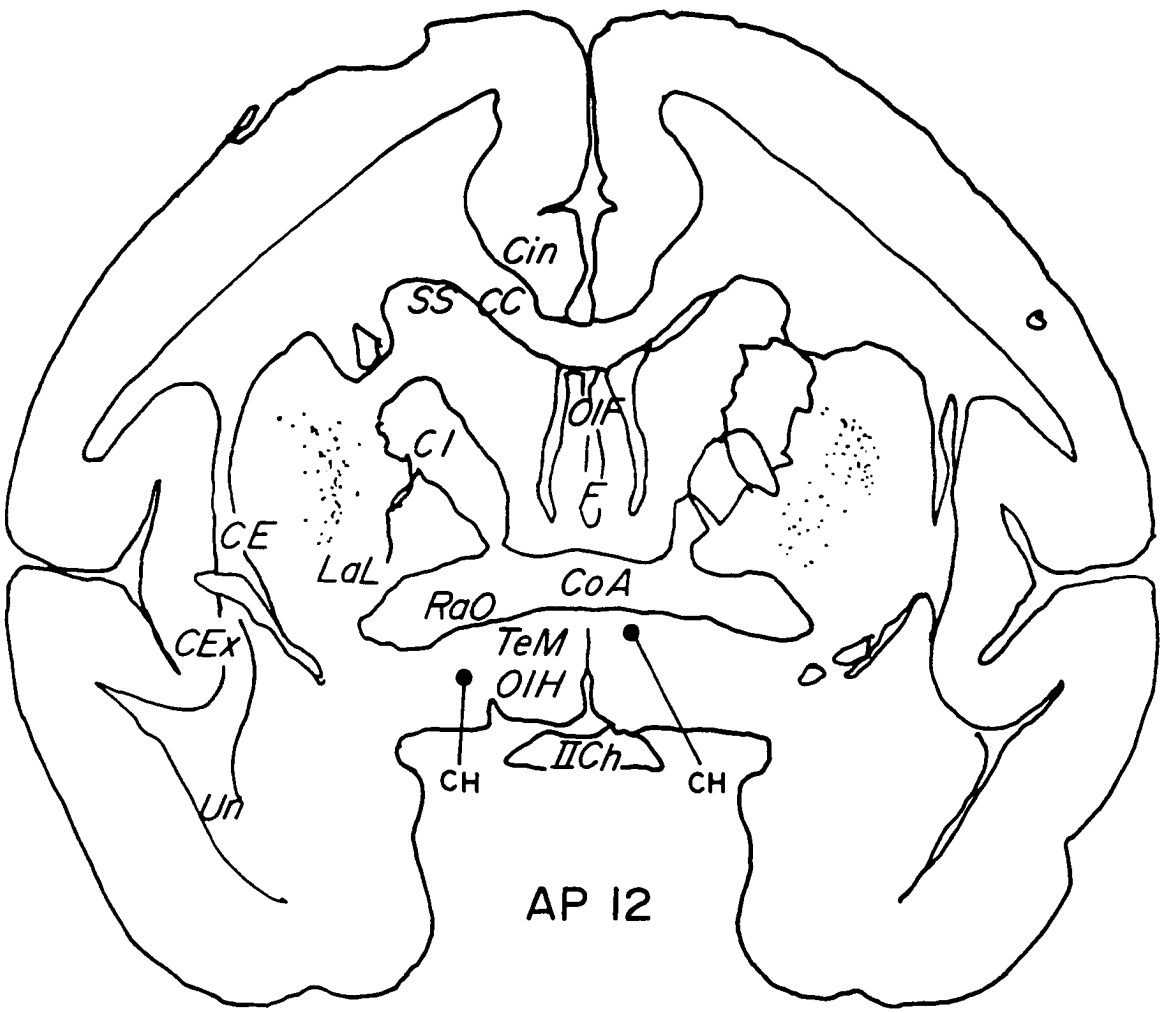
AL
PA
GE
AP 12.5

Figure 36 - Final placement of electrodes for one squirrel monkey (AP=12.0). Abbreviations: (+) positive with respect to ICSS; Ch-Chica (+); Cin-cingulum; CC-corpus calloaum; SS-stratum subcallosum; F-fornis; CoA-anterior commissure; RaO-olfactory radius; TeM-fasciculus telencephalic medialia (median forebrain bundle); DlH-diagonal band of Broca; CE-external capsule; CEx-extreme capsule; LaL-lateral medullary lamina; Un-uncinate fasciculus.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CI
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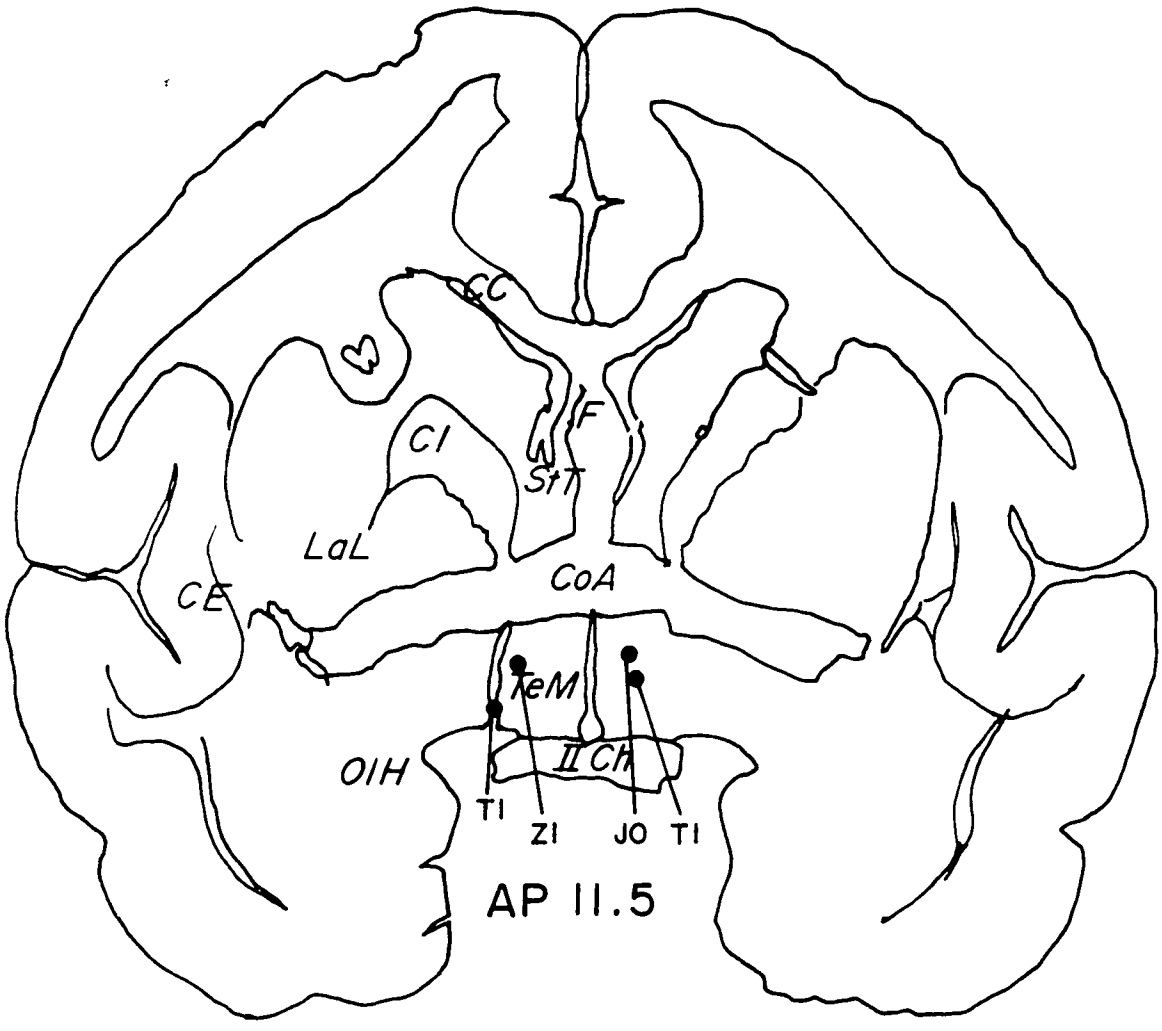
AP 12

Figure 37 - Final placement of electrodes for three squirrel monkeys (AP=11.5). Abbreviations: (+) positive with respect to ICSS; Ti-Tippy (+); Zi-Zip (+); Jo-Joco (+); CC-corpus callosum; F-fornix; CoA-anterior commisure; StT-stria terminalis; TeM-fasciculus telencephalic medialis (MFB); CI-Internal capsule; LaL-lateral medullary lamina; CE-external capsule; DlH-diagonal band of Broca.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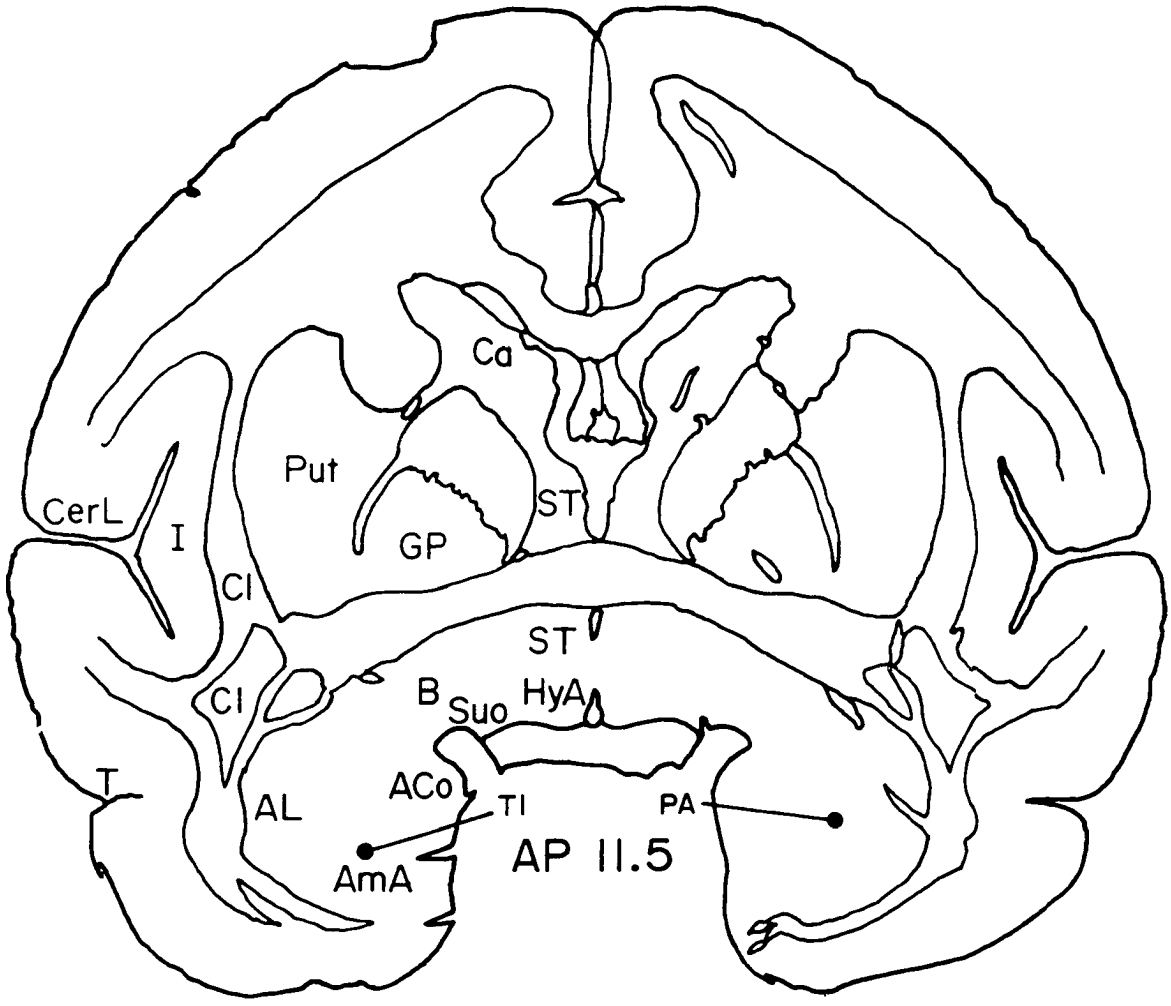
