THE EFFECT OF KINDLING DIFFERENT NUCLEI IN THE LEFT AND RIGHT AMYGDALA ON ANXIETY IN THE RAT

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by

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Abstract

The effects on rodent anxiety of kindling in the medial, lateral and central amygdaloid nuclei were measured using the hole board and elevated plus maze tests. Kindling has been suggested to model complex partial epilepsy with secondary generalization in humans. Kindling permanently increases the epileptic response of an animal to intracranial stimulation by repeated adminstration of high frequency electrical stimulation. The animals were kindled in medial or lateral amygdalas, of the left and right hemispheres, or in the right hemisphere Central amygdala. Controls had electrodes implanted but were not kindled. Post experimental analysis of electrode location showed that some of the animals were kindled in none of the above nuclei. These animals were labelled 'Outliers'. Kindling of the Medial/Lateral amygdala in the left hemisphere decreased anxiety in the elevated plus maze for at least one week after the last kindled seizure. Right hemisphere Medial/Lateral kindling did not affect anxiety significantly, though there was a trend toward an anxiogenic effect. The 'Outlier' kindled rats were less anxious than their controls regardless of hemisphere one week after their last kindled seizure. Central amygdala Kindled animals did not differ from their controls. Previous findings suggest that kindling of

specific loci in the right hemisphere may be anxiogenic. Clear anxiogenic effects were likely not seen in the right hemisphere in this study because of electrode locations. Correlations between anxiety and electrode location further pointed to the importance of kindled focus in the amygdala for behavioral effect. Future research should carefully control the location of kindled foci when investigating effects of amygdala kindling on anxiety and other behaviors.

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Epilepsy is a disorder which affects some 2 million North Americans (National Commission for the Control of Epilepsy, 1978). About 30 % of epileptics seem to experience more psychological problems than those without epilepsy (Adamec, 1990a; Dodrill and Batzel, 1986).

Recent investigations of human epileptics and of animal models of epilepsy (see Strauss, 1989, and Adamec, 1990 for thorough reviews) suggest that seizures with particular foci in the brain induce changes in emotional behavior. These behaviors can range from pleasure to depression.

One animal model of complex partial seizures of subcortical (limbic system) origin is amygdala kindling. Kindling of the amygdala and of pathways which lead to spread of seizures to the amygdala have been shown to produce lasting changes in animal anxiety (Adamec, 1990b; Adamec, 1991a,b). Anxiety has also been identified as one of the more common affective disturbances in epileptics (Hermann and Whitman, 1984). Recent data suggest that epileptics with seizures involving the limbic system may be particularly prone to anxiety (Adamec, 1990a). Most of this work has been done in the domestic cat, though a few studies have investigated affective change in rodents following kindling.

In the following sections the relationship between epilepsy and emotional disturbance in humans will be reviewed.

In addition, animal models of limbic system epilepsy including kindling will be reviewed. These will be discussed within the context of their suitability as models of human affective disturbance associated with epilepsy. The review will consider data from kindling studies in rats and cats, and the effect of kindling on anxiety. The importance of different amygdala nuclei to anxiety will also be addressed. Finally it will be suggested that more information is required on the location of the amygdala focus, duration of behavioral effects and the expected behavioral outcome.

Epilepsy and Psychopathology and Emotional Disturbance

Epilepsy was formerly thought to be associated with a variety of psychopathologies (Tizard, 1962). However, it was Gibbs and Gibbs (1964) who first demonstrated empirically that individuals whose epileptic seizures began in the temporal lobes had a significantly higher incidence of psychopathology relative to patients with other foci or with generalized seizure disorder. Emotional disturbances associated with epilepsy may occur either between seizures (interictal disturbances) or during a seizure (ictal disturbances). Ictal emotions (or related behaviors) displayed by epileptics can include pleasure, laughter, depression, crying, aggression, sexual behavior, and fear (Strauss, 1989). Fear is the most common ictal emotion and is experienced by about 3% of

epileptic patients. Ictal feelings of depression are somewhat less common, being reported by about 1% of epileptics. Experiences of pleasure, laughter, crying and aggression all appear to be rather rare ictal manifestations, occurring in less than 1% of the epileptic population. Ictal sexual sensations are probably infrequent although their prevalence is unknown (Strauss, 1989).

The case for interictal emotional disturbance is controversial. At the turn of the century investigators considered that all epileptic people underwent intellectual and personality deterioration because of their fits. This view was later challenged by Lennox and Lennox (1960). They argued that most epileptics were normal in personality, although some suffered psychological defects as a result of structural brain damage or the harmful effects of chronic anticonvulsant therapy. Furthermore, Gibbs and Stamps (1953) asserted that 'the patient's emotional reactions to seizures, to family and social situation are less important determinants of psychiatric disorder than the site and type of epileptic discharge'.

The 'global epileptic personality' was elaborated on by Bear (1979). He proposed the existence of 18 personality traits associated with Temporal Lobe Epilepsy (or TLE), which reflected alterations in behavior, thought and affect. These traits were hypothesized to be related to an underlying

mechanism - enhanced affective associations. These associations are formed to previously neutral stimuli, events or concepts. They are believed to be caused by a progressive change in limbic structure secondary to a temporal epileptic focus. Bear argued that an epileptiform focus in the limbic system produced new functional connections between neocortical and limbic structures (enhance and/or form new synaptic connections); he called this process 'sensory-limbic hyperconnection'.

The proposal that people with epilepsy originating in the temporal lobe are at a special risk for emotional disturbance, is controversial (for several reasons). The assessment of TLE presents considerable methodological problems. For example, it is difficult to classify under one general syndrome a disorder as heterogeneous as TLE. Moreover, there are imprecise criteria for diagnosis (Strauss, 1989).

However, a great deal of evidence implicates limbic structures in emotional behavior (Gloor et al., 1982; Kluver and Bucy, 1939). Moreover, certain limbic structures are more involved than others in the mediation of some emotional behaviors (Gloor et al., 1982; Halgren, 1982). Because of the close association between emotional behavior and temporal lobe or limbic function, it is reasonable to expect temporal lobe epilepsy which involves the limbic system to be associated

with a higher incidence of psychological dysfunctions (of an emotional nature).

Limbic System Epilepsy and Depression and Anxiety

One of the most common symptoms of an epileptic attack is the feeling of fear - not the fear of a fit but of an undirected fear (Jackson, 1879). It's intensity can vary from slight anxiety to stark terror, and can last from several seconds to minutes.

Hermann et al. (1982), comparing patients with TLE and generalized epilepsy, reported that temporal lobe epileptics with an aura of fear were more apt to display pathological elevations on MMPI clinical scale scores (of depression, paranoia, psychopathic deviation, schizophrenia, mania and social introversion). Ictal fear is thought to be related to spread of the discharge to limbic structures, particularly the amygdala (Gloor, 1972).

As noted by Betts (1981), depression and anxiety are among the most frequent interictal behavioral concomitants of the epilepsies. For example, depression is the most common diagnosis in hospitalized patients with TLE who manifest significant psychopathology (Betts, 1974; Dalby, 1971; Currie et al., 1971). Moreover, Trimble and Perez (1980) found 281 epileptics of mixed diagnosis to be more anxious than controls, and equal to or greater than psychiatric patients.

More recently, Robertson et al. (1987) reported high Beck Depression Inventory (BDI) and Spielberger State-Trait Anxiety Scale scores among epileptics of mixed seizure diagnosis. Although there have not been any investigations of the relationship between epileptic seizures with specific sub-cortical foci and anxiety and depression, anxiety and depression are associated with auras indicative of human limbic activation (Stark-Adamec et al., 1982). Also, recent work by Hermann et al., (1989) shows that temporal lobectomy aimed specifically at removal of the anterior temporal lobe and amygdala relieves anxiety in TLE. These findings imply some malfunction in the amygdala may be associated with heightened anxiety in human epileptics.

Epilepsy might directly alter limbic system functioning in such a way that anxiety and depression are induced and persist interictally. On the other hand, it is possible that social and genetic factors together interact with alterations in limbic function produced by limbic seizures to precipitate anxiety and depression (Adamec, 1990a). This former viewpoint is supported by the findings that 25% to 35% of epileptics are troubled, whereas 49%-65% experience psychiatrically relevant social stresses, and 11% of troubled epileptics have family histories of depression (reviewed in Adamec, 1990a). These percentages suggest that not all epileptics will respond

pathologically to social stress, nor are all troubled epileptics predisposed to do so.

Depression may occur as an aura of the epileptic seizure, during the seizure, or as a sequel to the seizure (Strauss, 1989) However, the mood usually persists (Weil, 1955; Williams, 1956). Weil (1956) proposed that the prolonged depressive episodes may be due to subclinical epileptiform activity in the hippocampal-amygdaloid-temporal lobe complex and/or due to afterdischarges following activation of these same structures. Unlike other emotions, depression usually lasts for days after an epileptic attack. In addition, there is usually evidence of temporal-limbic discharge (Strauss, 1989).

Hence, there is good evidence to suggest that there may be physiological brain changes associated with epilepsy that predispose the epileptic to emotional changes. Moreover, these changes are likely to involve the limbic system. So epileptics whose seizure disorder invades the limbic system may be at particular risk for emotional changes. One way to address this question is to use animal models to determine if experimental TLE (limbic epilepsy) can alter interictal affective states in animals. The next section examines some animal models of epilepsy and the behavioral changes which may model psychopathology associated with epilepsy.

Animal Models of Limbic System Epilepsy

There are a variety of different animal models of epilepsy including chemical lesions, audiogenic seizures, and photically induced seizures. One of the more widely used and studied is the kindling procedure. This model is of particular interest because lasting interictal affective changes have been observed following kindling. Kindling refers to a procedure where the brain of an experimental animal is electrically stimulated using short high frequency trains of biphasic pulse pairs. When the stimulations are repeated, there is a progressive increase in the strength of both the electrographic and behavioral response following termination of the stimulation. During the final stages of this treatment the animals respond with fully generalized electrographic and motor seizures (Racine, 1978). The change in response is long lasting and is not associated with tissue damage. Kindling also induces a form of long term potentiation of synaptic transmission in several limbic system and cortical pathways (Goddard, 1972; Racine, 1978).

Kindling as a Model of Temporal Lobe Epilepsy

Kindling is thought to be a useful model of human epileptogenesis. Kindling of limbic structures in animals is modulated most effectively by agents that are effective in treating complex partial seizure (CPS) disorder in humans

(Adamec, 1990a). These characteristics suggest that limbic kindling may model epileptogenesis of focal human limbic epilepsy (TLE).