AP 11.5

Figure 38 - Final placement of electrodes for two squirrel monkeys (AP=11.5). Abbreviations: (+) with respect to ICSS; Ti-Tippy (+); Pa-Paul (+); Ca-caudate nucleus; ST-striae terminalis nucleus; CP-globus pallidus; Put-putamen; Cl-claustrum; I-insula; CerL-lateral fissure; AL-lateral amygdaloid nucleus; AMA-anterior amygdaloid nucleus; ACo-cortical amygdaloid nucleus; T-temporalis fissure; B-basal nucleus; Suo-supraoptic nucleus; HyA-anterior hypothalamus.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TI
PA
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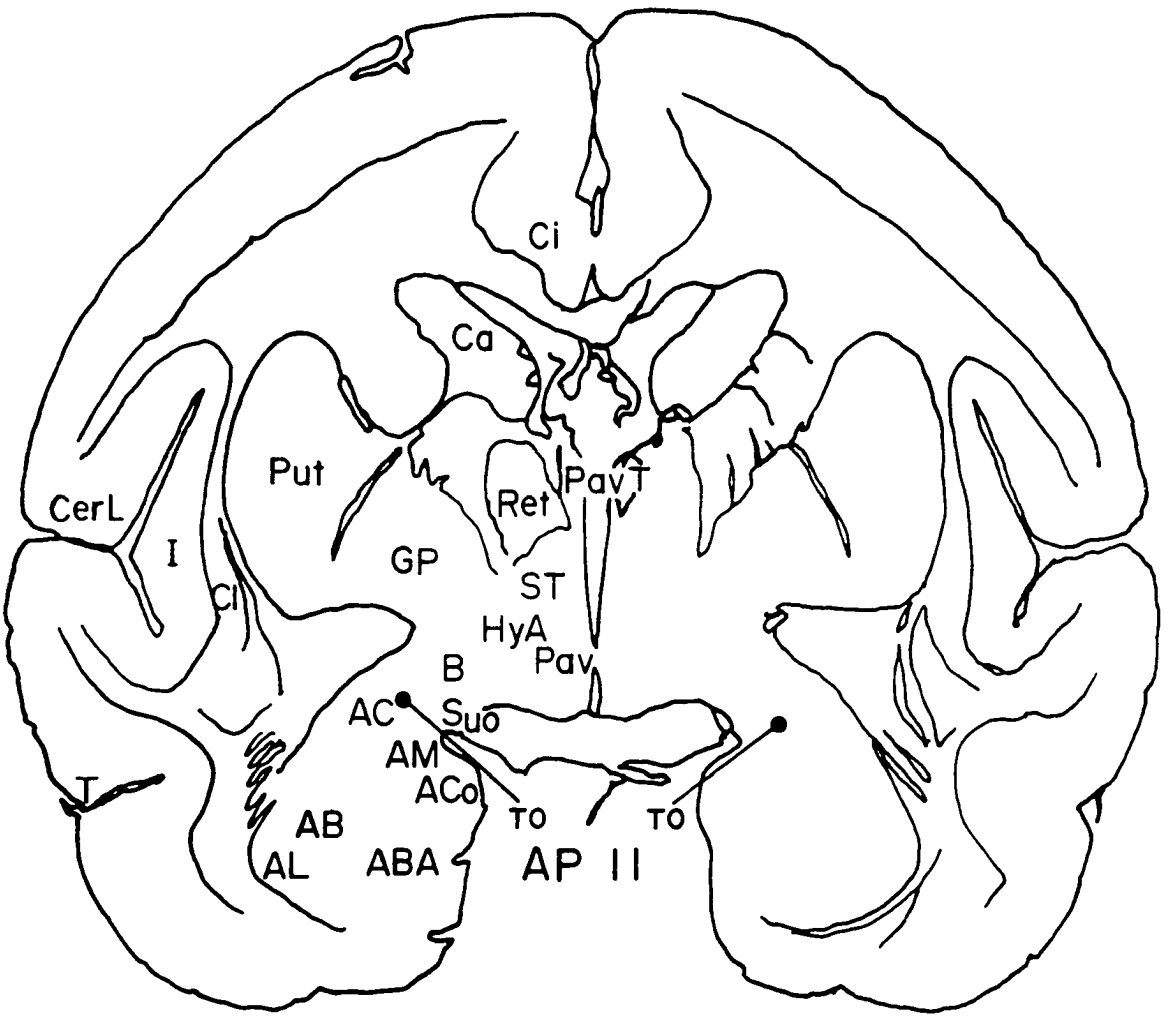
AP 11.5

Figure 39 - Final placement of electrodes for one squirrel monkey (AP=11.0). Abbreviations: (+) positive with respect to ICSS; To-Torius (+); Ci-cingulate gyrus; Ca-caudate nucleus; Ret-reticular nucleus; PavT-paraventricular nucleus (thalamus); CerL-lateral