There are two difficulties with this proposal. First, kindling has not been seen in humans. Second, kindling in animals is not associated with tissue damage, whereas limbic epilepsy often is. Several arguments may be made to support the existence of kindling in humans. First, kindling can be produced in many species, including primates. Logically, it should also occur in humans. Second, there are at least two reported cases where repeated electrical stimulation in the human hippocampus (Monroe, 1970) and thalamus (Sramka et al., 1977) produced an epileptic seizure where none was present before. Third, there is evidence of time-dependent spread of epileptic excitability independent of tissue pathology (Flor-Henry, 1976; Perrin and Hoffman, 1979), which is a property of kindling.

Regarding damage and human epilepsy, Goddard (1983) has suggested that scarring associated with post-traumatic epilepsy is an insufficient condition for the development of the seizure disorder. In his model, damage creates a kindling stimulus which then leads to a seizure disorder because neural pathways leading away from the damaged focus have been permanently modified. The delay between trauma and onset of epilepsy is consistent with this view (McNamara et al., 1980).

Goddard (1983) also suggests that removal of a focus could eliminate seizures while leaving the kindled changes distal to the focus intact. This is because the trigger (like brain stimulation in animals) has been removed. This formulation suggests that aggressive prophylactic anticonvulsant therapy following head trauma with neurological signs of brain damage should reduce the incidence of developing epileptic disorder. Servit and Musil (1981) have observed this to be the case.

As in human epilepsy, changes in emotional behaviors have been noted in kindled animals. Inducing a seizure focus with kainic acid in the feline dorsal hippocampus renders the cat more defensive (Griffin et al., 1987). Adamec and Stark-Adamec (1983a) found that spread of kindled seizure activity to the amygdala and the amygdala-ventromedial hypothalamic (AM-VMH) pathway is critical for the development of interictal enhancement of defensiveness. The enhanced defensiveness was accompanied by lasting long-term potentiation of evoked activity in the AM-VMH pathway. It has been suggested that the enhanced defensiveness following feline kindling models anxiety associated with epilepsy (Adamec, 1990a). In support of this contention is the observation that changes in feline defensiveness produced by kindling are mimicked nearly exactly by a β-carboline which induces anxiety in humans, FG-7142 (Adamec, 1991c).

Pinel et al. (1977) found that kindling of the rat amygdala resulted in an increase in reactive response to tail tap or resistance to handling, and Adamec (1990b) found that rats kindled in the medial amygdala were less likely to explore the open areas of an elevated plus maze. These studies suggest that kindling in the limbic system, and amygdala, in particular, may increase anxious behavior. In the next section the involvement of the amygdala and kindling in the susceptibility to anxiety will be discussed.

The Amygdala, Kindling and Susceptibility to Anxiety

A variety of experiments suggest that the amygdala is a central structure in the control or expression of anxiety (Graeff, 1984; Kuhar, 1986). Electrical stimulation of amygdaloid structures produces behavioral changes suggestive of the induction of fear or anxiety states. These include autonomic arousal, escape reactions, defensive postures, and verbalization of fear or anxiety. Amygdaloid lesions on the other hand are reported to suppress the typical affective reactions associated with fear- or anxiety-provoking stimuli (Grossman et al., 1975; Shibata et al., 1986; Ursin, 1965). The amygdala is rich in benzodiazepine receptors and intra-amygdaloid application of benzodiazepines (an anxiolytic drug in humans) reverses the effects of punished behavior in rats (Scheel-Kruger and Petersen, 1982).

The repeated spread of seizures into the amygdala induces a lasting hyperfunction of circuits (Racine, 1978). This hyperfunction is characterized by an increased sensitivity to neuronal activation (Goddard, 1972). The mechanisms of these changes are not entirely understood, although there are some clues. LTP can be produced by limbic kindling (Racine, 1978). In some circuits in the hippocampus, LTP is dependent on NMDA receptors (Collingridge and Bliss, 1987). The changes in amygdala kindling resemble LTP, and may also involve NMDA receptors (Adamec, 1990a). Furthermore, amygdala kindling is associated with a long lasting failure of GABA mediated inhibition in the basolateral amygdala (Gean et al., 1989). This condition leads to hyperexcitability of amygdala cells, and possibly heightened anxiety.

Previous work has shown that the amygdala is responsible for processing and evaluating aversive stimuli. Weiskrantz (1956) originally suggested that the effect of amygdalectomy in monkeys tested on avoidance tasks was to make it difficult for animals to identify reinforcing stimuli. Later recording and lesion studies in monkeys (Jones and Mishkin, 1972; Mishkin, 1978) and rats (Rolls and Rolls, 1973) have supported the idea of an amygdaloid mechanism for evaluating the motivational significance of sensory stimuli. Lesion and disruptive electrical stimulation of the amygdaloid complex in rats selectively decrease spontaneous and conditioned

avoidance behavior. These data suggest that the rodent amygdaloid complex is involved in the evaluation of aversive stimuli (Goddard, 1964; Grossman et al., 1975).

A body of evidence implicates the amygdala in regulation of emotional behavior (Gloor, 1972; Adamec, 1990a,b,c). However, there is some confusion in the literature with respect to the exact role played by this structure in emotion. Part of the difficulty lies in the lack of attention paid to possible functional differentiation within the rodent amygdala. There is clear evidence for functional differentiation in the feline amygdala (Egger & Flynn, 1963; Adamec, 1978; Adamec and Stark-Adamec, 1983a,b,c; Siegel, 1984).

Is the amygdala primarily involved in reducing or increasing anxious behaviors? Insight into the role of the amygdala was gained from the experiments of Egger and Flynn (1963). They showed that electrical stimulation of the magnocellular portion of the basal nucleus and adjacent portions of the lateral nucleus of the cat produced a marked suppression of quiet biting attack behavior. Facilitation of attack occurred when stimulation was applied to more lateral and dorsal portions of the lateral nucleus.

There is other evidence to suggest functional differentiation within the cat amygdala. Siegel (1984), using electrical stimulation, showed that the greater part of the

amygdala serves to inhibit attack behavior while a facilitatory function is associated with the lateral and central nuclei. There is also evidence for intra-amygdaloid differentiation in humans. Smith (1980), using electrodes to stimulate and record in the amygdalae of pathologically aggressive patients, found that fear based aggressive behavior was accompanied by changes in electrical activity in the medial aspects of the right amygdala. Stimulation of the medial amygdala provoked the behavior. In contrast, stimulation of the ipsilateral lateral amygdala produced a feeling of calm and relaxation in the patients.

In the light of these findings, it would seem particularly important to pay attention to the amygdaloid nuclei stimulated in kindling studies which look for interictal behavioral changes. In fact, Adamec and Stark-Adamec (1983b) suggested that the nature of the behavioral outcome of an experimental seizure focus would depend on the precise location of that focus in the limbic system. Siegel (1984) directly demonstrated this in the cat by showing that repeated seizures in the lateral amygdala of the cat lowered the electrical threshold for eliciting quiet biting attack from the hypothalamus. More medial amygdala seizures did the opposite and also lowered the threshold for eliciting defensive behavior from the medial hypothalamus. Given these findings in the cat, it would seem useful to map different

areas of the rodent amygdala. Some research has begun to do just that.

Lateral Amygdala Kindling Elicits an Anxiolytic Response

A growing body of evidence shows that the lateral amygdala is involved in reducing the anxious response (Adamec, 1990b; Siegel, 1984). Thomas et al. (1985) suggested that the lateral amygdala is an important component of the forebrain circuitry involved in the expression of anxiety and is sensitive to benzodiazepine drugs. They found that infusions of chlordiazepoxide (a benzodiazepine agonist) into the lateral amygdala of rats induced a release of responding measured during the component of a conditioned emotional response task previously associated with an aversive stimulus. These findings implicate the lateral amygdaloid complex in the anxiolytic action of the benzodiazepines.

Witkin et al. (1988) found that repeated electrical stimulation of the basolateral amygdala increased punished responding without concomitant changes in nonpunished behavior. It is of interest that the anxiolytic activity of basolateral stimulation was not prevented by the introduction of a benzodiazepine antagonist (Ro-15-1788 or flumazenil). These data argue against the effects of kindling being mediated by a benzodiazapine anxiolytic ligand.

In a later study, Adamec (1990c) found that defensive response to prey, and time spent biting prey, by domestic cats were changed by high-frequency electrical stimulation of the lateral part of the basomedial amygdala. Defensive response to rats decreased, and time spent biting rats increased.

The evidence suggests that the lateral/basolateral amygdala attenuates animal anxiety. Moreover, kindling of this sub-nucleus, has an anxiolytic effect in rats and possibly cats. Therefore, kindling may potentiate the anxiolytic function of the lateral amygdala. It seems to do so via a mechanism which does not involve benzodiazepine receptors, since flumazenil does not affect kindling induced anxiolysis.

Medial Amygdala Kindling Elicits an Anxiogenic Response

The medial amygdala facilitates defensive behavior in rodents (Luiten et al., 1985; Vochteloo & Koolhaas, 1987). Luiten et al. (1985) found that after bilateral lesions of the medial amygdala, rats showed deficits in avoidance of a dominant male. Also, lesioning the medial amygdala of male rats reduces aggressive behavior (Vochteloo and Koolhaas, 1987). In addition chronic adminstration of antidepressants (by microinjection) to the medial amygdala inhibits mouse-killing, and hyperemotionality in olfactory bulbectomized rats (Shibata et al., 1984). Pucilowski et al. (1985) demonstrated that bilateral microinjections of serotonergic receptor

agonists into the medial amygdala prolonged the attack latency and suppressed the incidence of aggressive postures in isolated killer rats. However, the effect did not seem to be dependent on changes in general activity and pain sensitivity.

Stoddard-Apter and MacDonnell (1980) found that chemical stimulation of the feline medial amygdala facilitated a strong (defensive) sympathetic nervous system response. Also, experimentally induced medial amygdala seizures in felines seem to sensitize neural substrates mediating fearful response to environmental threat or to direct electrical activation (Adamec and Stark-Adamec, 1983a,c). These effects persist interictally for weeks to months.