fissure; I-insula; Cl-claustrum; Put-putaman; GP-globius pallidus; ST-striae terminalis nucleus; HyA-anterior hypothalamus; Pav-paraventricular nucleus; B-basal nucleus; Suo-supraoptic nucleus; AB-basal amygdaloid nucleus; ABA-Basal accessory amygdaloid nucleus; AM-medial amygdaloid nucleus; AL-lateral amygdaloid nucleus; AC-central amygdaloid nucleus; T-temporalis fissure.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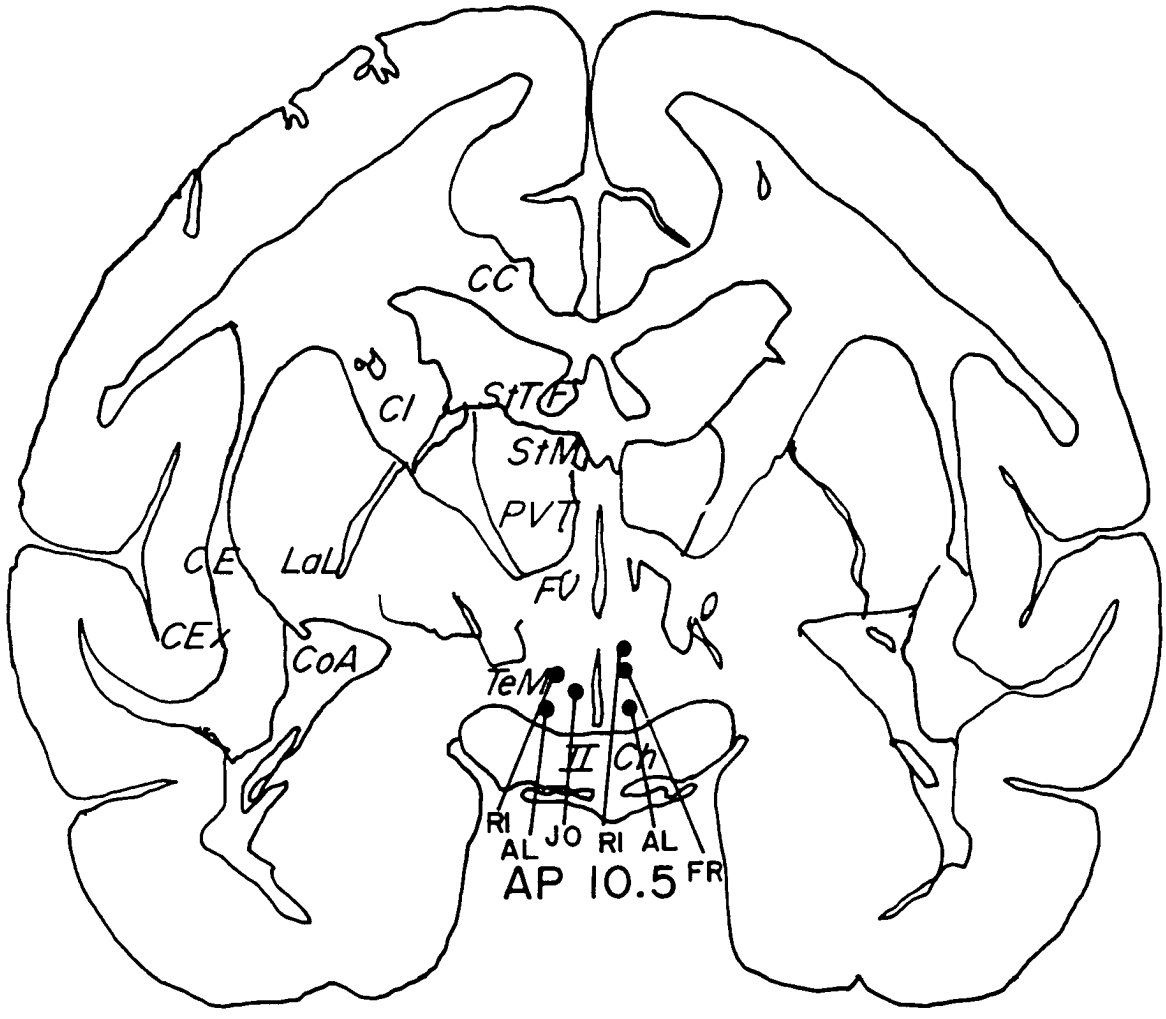
AP 11

Figure 40 - Final placement of electrodes for four squirrel monkeys (AP=10.5). Abbreviations: (+) positive with respect to ICSS; (-) aversive. Ri-Ringo (-); Al-Alvin (+); Jo-Joco (+); Fr-Fred (+); CC-corpus callosum; StT-stria terminals; F-fornix Cl-claustrum; PVT-ventral peduncle thalamus; CE-external capsule; CEx-extreme capsule; LaL-lateral medullary lamina; CoA-anterior commisure; TeM-fasciculus telencephalic; IICh-optic chiasma; StM-Striae medialis (median forebrain bundle) terminalis medialis.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AP 10.5