Adamec (1975) also found that cats that scored high on measures of predatory behavior (rat killers) had high afterdischarge thresholds in the medial amygdala compared to more fearful non-killers. He also found that lowering afterdischarge thresholds by partially kindling the amygdala turned rat killers into fearful non-killers.

There are few studies investigating the anxiogenic effects of medial amygdala kindling in rats. One recent report found that unilateral medial amygdala kindling in Wistar rats increased anxious response in the elevated plus maze for at least one week following the last seizure (Adamec, 1990a,b).

The evidence points to an anxiogenic role for the medial amygdala. Kindling, which is purported to strengthen synaptic

associations in the area stimulated, appears to enhance the anxious response of an organism. Ablation of the medial amygdala reduce fearful behavior, and in this regard may be anxiolytic.

The Involvement of the Central Amygdala in Anxiety

Direct projections from the central nucleus of the amygdala to the lateral hypothalamus (Price and Amaral, 1981; Shiosaka et al., 1980) and a variety of brain stem structures (Hopkins, 1975; Hopkins and Holstege, 1978) appear to be involved in activation of the sympathetic nervous system during fear and anxiety. Electrical stimulation of the amygdala can produce a complex pattern of behavioral and autonomic changes that highly resembles a state of fear. Stimulation of the amygdala can alter heart rate and blood pressure (Heinemann et al., 1973; Timms, 1981), respiration (Applegate et al., 1983; Harper et al., 1984), and produce a cessation of ongoing behavior (Applegate et al., 1983). In humans, electrical stimulation of the central amygdala elicits feelings of fear or anxiety, as well as similar autonomic reactions indicative of fear (Chapman et al., 1954; Gloor et al., 1981).

Direct application of benzodiazepines into the central nucleus of the amygdala are reported to have anticonflict effects (Shibata et al., 1986). Similarly, long lasting,

anticonflict effects are also reported resulting from lesions of the central nucleus of the amygdala (Nagy et al., 1979; Shibata et al., 1986). Yadin et al. (1991) tested the performance on a water licking conflict paradigm in rats with localized damage to the central amygdala. The result was a pronounced increase of punished responding.

Kindling in the central and medial amygdala seems to facilitate the subsequent development of restraint-induced stomach ulcers in rats (Henke and Sullivan, 1985). They suggest that neuronal hyperexcitability produced by the kindling procedure led to an increased susceptibility to gastric pathology in response to stress. Of interest is that as few as one amygdala afterdischarge without motor seizure was sufficient to increase stomach pathology. These findings suggest increased vulnerability to stress is produced independently of motor seizure mechanisms. Moreover, their data demonstrate that limbic seizures do enhance amygdala function, at least in stress reactions. It is known that cells in the centromedial amygdala increase their firing rates during restraint stress, and bilateral lesions of the centromedial amygdala attenuate restraint stress-induced stomach pathology (Henke, 1983; Innes and Tansy, 1980).

There is also evidence for functional differentiation within the central amygdala. Harrigan et al. (1991) have found that immunohistochemical labelling of CRF was primarily

localized in the lateral part of the central nucleus. In addition, Farb et al. (1991) found that direct projection of fibres from the lateral amygdala to the central amygdala was strongest caudally. More rostrally, the projection is greatest in the ventral and lateral aspects of the central amygdala.

The most intriguing data come from a study that looked at the effects of lesions in and around the rostral central amygdaloid nucleus of the rat on drinking passive avoidance (Coover et al., 1992). Lesions of the rostral half of the central amygdala appeared to have an anxiolytic effect that diminished as the lesions move farther away from the nucleus. In assessing his and others data, Coover states that the rostral third of the central amygdala continues to stand out as the most dramatic site for lesion-induced anxiolysis.

Evidence to date suggests that the central amygdala is important for producing anxious behavior. Together with the medial amygdala and as part of the centromedial amygdaloid axis, stimulation of the central amygdala appears to play a role in the enhancement of anxiety in the rat (but surprisingly enough not the cat - where it seems to play an anxiolytic role, Siegel, 1984). Destructions of the central amygdala, in turn, has an anxiolytic effect.

Possible Laterality of Emotional Effect

It has been suggested that processes and functions related to perception and expression of emotions are represented asymmetrically in the cerebral hemispheres (Smith et al., 1987; Joseph, 1988; Coffey, 1987). Emotion is generally thought to be right hemisphere dominant (Joseph, 1988; Coffey, 1987; Campbell, 1982). However, recently, some evidence has surfaced implicating the left hemisphere in processing of affect and cognition (Smith et al., 1987; Meyers and Smith, 1987). In these studies there appeared to be greater EEG measured neural activity in the left hemisphere upon presentation of nonverbal emotional stimulation.

Studies of persons with unilateral brain lesions provide additional information on the lateralization of emotional function. It was shown that the right hemisphere is involved in tasks requiring emotional analysis, particularly when the tone of the displayed emotion is negative (Campbell, 1982; Miller, 1988)

Silberman and Weingartner (1986) attempted to clarify the hemispheric lateralization of functions related to emotion. They describe three possible aspects of emotional lateralization: (1) emotions are better recognized by the right hemisphere; (2) control of emotional expression and related behaviors takes place principally in the right hemisphere; and (3) the right hemisphere is specialized for

dealing with negative emotions, while the left is specialized for dealing with positive emotions. Each hemisphere and their possible functional differentiations will be considered in turn.

The Right Hemisphere and Emotional Dysfunction

Studies of affective disorders suggest that the right hemisphere is particularly involved in the experience of depressive and unpleasant affects (Coffey, 1987; Swartzburg, 1983; Lenhart and Katkin, 1986). A popular model that explains these clinical states is that the right hemisphere has a general inhibitory function. This proposed inhibitory function is compatible with states of inactivity and withdrawal elicited by negative affects (Swartzburg, 1983).

Flor-Henry and Koles (1984) compared quantitative EEG characteristics of depressive psychotics, manics, schizophrenics and normal subjects. They statistically compared EEG power, coherence, and phase characteristics. Results suggested the presence of increasing disorganization (in EEG patterns) of the right hemisphere, in a progression that was parallel to the degree of psychopathological disorganization. Since profound emotional changes often accompany psychoses (Olbrich, 1987; Petho, 1987), this suggests that there may be asymmetrical electrophysiological changes with pathological emotional dysfunction.

Seizures with Left Hemisphere Foci Elicit Anxiety

Surprisingly, evaluating the effects of lateralization of epileptogenic lesions on mood changes and anxiety, show that left temporal foci subjects score significantly higher than those with right temporal foci and normals on tests for depression and trait anxiety scales (Perini and Mendius, 1984; Perini, 1986).

There are two lines of evidence which may help to explain this discrepancy. First, the work of Reiman et al. (1984;1986), using PET scanning in humans, suggests that an imbalance of activity with left<right limbic (parahippocampal gyrus) function might be associated with some forms of anxiety (panic). Other studies (Reiman et al., 1989) show a bilateral anterior temporal pole involvement in normal anticipatory anxiety. These data are controversial, however, and may reflect muscle artifact (Drevets et al., 1992). Secondly, PET scanning in temporal lobe epileptics indicates hypometabolism on the side of the focus (Kenichiro et al., 1989). If a left< right asymmetry is important for pathological anxiety, it might explain the above results. Patients with left foci, and interictally decreased left hemispheric activity would have a left<right asymmetry which might be related to their interictal anxiety.

It seems that EEG and PET studies suggest a greater role for the right hemisphere in depression and anxiety. Studies of

epileptic foci may be consistent with this view, or they may suggest that the left hemisphere is important for the expression of depression and anxiety. At least one PET study suggests that both hemispheres are involved in anticipatory anxiety, in normal subjects at least (Reiman et al., 1989).

Given the uncertainty in the literature regarding lateralization of negative affect, it would be of interest to investigate the effect of unilateral kindling in both hemispheres. To date very little evidence exists for lateralization of emotion in lower mammals. However, there is some data suggesting a lateralization of emotion in rats. Fride and Weinstock (1989) found prenatal stress induced alterations in rat cerebral asymmetries. They suggested a rightward bias of tail positioning in stressed rats induced by prenatal stress may underlie the decrease in the ability of the offspring to cope with anxiety-provoking situations.

Also, Mittleman et al. (1988) discovered that rats displayed an asymmetry in orientation reaction time to visual cues. They concluded that there is a functional lateralization of responding to visual stimuli in rats that is similar to that seen in humans. Finally, Denenberg et al. (1986) confirmed that in animals given handling stimulation in infancy, the right hemisphere is dominant for the occurrence of mouse killing and the left hemisphere acts to inhibit this

behavior. There is the possibility of an emotional component to the behavior measured in both studies.

Conclusions and Justification for the Present Study

Recent findings suggest that epileptics with seizures that invade the limbic system, also suffer from anxiety and depression (Adamec, 1990a). The search is on for the substrates of these pathologies using animal models. Work with cats has shown that limbic kindling in cats, and the behavioral changes which accompany it, represent a valid model of limbic epilepsy induced anxiety (Adamec, 1990a for review). Moreover, these behavioral changes in cats seem to be due to a long term change in the organization and transmission of neural activity within the limbic system circuits subserving emotions such as fear (Adamec, 1990a for review).

Recent work with rodents has attempted to show that similar changes in rodent anxiety occur following limbic kindling, with some success (Adamec, 1990b). It has been shown that limbic system kindling changes the behavior of rats in validated tests of anxiety. Kindling of a particular part of the rat amygdala, the medial amygdala, produces an anxiogenic (increased anxiety) effect which lasts at least one week after the last seizure (Adamec, 1990b). More recent work has shown left lateral amygdala kindling increases anxiety for two weeks after the last seizure (Nieminen et al., 1992). Since no

detail of electrode location is given in this study, the precise location of anxiogenic sites cannot be determined from the published report.

This study proposes to continue the localization of the anxiogenic effect of seizures that involve the limbic system. The following studies are designed to examine three questions. The first is to determine if the effects of kindling of the medial and lateral amygdala have different behavioral consequences. There are data to suggest that lateral kindling should have an anxiolytic rather than an anxiogenic effect (Adamec, 1990a; Witkin et al., 1988). The second is to test whether kindling of the left or right hemisphere produce different effects. There is evidence in humans that emotional dysfunction may involve one hemisphere (the right) more than the other. And third, to determine if kindling of the central amygdala enhances its anxiogenic function in the rodent.

In addition the findings will provide useful data on the importance of the location of the epileptic focus for emotional disturbance. Moreover, these data will help to validate this animal model for human anxiety associated with epilepsy.