Figure 41 - Final placement of electrodes for one squirrel monkey (AP=10.0). Abbreviations: (+) positive with respect to ICSS; To-Torius (+); Ci-cingulum; Ca-caudate nucleus; Ret-reticular nucleus; PavT-paraventricular thalami nucleus; VA-ventral anterior nucleus (thalamus); Put-putamen; GP-globius pallidus; Pav-paraventricular nucleus; HyL-lateral hypothalamus; CerL-lateral fissure; I-insula; AC-central amygdaloid nucleus; AM-medial amygdaloid nucleus; AB-basal amygdaloid nucleus; ABA-basal accessory amygdaloid nucleus; Aco-cortical amygdaloid nucleus.

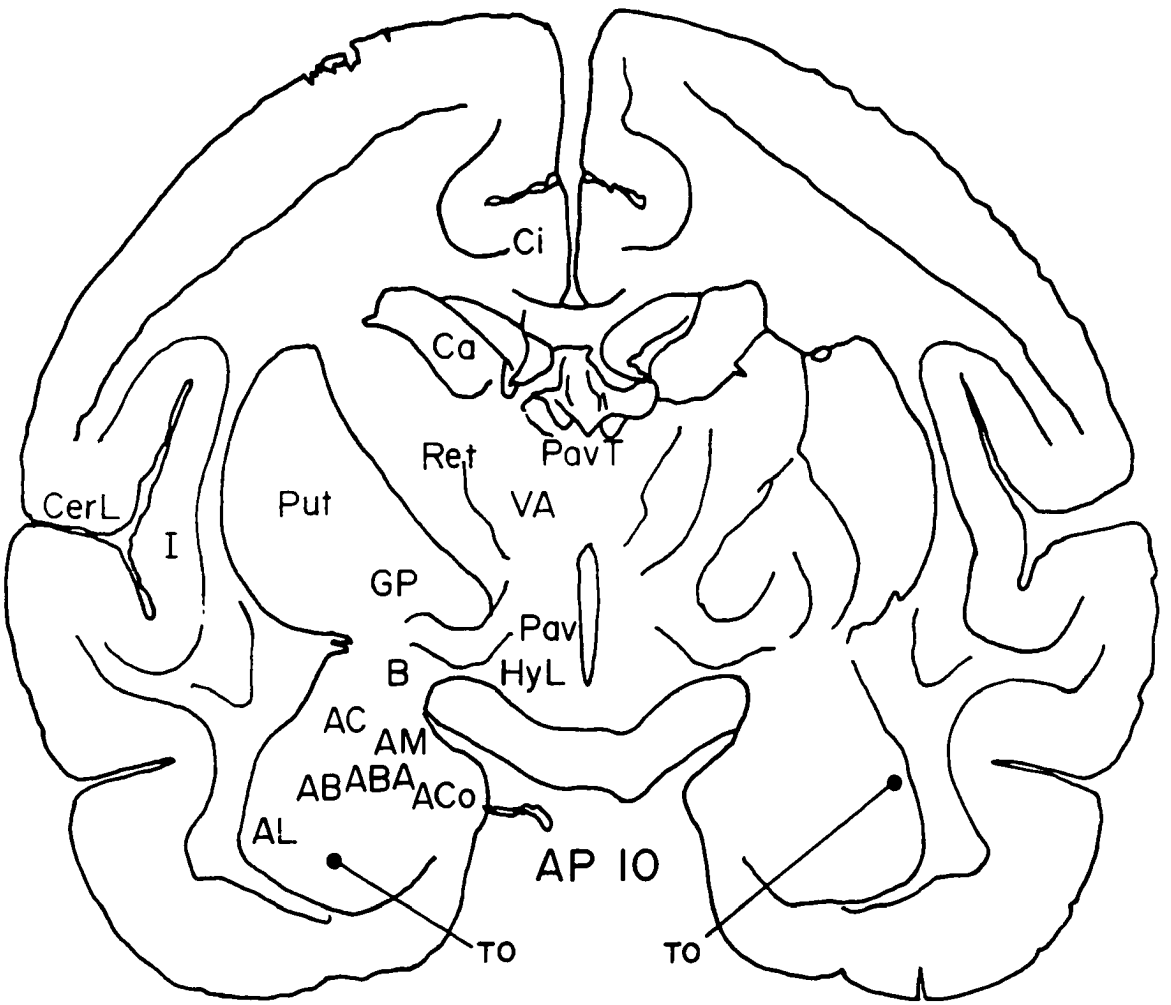
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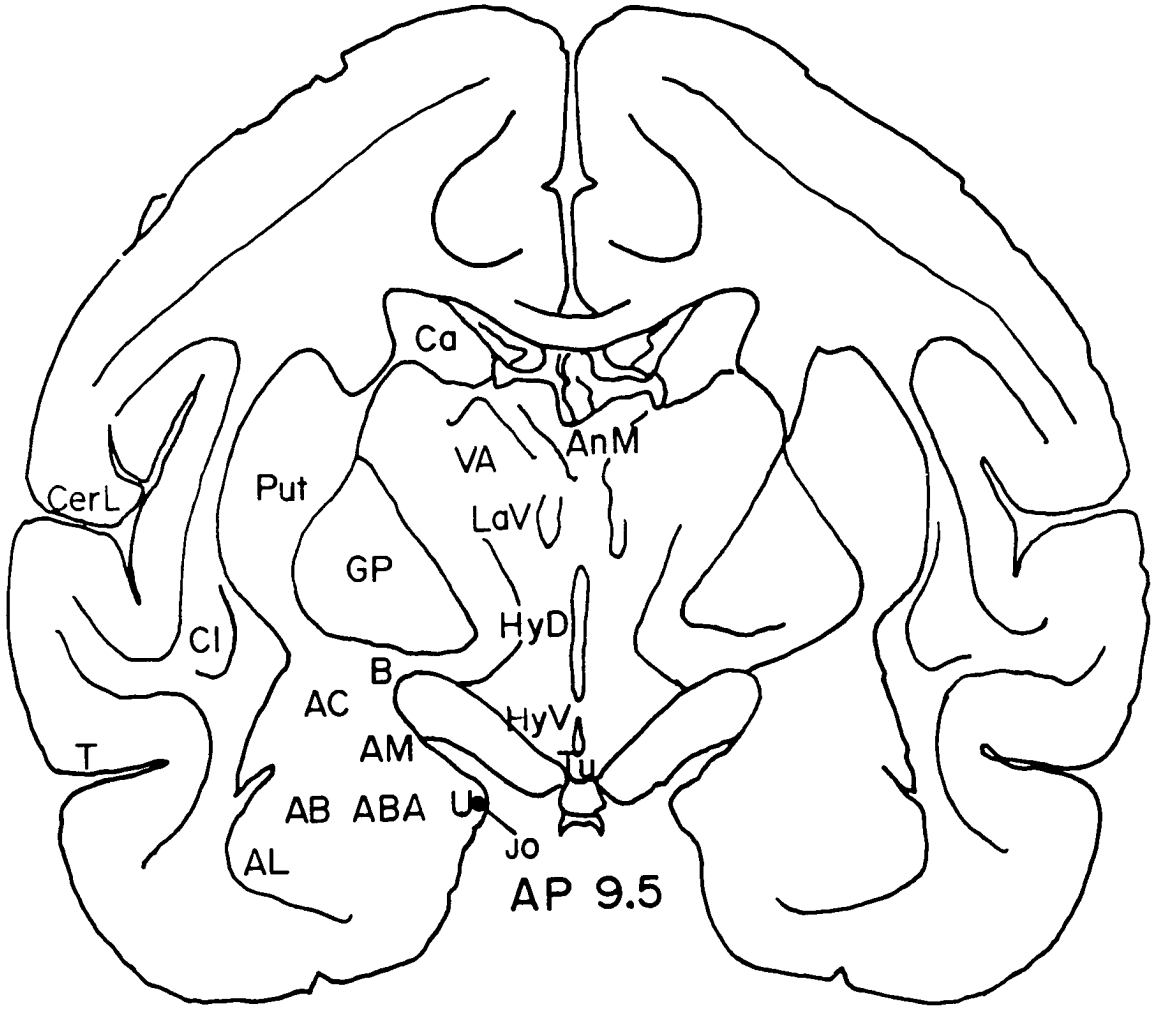
Figure 42 - Final placement of electrodes for one squirrel monkey (AP=9.5). Abbreviations: (0) nueutral with respect to ICSS; Jo-Joco (0); Ca-caudate nucleus; VA-ventral anterior nucleus; AnM-anterior medial nucleus (thalamus); LaV-Ventral lateral nucleus (thalamus); CerL-lateral fissure; Put-putamen; GP-globus pallidus; HyD-Dorsal