Methods

Subjects

One hundred and ninety two male Wistar rats (Charles River Canada) weighing between 200 and 250 grams at the beginning of the experiment were used. Rats were housed individually in transparent plastic cages on racks holding 15 cages in the same holding room. They were maintained on a 12 hour light-dark cycle (lights on at 7:00 hrs). Rats had water and rat chow available at all times.

Groups

Medial/Lateral amygdala placements. Rats were randomly assigned to one of eight groups, which were a combination of three conditions: Medial/Lateral amygdala (M/L), Left/Right hemisphere (L/R) and Kindling/No Kindling (K/NK) (Table 1). All animals were implanted with stimulating electrodes, however only half were kindled: group 1 consisted of animals that had electrodes implanted into the Right Medial amygdala then Kindled (RMK), group two's electrode placement was in the Right Lateral amygdala then Kindled (RLK). The remaining 2 kindled groups consisted of the following combinations: Left Medial Kindled (group 3, LMK) and Left Lateral Kindled (group 4, LLK). The same combinations of Left/Right and

Medial/Lateral were used for the non-kindled rats: group 5, (RMNK); group 6, (RLNK); group 7 (LMNK); and group 8, (LLNK).

Note that the words 'lateral', 'medial', 'outlier', 'left', 'right', 'kindled', 'not kindled', 'control', 'present', 'previous', 'on target', 'off target' and 'central' are capitalized when used as proper nouns to identify subject groups.

Because of the large numbers of subjects, the rats were run in cells or groups, ranging from 16-40 at a time. In each replication, rats were randomly assigned to one of the eight groups.

Central amygdala placements. Two additional groups of rats were used to examine the effect of central amygdala kindling on anxiety. Rats were randomly assigned to either Central amygdala Kindled or Not Kindled groups (Table 1). Electrode placement in both cases was in the central amygdala of the right hemisphere.

Procedures

Handling procedures. All rats were handled in the rat holding room. Pre-handling was carried out for three days prior to the surgery. The procedure consisted of picking up the rat from its home cage with a gloved hand. The rat was gently restrained around the shoulders, while using one arm as a platform on which the rat could rest its feet. When the rat

struggled or tried to escape, the grip was tightened to keep the rat still. When the rat was immobile, the grip was relaxed. Rats were held this way for 1 minute and then returned to their home cages.

The rats were also handled every other day after recovery from surgery, up to the day of adaptation to the kindling apparatus.

Surgical procedures. Surgery was performed, aseptically, under sodium pentobarbital anaesthesia (60 mg/kg ip). Twisted bipolar stainless steel stimulating electrodes (.125 mm in diameter, Plastics One) were implanted in the rats (according to group assignment) using stereotaxic technique. Coordinates were used according to the atlas of Pellegrino and Cushman (1979) for Medial placements (0.6 posterior to bregma, 4.0 lateral to midline, 8.6 ventral to dura, skull elevated 5 mm above the horizontal) in order to match those reported in Adamec (1990b). The atlas of Paxinos and Watson (1986) was used for Lateral placements (2.2 posterior, 4.7 lateral, 6.7 ventral) and Central (2.12 mm posterior, 4.1 mm lateral and 8.0 ventral) electrode placements. This was done to match the coordinates of Witkin et al. (1988). Rats heads were positioned horizontally in the stereotaxic for both lateral and central placements. Wound edges were locally anaesthetized during surgery with lidocaine (2%) infusion. The skull and wound edges were sprayed with antibiotic (Neosporin antibiotic

spray) prior to closing. Electrodes were fixed in place with dental acrylic cement secured to the skull with four stainless steel skull screws. Following surgery, rats were given 10 mg of chloramphenicol subcutaneously. Rats were allowed one week of recovery from surgery.

Kindling. Before kindling commenced, the rats were adapted to the kindling apparatus. The rats were placed in the boxes in which they would be kindled and connected to the electrode leads on the two days before kindling.

Rats in Kindled groups were stimulated twice per day between 9 and 11 AM and again between 2 and 4 PM, with at least 3 hours between stimulations. Stimulation consisted of 400μA peak to peak constant current square wave pulses of 1 msec pulse width delivered in a train at 62.5 pulses per second. Train duration was set to 1 sec for the first two stimulations; then it was increased to 2 sec for the remaining stimulations. Stimulus intensity was kept constant, though in some instances it was increased to as much as 800μA peak to peak pulses in a 3 second train. Stimulation was repeated until a rat showed three stage five convulsions outlasting the stimulus, as defined by Racine (1978). Then Kindled rats (and their Not Kindled controls) were not stimulated for 1-4 days to allow other rats in the group to achieve three stage 5 seizures. Then a fourth stage 5 seizure was triggered in all kindled group rats and the rats were left unhandled for one

week. At the end of the week, the behavior of all rats was tested.

Behavior Testing

The elevated plus maze test of anxiety was chosen because it is a simple and a relatively motivation artifact free test of rodent anxiety (Chopin and Briley, 1987). This test has been validated on pharmacological grounds to be a model of benzodiazepine sensitive anxiety (Pellow et al., 1985). The test measures strength of antagonism between exploratory tendency and avoidance of open novel spaces (Chopin and Briley, 1987).

All rats were tested once in the elevated plus maze between 9:30 AM and 5:00 PM. Behavior of the rats was observed and videotaped remotely in an enclosed room. Reactions of the rats to a novel hole board apparatus were examined first in order to measure activity and exploratory tendencies which might influence behavior in the plus maze (File and Wardill, 1975a,b). The hole board was a square wooden box, 60 cm on a side, with four sides rising 35 cm above the floor of the box. There were four evenly spaced holes drilled in the floor of the box, which was elevated 12 cm above the ground. The holes were drilled at the corners of a square drawn on the inside of the box whose sides were 14 cm from the walls of the hole board. The box was painted with flat grey enamel paint. Rats

were placed in the center of the hole board and observed and videotaped for 5 minutes.

Rats were then transferred by gloved hand to the novel elevated plus maze apparatus. This consisted of four arms arranged in the shape of a plus sign. Each arm was 10 cm wide, 50 cm long and elevated 50 cm above the ground. The four arms were joined at the center by a 10 cm square platform. Two of the arms opposite each other had no sides. The other two arms were closed on the sides with walls 40 cm high, but open on the top. The walls did not extend into the center of the maze. The maze was painted with flat grey enamel paint. Rats were placed in the center of the maze facing the same open arm of the maze and their behavior videotaped for 5 minutes. At the end of the testing, rats were returned to their home cages.

A number of behavioral measures were taken. From the hole board, activity was measured as time spent in motion and frequency of rearing (File and Wardill, 1975a). Exploratory tendency was measured as Head Dipping (defined as placing the snout or head into a hole in the hole board (File and Wardill, 1975b). Finally, number of faecal boli left in the hole board were counted.

Several measures taken in the hole board were also taken in the plus maze. Number of boli were counted. In addition, the total number of entries into any arm of the plus maze was used as a measure of exploratory activity.

A commonly used measure of anxiety was taken from the elevated plus maze: the ratio of the time spent in the open arms divided by the total time spent in both arms of the maze (Ratio Time). A rat was considered to have entered an arm of the maze when all four feet were within the arm. The smaller the ratio, the more 'anxious' the rat (File and Wardill, 1975a).

Another behavioral measure observed in the plus maze was 'Risk Assessment' behavior. This was defined as the rat's willingness to poke it's head out into the open arm but not actually enter it. Both time and frequency of this behavior were measured. Risk Assessment was originally defined and investigated as a measure of rodent anxiety by Blanchard and Blanchard (1989).

Histology

At the end of the experiment, rats were deeply anaesthetized with Somnotol, perfused transcardially with 10% formalin in phosphate buffered saline and the brains removed. Frozen sections (37 μM) were taken through the electrode tracks and the tissue mounted and stained with metachromatic cresyl violet and stained for acetylcholinesterase. Since the lateral amygdala stains darkly for acetylcholinesterase, the latter stain permitted more precise localization of lateral amygdala electrodes.

Stereotaxic coordinates of tip location were found with the aid of an image analyzer. The rat brain section being analyzed was normalized to the corresponding atlas section. Normalizing factors were found by dividing the width of the rat section being examined by the width of the same cross-section through the atlas. The vertical and lateral position of the electrode tip measured in the section was multiplied by this factor. These normalized coordinates were recorded and also plotted on rat atlas sections. Animals were sorted into groups with electrodes in the same anatomical location (according to Paxinos and Watson (1986) and Pellegrino and Cushman (1979) atlases, see Appendices A & B.)

Statistical Analysis

The effects of the manipulations on behaviors measured in both the hole board and plus maze were assessed using three-way or one-way univariate analyses of variance (BMDP for PC-SOLO program). Post-hoc Duncan's or a priori t-tests (planned comparisons) were used to analyze the differences between various subgroups.

Three-way analysis of variance) was performed on animals with a medial or lateral (amygdala) focus of stimulation. The independent variables of this analysis were Left/Right hemisphere, Medial/Lateral nucleus and Kindled/Not Kindled. Animals with foci outside of these two nuclei were considered

to be either medial or lateral Outliers (respectively), and were grouped into a subset labelled 'Outliers'. Finally, animals with electrode placements clearly in the central amygdala were analyzed separately using a one-way analysis of variance (Kindled vs. Not Kindled).

Since this is the continuation of ongoing research, some of the findings of this study were compared with a previous study when appropriate.

Also, correlations were done between electrode location and scores of relative anxiety.

Finally, the total amount of charge (in micro coulombs) that the animal received during kindling was calculated. This was done by multiplying together the peak to peak amplitude (μA), pulse width (msec), and total number of pulses in the train for each kindling session Since kindling stimulation was biphasic, the entire product was multiplied by two. The total amount of charge that passed through a rat's brain was found by summing the charge passed in all of the kindling sessions. Differences between groups were assessed using a three-way analysis of variance. The independent variables, again, were hemisphere, amygdala nucleus, and kindling.

Results

Present Findings

Medial and Lateral placements. In the plus maze there was a kindling by hemisphere interaction for Ratio Time [$F(1,82)=6.30$, $p<.05$, Figure 1]. Left hemisphere Kindled animals spent relatively more time in the open arms than the Left hemisphere Not Kindled animals [$t(82)=2.08$, $p<.05$]. There was a trend in the opposite direction in the right hemisphere. Rats kindled in the right hemisphere tended to spend relatively less time in the open arms than Not Kindled animals, although this was not significant [$t(82)=1.49$, $p<.1$]. Within either hemisphere there were no differences or interactions between Medial and Lateral Kindled or between Medial and Lateral Control animals.