hypothalamus; HyV-ventral hypothalamus; Tu-pars tuberalis (hypothalamus); T-temporalis fissure; Cl-claustrum; B-basal nucleus; AC-central amygdaloid nucleus; ABA-basal accessory amygdaloid nucleus; AL-lateral amygdaloid nucleus; U-uncinate gyrus.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AP 9.5

Figure 43 - Final placement of electrodes for two squirrel monkeys (AP=9.0). Abbreviations: (+) positive with respect to ICSS; (0) neutral with respect to ICSS; Jo-Joco (+); Ch-Chico (0); Ci-cingulum; Ca-caudate nucleus; AnV-anterior ventral nucleus (thalamus); Pat-para ataeniales nucleus (thalamus); AnM-anterior medial nucleus (thalamus); Pac-paracentral nucleus; Ret-reticula nucleus; LaV-ventral lateral nucleus (thalamus); Put-putamen; GP-globius pallidus; HyD-dorsal hypothalamus; HyL-Lateral hypothalamus; HyV-ventral hypothalamus; Tu-pars tuberalis (hypothalamus); Cl-claustrum; B-basal nucleus; Aco-cortical amygdaloid nucleus; AL-lateral amygdaloid nucleus; AB-basal amygdaloid nucleus; U-uncinate gyrus.