Controls with electrodes in the right and left hemisphere did differ, however (Figure 1). Right hemisphere Controls were less anxious than Left hemisphere Controls (Duncan's, $p<.05$).

There was a kindled by hemisphere interaction for the frequency of Risk Assessment [$F(1,82)=4.53$, $p<.05$, Figure 2]. Animals kindled in the left hemisphere engaged in Risk Assessment more frequently than Left hemisphere Not Kindled animals [$t(82)=2.01$, $p<.05$]. Right hemisphere Kindled and Not Kindled animals did not differ from one another, or from Left hemisphere Kindled rats. However, both Right hemisphere groups

showed less Risk Assessment than Left hemisphere Kindled rats (Duncan's, $p<.05$).

A completely different interaction emerged for the analysis of time an animal spent in Risk Assessment. There was a Medial/Lateral by Left/Right interaction [$F=(1,82)=4.56$, $p<.05$, Figure 3]. Duncan's tests showed that animals with Right Lateral placements were spending less time in Risk Assessment than Right Medial or Left Lateral animals, which did not differ. Also Left Medial animals fell between Right Lateral and Medial and Left Lateral animals (Duncan's, $p<.05$). Nevertheless, kindling was without effect on Time Spent Assessing Risk.

There was an interaction of kindling effect with Medial/Lateral placement for Head Dipping in the hole board [$F(1,82)=3.97$, $p<.05$, Figure 4]. Lateral Kindled animals Head Dipped more than Lateral Not Kindled animals [*a priori* $t(82)=2.13$, $p<.05$]. Medial Kindled and Not Kindled animals did not differ (Duncan's, $p<.05$). In addition, Lateral amygdala Kindled animals Head Dipped more than all the other groups (which did not differ, Duncan's, $p<.05$).

A three-way analysis of variance (with the same independent variables as above) was used to examine possible differences in electrode placements in the three planes (Table 2). A hemisphere by Medial/Lateral interaction was found for the anterior/posterior (AP) plane coordinate [$F(1,82)=14.83$,

$p<.01$, Figure 5]. Both Left Medial and Right Medial placements were more anterior than Left Lateral placements [$t(82)=9.16$, $p<.01$] and Right Lateral $t(82)=5.68$, $p<.01$]. Also, post-hoc tests showed that Left Medial placements were more anterior than Right Medial placements (Duncan's, $p<.01$). In the lateral plane, Lateral group placements were more lateral than Medial group placements [Medial/Lateral Effect, $F(1,82)=88.3$, $p<.01$], $4.65 \pm .06$ mm vs $3.90 \pm .05$ mean $\pm$ SEM lateral coordinates for Lateral and Medial animals respectively]. In the ventral plane Medial placements were more ventral than Lateral placements [Medial/Lateral Effect, $F(1,82)=20.62$, $p<.01$], $9.31 \pm .09$ vs $8.98 \pm .09$ mm mean $\pm$ SEM vertical coordinates ventral to the dura for Medial and Lateral placements respectively]. In addition, Left placements were more ventral than Right placements [Hemisphere Effect, $F(1,82)=12.10$, $p<.05$; mean $\pm$ SEM mm $9.24 \pm .11$ vs. $8.76 \pm .83$, Left vs. Right respectively]. The differences between the Medial and Lateral groups were due to the different coordinates used for the different target sites. The hemispheric differences in electrode location, however, are due to experimental variability. It is important to note that Kindled and Not Kindled controls in the various subgroups did not differ from each other.

Finally, similar three way analyses of variance were performed on the various parameters of kindling (Table 3).

Parameters analyzed were: the number of stimulations to the first stage five seizure (for Kindled animals), duration of the fourth stage five seizure (for Kindled animals only), the length of pause between the third and fourth stage five seizure (for Kindled and Not Kindled animals), and current passed during kindling.

There was a hemisphere by Medial/Lateral interaction for number of stimulations to first stage five seizure [$F(1,36)=6.91$, $p<.02$, Figure 6]. Post-hoc Duncan's testing showed Left Medial animals required more stimulations to stage five than all other groupings, which did not differ (Duncan's, $p<.05$). There were no differences in either the duration of the last seizure or pause data. Because there was a difference in number of stimulations to kindle, total current passed during kindling was divided by the total number of stimulations to yield average current passed per train. Analysis revealed an hemisphere effect, where Left Kindled rats had more current passed than Right [$F(1,77)=16.45$, $p<.001$, Table 3].

Since there was an hemispheric bias in current passed, it was necessary to reanalyse the behavioral data showing hemispheric effects. Analysis of covariance was used to remove the effects of average current from Ratio Time, frequency and time of Risk Assessment and Head Dipping. The interactions and pattern of mean contrasts reported above were unchanged for

Ratio Time and Frequency of Risk Assessment. So differences in current passed did not influence anxiety or one measure of Risk Assessment. The Medial/Lateral by Left/Right interaction for time spent in Risk Assessment was no longer significant, however. Finally, the Kindling by Medial/Lateral interaction for Head Dipping remained [$F=(1,76)=4.25.p<.05$], but the pattern of mean contrasts changed. Medial Kindled rats now Head Dipped less than controls [$t(76)=3.89$, $p<.01$; mean $\pm$ sem: $3.30 \pm .88$ vs. $8.15 \pm .88$, Kindled vs. Not Kindled Medial rats respectively]. Lateral Kindled rats did not differ from their controls (mean $\pm$ sem: $8.11 \pm .98$ vs. $9.08 \pm .86$, Lateral Kindled vs. Lateral Not Kindled animals respectively). Moreover, Medial Kindled rats Head Dipped less than all other groups, which did not differ (Duncan's, $p<.01$)

The Outliers. Three way analysis of variance was applied to the behavior of all the animals whose electrodes fell in neither the medial nor lateral sub-nuclei of the amygdala. The animals were grouped according to their original Medial/Lateral and hemisphere assignments for this analysis. Independent variables were Medial/Lateral amygdala, Left/Right hemisphere and Kindled/Not Kindled.

Analysis of Ratio Time in the plus maze was done after the data were transformed (square root) to normalize them. The raw data deviated from normality (D'Agostino-Pearson Omnibus K^2 Normality Test, $K^2=11.1$, $p<.05$). After transformation the

data appeared more normally distributed (K^2=0.1, p<.95). There was a main Kindled/Not Kindled effect [F(1,77)=4.16, p<.05, Figure 7]. Kindled animals spent relatively more time in the open arms than Not Kindled animals regardless of electrode placement.

Analysis of the Number of Boli in the hole board yielded a three way interaction of kindling, hemisphere, and Medial/Lateral electrode placement [F(1,77)=4.35, p<.05, Figure 8]. Subsequent post-hoc analysis showed that Left Medial Kindled animals left fewer boli in the hole board than all of the other groups of animals (which did not differ from each other, Duncan's, p<.05).

An analysis was done on the differences in kindling parameters between the groups of Outlier animals (Table 3). No significant differences were found.

Central amygdala kindling. A one-way analysis of variance (Kindling/Not Kindling) yielded no significant effects for any of the behaviors measured (Ratio time means ± sem: .271 ± .052 vs .290 ± .062, for Kindled and Not Kindled respectively). Also, there were no significant differences in electrode locations between Kindled and Control groups (Table 2).

Effects of Kindling on Other Behavioral Measures

Kindling in the Medial/Lateral amygdala, in Outliers or in the Central amygdala was without effect on the following behaviors: boli produced in the plus maze, and activity in the hole board (Table 4). Medial/Lateral and Central amygdala kindling had no effect on boli produced in the hole board. Finally, kindling in Outliers and in the Central amygdala did not affect Risk Assessment in the plus maze, or Head Dipping in the hole board (Table 4).

Comparisons with Previous Findings

Since similar experiments have been done in this laboratory, comparisons of the results of this study with previous findings are of interest. Of particular interest is the fact that in previous studies right Medial amygdala kindling was clearly anxiogenic (Adamec, 1990b). In the present study there was only a trend. Previous work (Adamec, 1990d) showed the importance of electrode location for behavioral outcome of kindling. So it was of interest to compare electrode locations in Right Medial amygdala kindled rats in the present and past studies.

A three way analysis of variance compared electrode locations from a previous study in this laboratory (Adamec, 1990d) with those of the present study. The independent variables considered were Study (Previous/Present),

Kindled/Not Kindled, and On Target/Off Target. Target sites in the Previous study were defined as whether the electrode was in the right medial amygdala nucleus (On Target) or not (Off Target). In the Present study there were Right Medial amygdala (On Target) and Right Medial Outliers (Off Target) rats.

The average electrode location of the two studies was different. AP plane data were log transformed to normalize them (raw data, K^2=52.8, p<.01; transformed, K^2=3.8, p<.15). Analysis of transformed AP plane data revealed a study by target interaction [F(1,56)=4.28, p<.05, Figure 9]. Duncan's mean testing (post-hoc) showed that in the Previous study On Target animals were more anteriorly placed than Off Target animals (p<.05). However, in the Present study On and Off Target animals did not differ, and fell between the Previous study On and Off Target animals.

There was a Study by Target interaction for lateral coordinates as well [F(1,57)=8.71, p<.01]. Previous study Off Target animals were more medial than the Present study Off Target rats (mean ± SEM mm: 3.76 ± .17 vs 4.34 ± .09, Previous vs Present study lateral coordinates respectively, Duncan's Test, p<.05). Previous and Present study On Target animals did not differ in the lateral plane. The two studies differed with respect to vertical plane location of electrodes. Electrodes of animals in the Previous study were not located as deep as those of rats in the Present study [main Study Effect for the

vertical plane, $F(1,64)=11.32$, $p<.01$; $9.15 \pm .11$ vs $9.70 \pm .09$, mean $\pm$ SEM mm below the dura for Previous and Present studies respectively].

Correlations

The importance of electrode location was further assessed using correlations between electrode location and a score of 'relative anxiety' over all rats. Relative anxiety scores were calculated as follows. Ratio Time for each kindled rat was divided by the average ratio time of the appropriate control (Not Kindled) group. For example, each Right Medial Kindled rat's Ratio Time score was divided by the mean Ratio Time of Right Medial Not Kindled rats. This was necessitated by the fact that in some cases control groups differed from each other in anxiety.