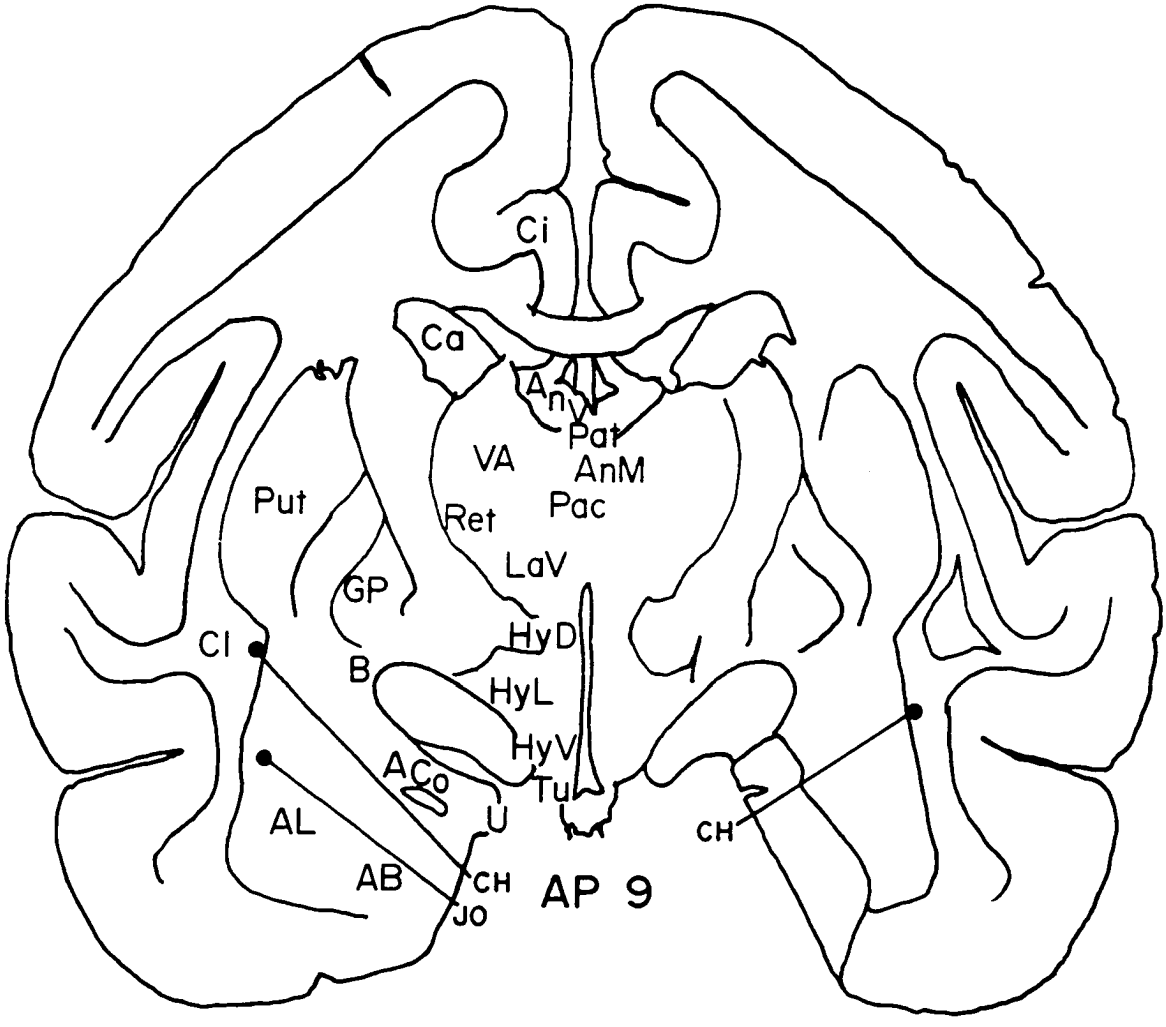
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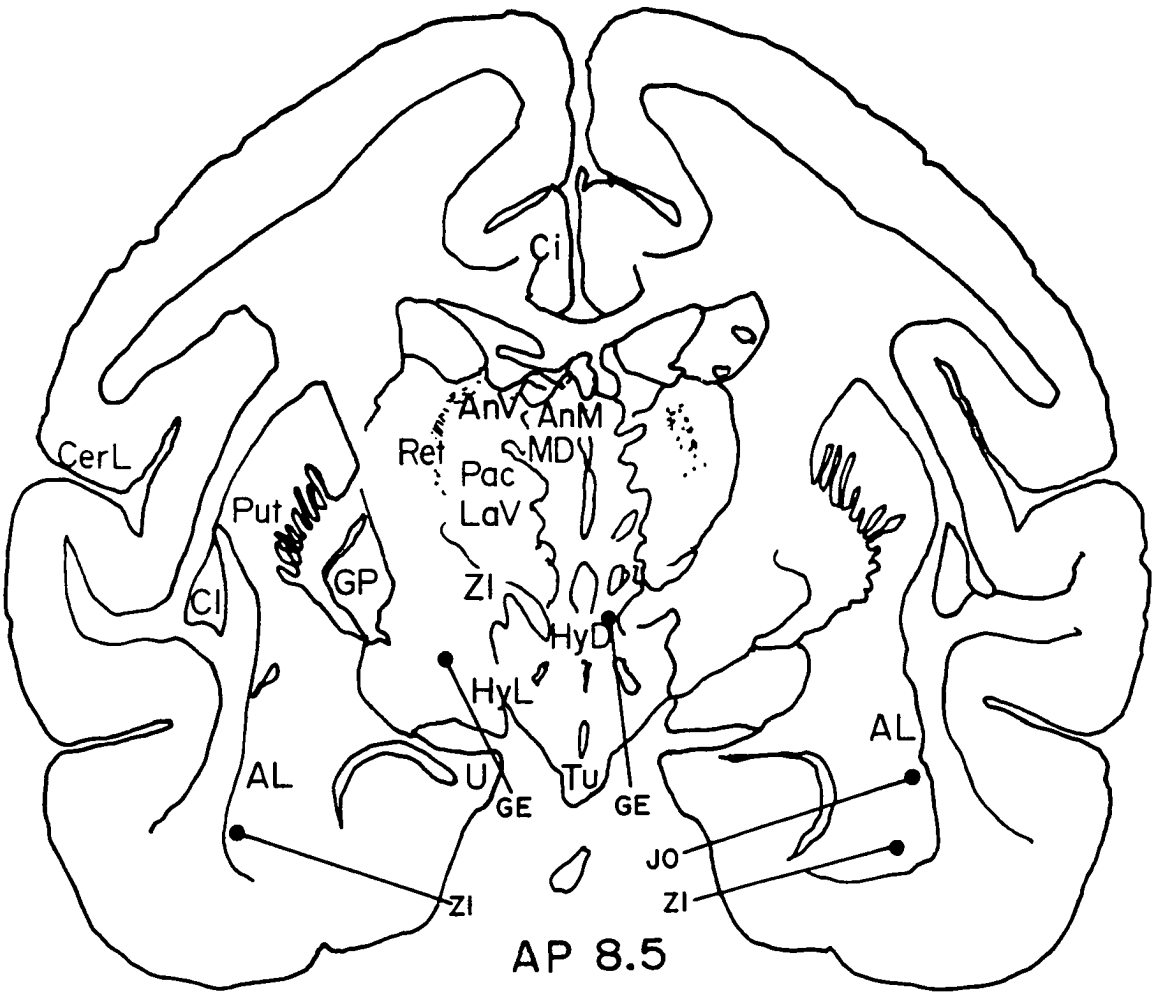
Figure 44 - Final placement of electrodes for three squirrel monkeys (AP=8.5). Abbreviations: (+) positive with respect to ICSS; Zi-Zip (+); GE-George (+); Jo-Joco (+); Ci-cingulum, AnV-anterior ventral nucleus (thalamus); AnM-anterior medial nucleus (thalamus; MD-medial dorsal nucleus (thalamus); Pac-paracentral nucleus (thalamus); LaV-ventral lateral nucleus (thalamus); ZI-zona incerta; HyD-dorsal hypothalamus; HyL-lateral hypothalamus; Tu-pars tuberalis (hypothalamus); CerL-lateral fissure; Put-putamen; GP-globius pallidus; Cl-claustrum; U-uncinate gyrus.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AP 8.5

Figure 45 - Final placement of electrodes for two squirrel monkeys (AP=8.0). Abbreviations: (+) positive with respect to ICSS; Bu-Buttons (0); La-Larry (+); Ci-cingulum; AnD-anterior dorsal nucleus (thalamus); AnV-anterior ventral nucleus (thalamus); MD-medial dorsal nucleus (thalamus); Pac-paracentral nucleus (thalamus); LaV-ventral lateral nucleus (thalamus); Ret-reticular nucleus; VPL-ventral posterior lateral nucleus (thalamus); Put-putamen; HyD-dorsal hypothalamus; HyL-lateral hypothalamus; TU-pars tuberalis (hypothalamus); Hip-hippocampus.

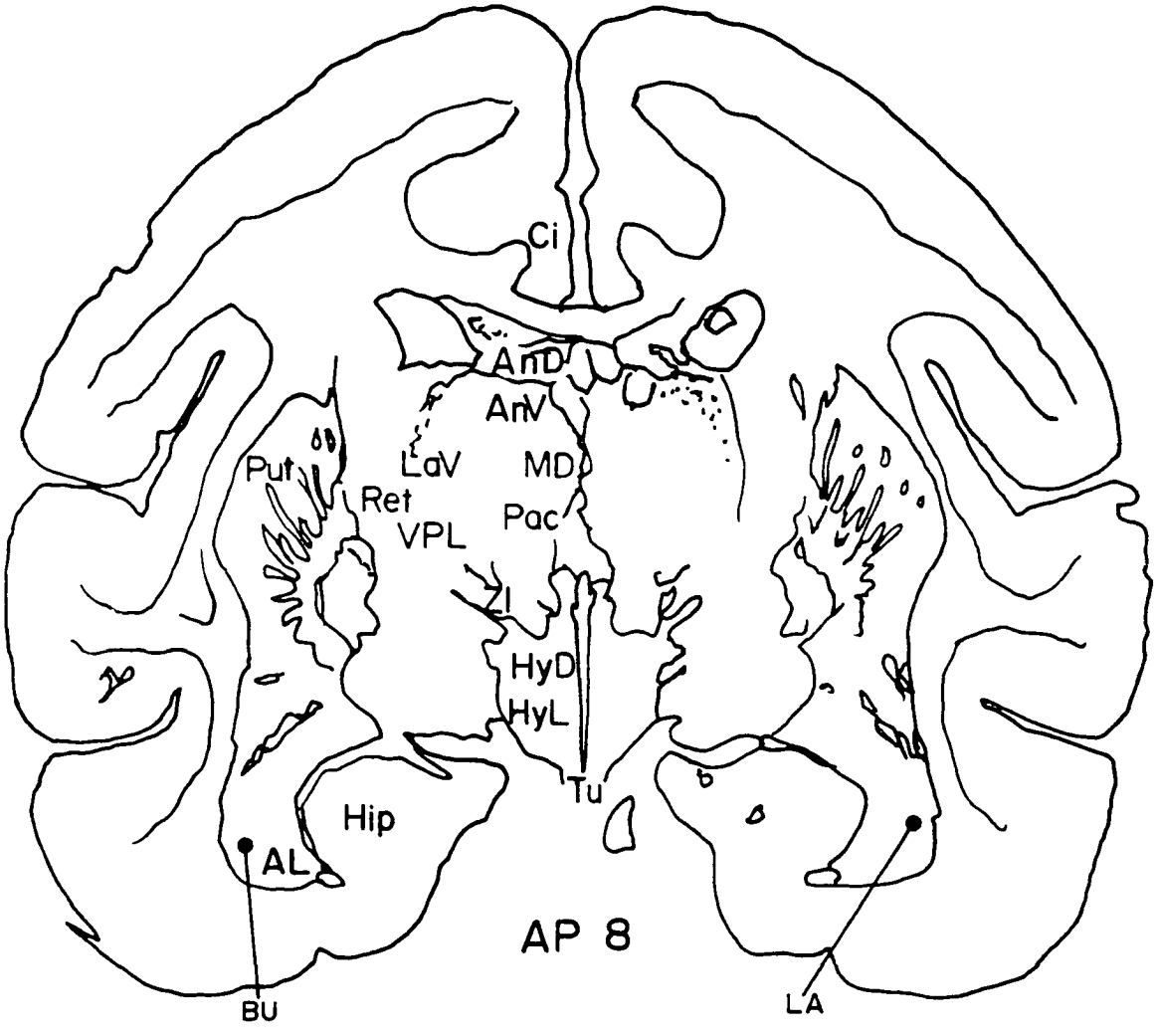
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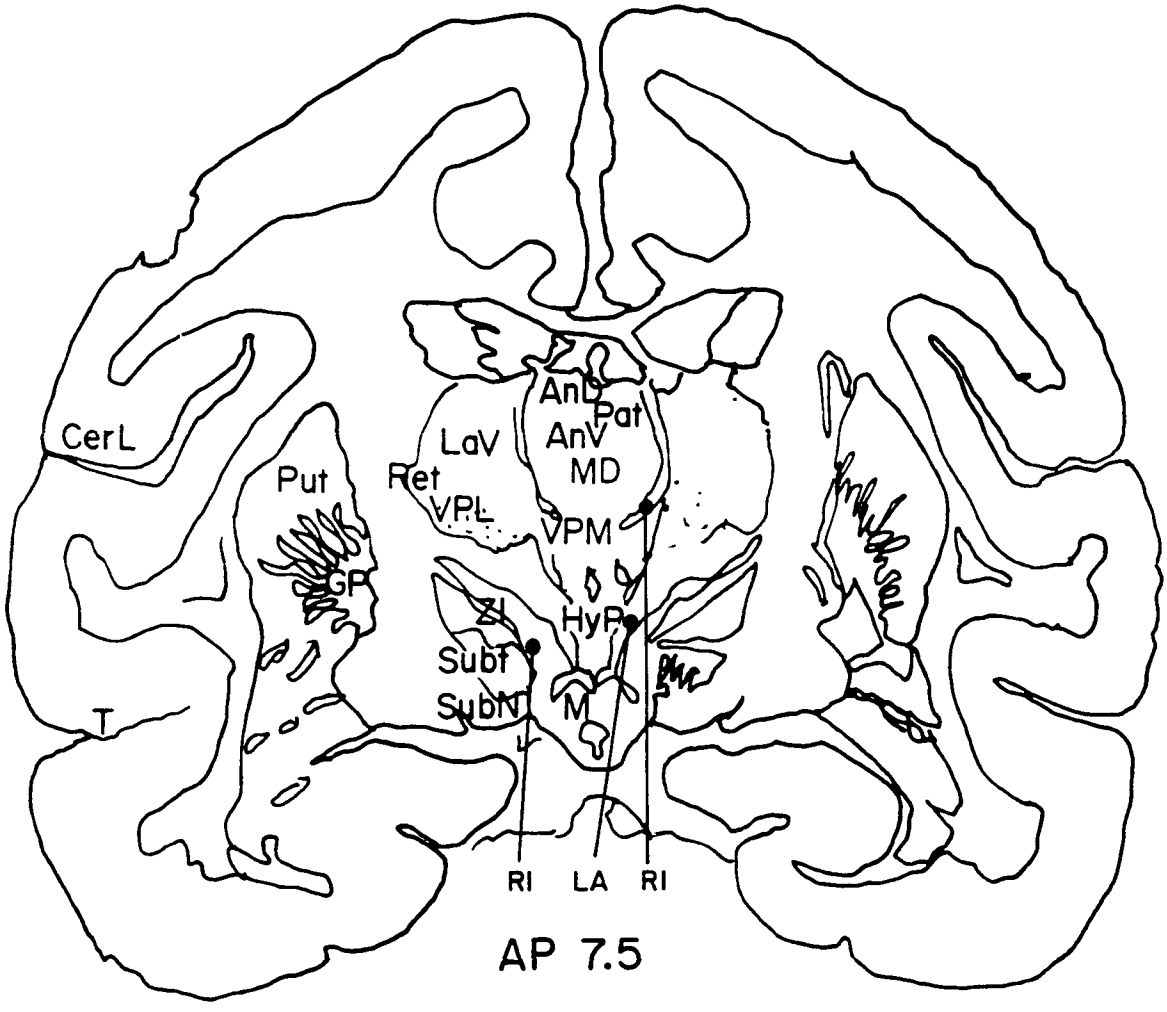
Figure 46 - Final placement of electrodes for two squirrel monkeys (AP=7.5). Abbreviations: (-) aversive; RI-Ringo (-); La-Larry (-); AnD-anterior dorsal nucleus (thalamus); Pat-para ataeniales nucleus (thalamus); AnV-anterior ventral nucleus (thalamus); MD-medial dorsal nucleus (thalamus); LaV-ventral lateral nucleus (thalamus); Ret-reticular nucleus; VPL-ventral posterior lateral nucleus (thalamus); VPM-ventral posterior medial nucleus (thalamus); HyP-posterior hypothalamus; M-Corpus mammillary; ZI-zona incerta; Subt-corpus subthalamicum; SubN-substantia nigra; CerL-lateral fissure; Put-putamen; GP-globius pallidus; T-temporalis fissure.