In addition, Lateral coordinates were adjusted to the coordinate system used for the Medial placements. The electrode locations of Lateral amygdala groups, which were expressed relative to the coordinate system of Paxinos and Watson (1986), were converted to Pellegrino et al. (1981) atlas coordinates using correction factors described in Appendix C.

In the right hemisphere, there was a significant negative correlation of relative anxiety with the AP plane ($r=-.509$, $p<.01, n=53$). As the electrode placement moved backwards from

bregma relative anxiety scores, of Kindled rats, increased (or anxiety decreased). In addition, there was a positive correlation of relative anxiety with the vertical coordinates ($r=.876$, $p<.01$, $n=53$, Table 5). The deeper the electrode (more negative vertical location), the lower the anxiety score (or the more anxious Kindled rats were relative to Controls).

Left hemisphere coordinates showed a weak positive correlation of relative anxiety with vertical plane coordinates ($r=.362$, $p<.05$, $n=29$, Table 5). The deeper the electrode the lower the relative anxiety score or the greater the anxiety.

Discussion

The Effect of Kindling on Anxiety

Contrary to the original hypothesis, the medial and lateral amygdalas have similar effects on anxiety, though the nature of that effect depends on the hemisphere. Kindling of the left medial or lateral amygdala had an anxiolytic effect. In contrast, right medial or lateral amygdala kindling tended to be anxiogenic.

Also, simply placing the electrodes in the left or right hemispheres may have affected anxiety levels. Hemispheric differences were found in Control rats with electrodes in the Medial/Lateral amygdala targets. Left hemisphere Control rats

were more anxious than right hemisphere Controls (Figure 1). This suggests that electrode damage in the left hemisphere may be anxiogenic, and possibly anxiolytic in the right hemisphere. In the absence of an unoperated control, it cannot be said for certain if this is true. Nevertheless, it has recently been shown that placing an electrode in the right medial amygdala is anxiolytic (Adamec and McKay, 1992, submitted).

There were different effects of kindling of other than the medial or lateral amygdala on anxiety. The Outlier Kindled rats showed less anxiety than their Controls regardless of hemisphere. Central amygdala kindling, however, had no effect on anxiety.

The present results suggest that the left medial/lateral amygdalas may be responsible for 'generating' or 'mediating' an anxiolytic response. The right medial/lateral amygdalas may be responsible for generating an anxiogenic response. Outside the medial/lateral nuclei the amygdala appears to mediate an anxiolytic response, or have no effect on anxiety at all (Central amygdala).

For these effects to be considered selective to anxiety, the effects of kindling on exploratory tendency and activity must be shown to be independent of the effects on anxiety measures. Kindling does influence exploration in the hole board. Lateral amygdala Kindled animals Head Dipped more than

all other groups (which were equal). Head Dipping is considered to be a measure of exploratory behavior (File and Wardill, 1975b), therefore, Lateral Kindled animals show more exploratory behavior than other groups of animals. Increased exploratory behavior could explain the anxiolytic-like effects in the plus maze, since increased exploration of the open arms could increase Ratio Time. However, if exploration of the plus maze were increased, one would also expect more total entries into the arms of the maze in kindled rats. This was not observed. Also, the pattern of results for raw Head Dipping is not the same as that for Ratio Time. Neither is it the same when average current was covaried out of the data. Therefore, Head Dipping and anxiety measures appear unrelated.

Furthermore, Adamec (1990b) found kindling reduced Head Dipping as well as Ratio Time. To control for the possibility that behavioral changes in the plus maze did not reflect a change in exploratory motivation, he covaried Head Dipping from anxiety measures. Removing the effects of Head Dipping did not alter the main effects of kindling and anxiety. He concluded that kindling-induced changes in exploratory motivation did not account for the effect of kindling on anxiety measured in the plus maze (Adamec, 1990b).

Finally, kindling was without effect on rearing or time active in the hole board. Rearing is considered to be a measure of activity (File and Wardill, 1975a). Therefore,

changes in activity cannot account for the effects of kindling on behavior in the plus maze. The kindling effects on anxiety measures appear to be selective for anxiety.

Left hemisphere Kindled animals engage in more frequent Risk Assessment than Left Not Kindled animals. This parallels the effects of kindling on Ratio Time and suggests that frequency of Risk Assessment is a valid measure of anxiousness. Left Kindled animals are willing to take more risks than Controls probably because they are less anxious than Controls. However, the Right Kindled and Not Kindled rats do not differ (and are the same as the left Not Kindled animals). The lack of a trend toward less Risk Assessment in the right hemisphere does not parallel the trend toward increased anxiety (decreased Ratio Time).

The work of Blanchard's research group also suggests that risk taking behavior is a valid measure of anxiety. They found that the frequency with which rats would extend their head out of a tunnel decreased after the presentation of a cat (Blanchard & Blanchard, 1989). This decrease in risk taking reflects an increase in defensive behavior in the rat. In a follow-up study they tested the effects of diazepam (an anxiolytic drug) on Risk Assessment. Diazepam increased Risk Assessment behavior (Blanchard et al., 1990). These findings suggest that risk taking behavior is a valid measure of anxiety.

In contrast, time spent in Risk Assessment was unaffected by kindling. In addition, when total current passed is covaried out of time in risk assessment the hemisphere by amygdala nucleus interaction disappears. Time spent in risk assessment seems to be unrelated to anxiety measured in the plus maze.

There were also differences in the Number of Boli measured in the hole board for Outlier rats. The Number of Boli might be considered a measure of anxiety (File and Wardill, 1975b). Left hemisphere Medial amygdala Kindled animals left fewer boli than all other groups of rats (which were equal, Figure 8). Since, this measure doesn't vary in the same pattern as Ratio Time, Number of Boli appear not to be related to measures of anxiety in the plus maze. Moreover, On Target animals showed no differences in number of boli in the hole board, but there were differences in Ratio Time.

A possible explanation for the change in behavior caused by kindling may be a change in the availability of the inhibitory neurotransmitter - GABA. In 1989, Gean et al. found that <u>in vivo</u> kindling of the basolateral amygdala resulted in a lasting decrease of GABAergic inhibitory post synaptic potentials contralateral to the kindling site. This loss of inhibition in the basolateral amygdala was accompanied by an increase in excitability in that nucleus. They also found spontaneous and evoked epileptiform bursting and extra evoked

synaptic potentials, which were depressed by NMDA receptor antagonists. This reduction in inhibition and the increase in excitability of amygdala cells in different nuclei could mediate the changes in anxiety following kindling. Data from the present study, and those of Adamec (1990b) for Right Medial Kindled rats, suggest that increased sub-nucleus excitability following kindling may be anxiogenic or anxiolytic.

Comparisons with Previous Studies

The present findings are both consistent and inconsistent with previous work. Adamec (1990b) and Henke and Sullivan (1985) found that kindling the right medial amygdala produced both anxiogenic effects and increased susceptibility to stress ulcers. Consistent with these findings is the trend toward an anxiogenic effect of kindling the Right Medial amygdala observed here. In contrast Nieminen et al. (1992) reported that left lateral amygdala kindling produced an anxiogenic effect. The present study found an anxiolytic effect of kindling of the Left Lateral amygdala. Also, Witkin et al. (1988) observed that right hemisphere lateral amygdala kindling was anxiolytic. This report is inconsistent with the present findings that Left (and not Right) Lateral amygdala kindling is anxiolytic.

Electrode placement may be an important factor in these differences in results. The findings of this thesis suggest that electrode placement is linearly related to the effects of kindling on anxiety. There are likely very specific anatomical areas where kindling may have anxiolytic, anxiogenic or no effects on anxiety (Adamec, 1990d).

Anatomical location of stimulating electrodes may explain the fact that only a trend toward an anxiogenic effect of Right Medial amygdala kindling was found in the present study. Comparisons were made between electrode location in these animals with electrode location of On and Off Target animals in Adamec (1990b). On and Off Target were defined as in the Medial amygdala or not, respectively. Adamec (1990b) found anterior Medial amygdala kindling to be anxiogenic, while more posterior Medial-cortical amygdala kindling was anxiolytic. In fact, there was a negative correlation between anxiety and AP plane in Adamec's (1990b) study ($r=-.506$, $p<.05$). Electrodes of Right Medial and Right Outlier Kindled rats in the present study fell between Adamec's On and Off Target groups in the AP plane. Moreover, a similar correlation between anxiety and AP plane location of kindling electrodes was seen in the present study ($r=-.509$, $p<.05$). Therefore the trend toward (right hemisphere) anxiogenic effects in the present study could be explained by the more posterior location of the kindling electrodes. Furthermore, the lack of significant effect on

anxiety might represent a cancellation of both anxiogenic and anxiolytic effects of kindling these animals.

There were also differences between the two studies in electrode placements in the lateral and vertical planes. In the lateral plane, Off Target animals in Adamec, 1990b were more medial than Off Target animals in the present study. Rats in this study also had electrodes deeper than in Adamec (1990b).

Correlations of electrode location with (relative) anxiety scores and vertical plane coordinates of the right hemisphere in this study showed that the deeper the electrode the more anxious kindled rats were relative to controls. Again, there is a graded change in anxiety as electrode location changes. Though the present study rats' electrodes were deeper than in Adamec's (1990b) study, they were less anxious. This suggests the AP plane bias is the determining factor.

The discrepancy in the effects of kindling on anxiety between this study and Adamec (1990b) seem to be due to the precise location of the stimulating electrode. It is possible, then, that the discrepancies between this study and other findings (Witkin et al., 1988; Nieminen et al., 1992) could also be due to differences in anatomical location of the stimulating electrode. Another look at Witken et al. (1988) shows that their target site, within the amygdala, could bring

the electrodes close to the intra-amygdaloid zone. This is an area which this study found to be an Outlier anxiolytic site. Unfortunately Witkin et al. (1988) did not give the exact location of their electrodes.

The finding that kindling the left lateral amygdala produced an anxiogenic effect (Nieminen et al., 1992) is a little more puzzling, in view of the present finding that left amygdala kindling is anxiolytic. Nieminen et al. (1992) did not specify the exact location of their electrodes either. They may have found an anxiogenic area in the left amygdala which was not detected in the present study.

One explanation for such anatomical specificity of kindling effects on behavior may be in differences in efferent pathways engaged by stimulating different foci. Anatomical data from the hamster show that there are different efferent pathways from the anterior/posterior medial amygdala (Gomez and Newman, 1992). An anterograde neuronal tracer injected into the hamster medial amygdala showed that the anterior (but not the posterior) Medial amygdala projects to the olfactory bulb, the intermediate part of the posterior bed nucleus of the stria terminalis, the lateral part of the medial preoptic areas, and the core of the ventromedial hypothalamus (VMH). The posterior region of the medial nucleus projects to the medial parts of all of these areas except the VMH. The

posterior region projects to the shell around the VMH (Gomez and Newman, 1992).

Together these data suggest that the anterior medial amygdala of the right hemisphere mediates some of the anxiogenic effects of right hemisphere kindling.

The differences between hemispheres in kindled animals might be explained by difference in electrode location. Electrodes of animals with implants in the left hemisphere were more ventrally placed than electrodes of animals with right hemisphere electrodes. On the other hand, kindling of more deeply placed electrodes was associated with greater anxiety in both hemispheres. So some other factor likely accounts for the hemispheric difference. It is not clear what that might be.

Although the left medial amygdala took slightly longer to kindle than any of the other groups of animals, it seems that none of the kindling parameters had any differential effects on anxiety. The greater amount of time it took the Left Medial animals to kindle was not reflected in any significant difference in anxiety between this group and the left Lateral animals. There were, otherwise, no differences between groups with Medial/Lateral, Outlier and Central amygdala electrode placements for any of the kindling parameters. Therefore variation in kindling parameters did not contribute to any of the group differences in anxiety.

The testing environment is another factor which may have reduced the anxiogenic effects of right medial amygdala kindling in the present study. Adamec (1990b) tested his rats in a plus maze which was in a room that often doubled as a testing room for cats. In the present study, behavioral testing was conducted in a room where cats had never been. It is possible that the presence of cat odours enhanced the effects of kindling on behavior in Adamec (1990b). It has been shown that cat odours increase anxiety-like behaviors in rats (Blanchard and Blanchard, 1989). Moreover, amygdala kindling in the cat increases defensive responsiveness to exogenous (rats - Adamec, 1991a) and endogenous stimuli (Hiyoshi et al., 1990) which elicit anxiety.

There is well documented evidence to show that kindling involves a long term change at the cellular level (Goddard, 1972; Goddard, 1983; Racine, 1978). Evidence now exists to suggest that these neuronal changes may contribute to changes in emotional behavior. The observation of amygdala kindling-induced changes in anxiety seen here complement and extend other studies: in the cat (Adamec and Stark-Adamec, 1983a; Adamec, 1990b) and the rat (Adamec, 1990b).

The discussion thus far has concentrated on areas other than the central amygdala. Kindling of the Right Central amygdala had no effect on behavior. There are several possible reasons for this finding: 1) the central amygdala is not

involved in mediating any aspect of anxiety in the rat; 2) Kindling does not affect the central amygdala in the same way as in other amygdaloid nuclei in the rat; or 3) kindling could be producing mixed effects in this area which cancel each other out.

Although several studies have implicated the Central amygdala in the anxious response (Yadin, et al., 1991; Grijalva et al., 1990), there is reason to believe that anatomical specificity and electrode location are crucial here also. Coover (1991) found that electrolytic lesions of the rostral third of the central amygdala produced a marked deficit in drinking passive avoidance, however lesions in the caudal third produced no deficit at all. In fact, lesions as little as 0.7mm dorsal to the middle third of the central amygdala or 0.7mm ventral to either the middle third or caudal third of the central amygdala did not produce any deficits. This suggests that kindling might induce anxiety if electrodes were restricted to the rostral third of the central amygdala.

If the rostral one third of the central amygdala mediates fearful and anxious response, then it might explain the lack of effect of kindling of the Central amygdala on anxiety in the present study. The central amygdala spans the AP coordinates of -1.46 to -3.0 from bregma (Paxinos and Watson, 1986). Therefore, the rostral one third spans the AP coordinates of -1.46 to -1.9. Central amygdala placements in

this study had an average AP plane location of -2.19 with a standard error of .06. Coover et al.'s (1992) lesions were centered on a location -2.12 from bregma with a standard error of .27. While these lesions would overlap with the area stimulated in the present study, they also involve tissue considerably anterior to that kindled. Therefore the electrodes in this study may well have been placed too posteriorly to induce an anxiogenic effect.

There is also other evidence for functional differentiation within the central amygdala. Harrigan et al. (1991) found immunohistochemical labelling of CRF was localized to the lateral part of the central nucleus. Also, Farb et al. (1991) showed that fibres from the lateral amygdala project differentially to the rostral and caudal parts of the central nucleus. The strongest projection was to the caudal central amygdala. More rostrally the greatest projections were in the ventral and lateral aspects of the central amygdala. Electrodes in the present study straddled the medial and central nuclei. If one or the other were more important in anxiogenic effects, then the straddled position might also contribute to the lack of behavioral effects.

Together the evidence indicates an intra-nucleic differentiation of the central amygdala with respect to neurochemistry and afferent and efferent projections. Moreover, there may be a functional differentiation in the

anterior-posterior plane like that suggested in the medial amygdala. Once again, careful attention should be paid to electrode placement in future studies.

Contribution of Electrode Damage and Current Spread to Behavioral Effects of Kindling

Given the apparent localization of effects of anxiety to particular regions of the amygdala, it is important to consider how damage due to electrode implantation and current spread during electrical stimulation might affect this localization. Diameter of the stimulating electrodes used (0.125 mm x 2 = 0.25 mm) is not trivial with respect to the size of some of the structures stimulated. Considering the care taken to locate each electrode to the centre of the medial and lateral amygdala in the lateral plane (of which the minimum size is 1 mm^3), it is not likely that the electrodes straddled any areas other than those targeted in On Target rats. Electrodes of this size did produce considerable damage relative to the size of the nucleus, however. Nevertheless, controls were carefully matched in electrode location to kindled rats. Since the main measure of anxiety showed a clear difference between Kindled and Not Kindled animals, damage alone could not account for the kindling induced changes in the behavior.

The issue of current spread is a more complex one. It relates to the question of degree of localization of stimulus effects. A number of studies have addressed the relationship between current intensity and distance of spread of excitatory effects. Ranck (1975) found that currents of 400-600 μA produced excitation of single myelinated axons only as far as 1-2 mm away. In addition, he demonstrated that smaller unmyelinated axons and cell bodies require currents of greater strength to excite them than myelinated axons. This finding was confirmed by Bagshaw and Evans (1976), who showed that excitation to conduction in an unmyelinated axon required as much as 700 μA of current only 1 mm away.

Of particular relevance is an extensive study by Watson et al. (1983). Spread of excitation from the tip of a stimulating electrode within the rat medial and lateral amygdala was visualized with ^{14}C-2-deoxyglucose (2-DG) autoradiography.

Electrode and stimulus parameters used by Watson et al. are very similar to those used in the present study. Their stimulating electrodes were .2 mm in diameter compared to .125 mm per pole in the present study. The current used was 200 μA peak, the same as the present study. The frequency of stimulation was 60 Hz, whereas 62.5 Hz was used in the present study. One msec biphasic pulse pairs were used, by Watson et al. and in the present study. Watson et al. found that

excitation decreased rapidly within fractions of a millimetre from the centre of the electrode tip. There was a 90 percent reduction within a sphere of .3 mm radius in the medial amygdala, and 70 percent reduction within a sphere of similar radius in the basolateral amygdala. Spread of excitation due to current spread is likely even more restricted in the present study. Monopolar stimulation was used by Watson et al., whereas bipolar stimulation was used in the present study. Given the wider diameter of their monopolar electrodes, and the use of a single electrode to stimulate, spread of current might be expected to be more widespread and diffuse in the Watson et al. study than in the present study.

Finally, Watson et al. found little overlap in areas activated by lateral and medial amygdala stimulation. This finding further supports the view that localized effects of stimulation do occur.

All of this information would suggest that spread of current in this study is not likely to have caused the activation of any structures other than the intended focus and its efferent pathways. Since the centres of various nuclei involved herein are at least 1 mm distance from each other, and are likely to be structurally different, the spread of activation from one to the other is probably of little concern. Finally, since there is a correlation between electrode distance from target site and change in anxiety

level for the medial amygdala, it seems likely that the effects of stimulating a precise location are particular to that location at the intensities used in the present study.

Implications for Human Epilepsy

It is an open question whether the long term changes (in brain function and behavior) seen following kindling models physiological and behavioral aspects of human epilepsy. Evidence exists to suggest that they do (Adamec, 1990a; Racine, 1978; Goddard, 1983). Moreover, the change in functioning of amygdala cells following kindling is consistent with processes hypothesized to underlie the 'global epileptic personality' (Bear, 1979). Bear (1979) suggested that alterations in behavior that accompanied temporal lobe epilepsy were caused by a progressive change in limbic structures secondary to a temporal epileptic focus. He argued that an epileptiform focus in the limbic system produced new functional connections between neocortical and limbic structures; he called this process 'sensory-limbic hyperconnection'.

Hyperexcitability in response to input is an effect of kindling. Moreover, kindling strengthens many synaptic pathway efferents to the kindled focus (Racine et al., 1983), with behavioral consequences (Adamec, 1991a).

If the changes involved in kindling are representative of the 'global epileptic personality' then the present study may serve as an interesting model. It seems that kindling of the Medial/Lateral amygdala changes the anxious state of the rat. These changes may be relatively stable (at least 1 week in this study, and Adamec 1900b, and two weeks in Nieminen et al., 1992). These kindling induced changes in anxiety may model aspects of anxiety in the human epileptic (Adamec, 1990a). This study adds further evidence that there is a link between limbic epilepsy which involves the amygdala and changes in anxiety levels.

Perhaps the most interesting finding in this report is the observation that kindling had differential effects in the two hemispheres. Very few studies have demonstrated hemispheric differentiations in rats (Denenberg et al., 1986; Fride and Weinstock, 1989 Mittleman et al., 1988). This may reflect an asymmetrical representation of emotion in rats, similar to the one seen in humans. Indeed, the pattern of results in this study fits well with the lateralization of emotion in humans proposed by Silberman and Weingarten (1986). An anxiogenic trend was noted for right hemisphere kindling, but not left. This may reflect the observation, in humans, that the right hemisphere (and not the left) is involved in depressive and unpleasant affects (Coffey, 1987; Swartzberg, 1983). The anxiolytic effects of left hemisphere kindling are

consistent with the view that the left hemisphere is specialized for positive affects (Silberman and Weingarten, 1986). Of course, it must be recalled that Outlier placements in both hemispheres were anxiolytic, so a strict separation of positive and negative affect modulation by kindling is not consistent with the data.