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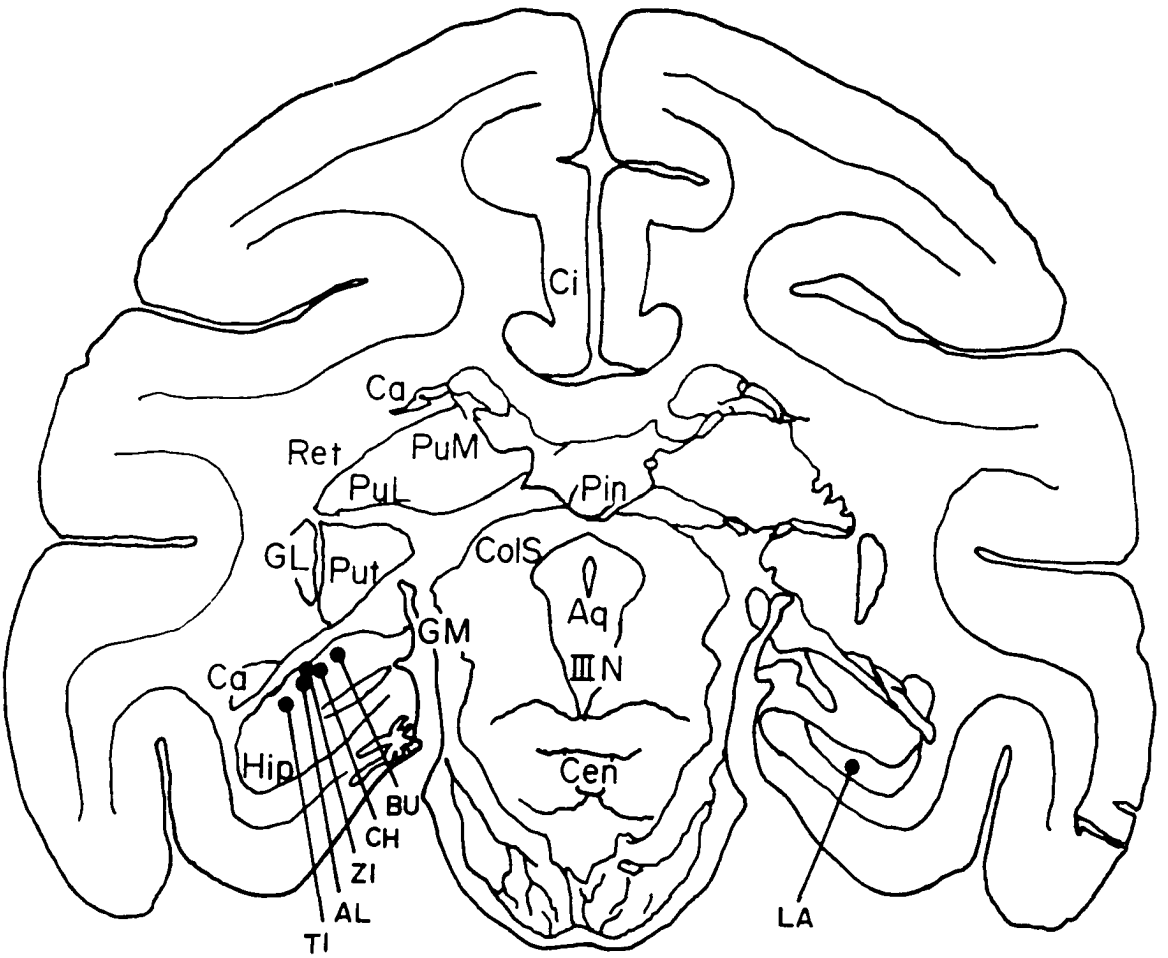
AP 7.5

Figure 47 - Final placement of electrodes for six squirrel monkeys (AP=2.0). Abbreviations: (0) neutral with respect to ICSS; Ti-Tippy (0); Al-Alvin (0); Zi-Zip (0); Ch-Chico (0); Bu-Buttons (0); La-Larry (0); Ci-cingulum; Ca-caudate nucleus; PuM-medial pulvinar nucleus; PuL-lateral pulvinar nucleus; Pin-corpus pineale; GL-lateral geniculate; Put-putamen; ColS-superior colliculus; Aq-anulus aquaeductus; IIIN-occulomotor nerve; Cen-central superior nucleus; GM-medial geniculate; Hip-hippocampus.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AP 2.0