To sum up, the behavioral changes associated with the kindling phenomenon may model behavioral changes associated with human epilepsy. This study suggests, further, that precise location of the limbic focus is critical for the nature of the behavioral change induced by kindling. It would be of interest to determine if the same is true in human epilepsy.

Conclusions

This study supports the findings of previous studies that the amygdala modulates anxiety. Amygdala kindling, which induces neuronal hyperactivity changes the anxious response of rats. The effects are anxiolytic in the left amygdala over a broad range of nuclei. In the right amygdala the effects of kindling tend toward anxiogenesis in the Medial and Lateral nuclei. Anxiolysis is observed following kindling of tissue between the two nuclei.

The trend toward an anxiogenic effect of kindling the right medial or lateral amygdala may have been due to electrode placement. It seems that the locus of control of anxiety in the amygdala may be very localized.

Together the data suggest that large parts of the amygdala bilaterally function to reduce anxiety. Smaller areas which increase anxiety are interleaved within.

In addition, there was no effect of kindling in the central amygdala. It is suggested that there may be anxiolytic and anxiogenic control sites within the central amygdala. The results of this study may be due to activation of both of these loci.

Finally, future research in this area should closely control, and carefully describe, the locus of stimulation.

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TABLES

Table 1: Original Medial/Lateral and Central experimental groupings. The number of animals considered on target are indicated in each cell grouping (with the number of off target 'Outliers' in brackets).

KINDLED

Hemisphere:	Left	Right
Medial	n=10 (n=6)	n=13 (n=13)
Lateral	n=6 (n=7)	n=13 (n=14)
Central	--	n=10

NOT KINDLED

Hemisphere:	Left	Right
Medial	n=11 (n=9)	n=13 (n=13)
Lateral	n=7 (n=8)	n=17 (n=8)
Central	--	n=14

Table 2: Mean ± standard error of the mean (sem) of electrode location for Medial/Lateral, Outlier and Central amygdala rats for each Hemisphere (Left/Right).

Posterior to Bregma:		Left	Right
ON TARGET	MEDIAL	0.68 ± .11	1.33 ± .10
ON TARGET	LATERAL	2.37 ± .14	2.12 ± .10
RIGHT	CENTRAL		2.19 ± .04
OUTLIER	MEDIAL	0.85 ± .20	0.85 ± .15
OUTLIER	LATERAL	2.58 ± .20	2.13 ± .17

Lateral to Midline:		Left	Right
ON TARGET	MEDIAL	3.86 ± .08	3.93 ± .07
ON TARGET	LATERAL	4.68 ± .10	4.63 ± .07
RIGHT	CENTRAL		4.06 ± .04
OUTLIER	MEDIAL	3.95 ± .13	4.30 ± .10
OUTLIER	LATERAL	4.42 ± .13	4.4[illegible] ± .11

Ventral to Dura			
ON TARGET	MEDIAL	9.57 ± .14	9.05 ± .12
ON TARGET	LATERAL	8.90 ± .17	8.47 ± .11
RIGHT	CENTRAL		7.98 ± .06
OUTLIER	MEDIAL	9.75 ± .16	9.62 ± .12
OUTLIER	LATERAL	8.79 ± .17	8.50 ± .14

Table 3: Mean ± standard error of the mean (sem) of kindling parameters for Medial/Lateral, Outlier, and Central amygdala rats.

Electrode Location	Number of Stimulations to Stage 5 Seizure	Duration (sec)	Pause (days)
Medial/Lateral	(see Fig 6)	61.04 ± 4.2	3.88 ± 0.2
Outliers	11.81 ± 0.8	60.22 ± 4.6	3.44 ± 0.3
Central	9.46 ± 1.1	57.66 ± 6.2	2.93 ± 0.3

	Average Current Passed During Kindling (μC)	
	Left Hemisphere	Right Hemisphere
Medial/Lateral	86.2 ± 4.3	54.6 ± 3.5
Outliers	41.5 ± 4.3	37.7 ± 3.6
Central		28.17 ± 6.5

Table 4: Mean ± standard error of the mean (sem) of behaviors in the Hole Board and Plus Maze which were unchanged for Medial/Lateral, Outlier, and Central amygdala rats.

Time Active in the Hole Board

	Kindled Animals	Control Animals
Medial/Lateral	294.47 ± 2.08	293.13 ± 1.95
Outliers	294.83 ± 0.76	296.82 ± 0.78
Central	293.08 ± 2.01	290.41 ± 2.38

Head Dipping in the Hole Board

	Kindled Animals	Control Animals
Medial/Lateral[1]	-	-
Outliers	6.95 ± 0.60	7.54 ± 0.61
Central	5.77 ± 0.98	7.00 ± 1.15

Number of Boli in the Hole Board

	Kindled Animals	Control Animals
Medial/Lateral	.19 ± 0.15	.50 ± 0.14
Outliers[2]	-	-
Central	.42 ± 0.13	.56 ± 0.13

Number of Boli in the Plus Maze

	Kindled Animals	Control Animals
Medial/Lateral	.19 ± 0.12	.42 ± 0.11
Outliers[2]	.02 ± 0.10	.24 ± 0.10
Central	.00 ± 0.00	.00 ± 0.00

Frequency of Risk Assessment in the Plus Maze

	Kindled Animals	Control Animals
Medial/Lateral[3]	-	-
Outliers	13.24 ± 0.59	12.75 ± 0.60
Central	12.29 ± 0.73	11.30 ± 0.87

Table 4 (continue): Mean + standard error of the mean (sem) of behaviors in the Hole Board and Plus Maze which were unchanged for Medial/Lateral, Outlier, and Central amygdala rats.

Time Spent in Risk Assessment in the Plus Maze

	Kindled Animals	Control Animals
Medial/Lateral[4]	-	-
Outliers	41.08 ± 3.16	41.01 ± 3.24
Central	48.39 ± 6.02	48.71 ± 0.71

[1]·See Figure 4

[2]·See Figure 8

[3]·See Figure 2

[4]·See Figure 3

Table 5: Correlations of electrode location (AP, lateral, and vertical planes) and relative anxiety (RANX) score (as defined in the text).

Right Hemisphere

	AP	Lateral	Vertical
RANX	-.509	.050	.876
p<	.001	ns	.001

Left Hemisphere

	AP	Lateral	Vertical
RANX	.053	.062	.362
p	ns	ns	.05 (1 tailed)

ns = not significant

FIGURES

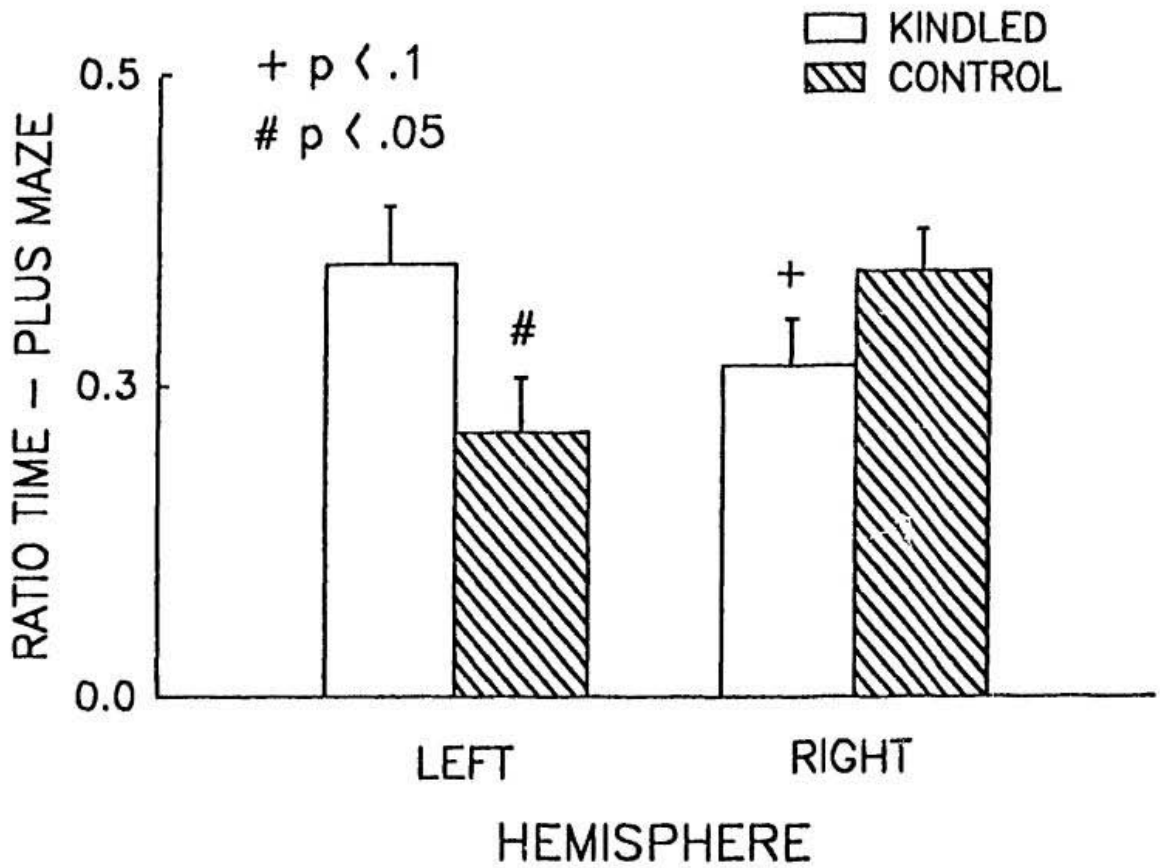


Figure 1. Plotted in the figure are mean ± SEM (standard error of the mean) Ratio Time in the elevated plus maze for rats with electrodes verified to be within the Medial or Lateral amygdala. Means are collapsed over amygdala nucleus and plotted separately for Kindled and Control animals with electrodes in the right and left hemisphere. Marked means differ from unmarked means. The right Kindled mean marked with a '+' tends to differ from the right hemisphere Control.

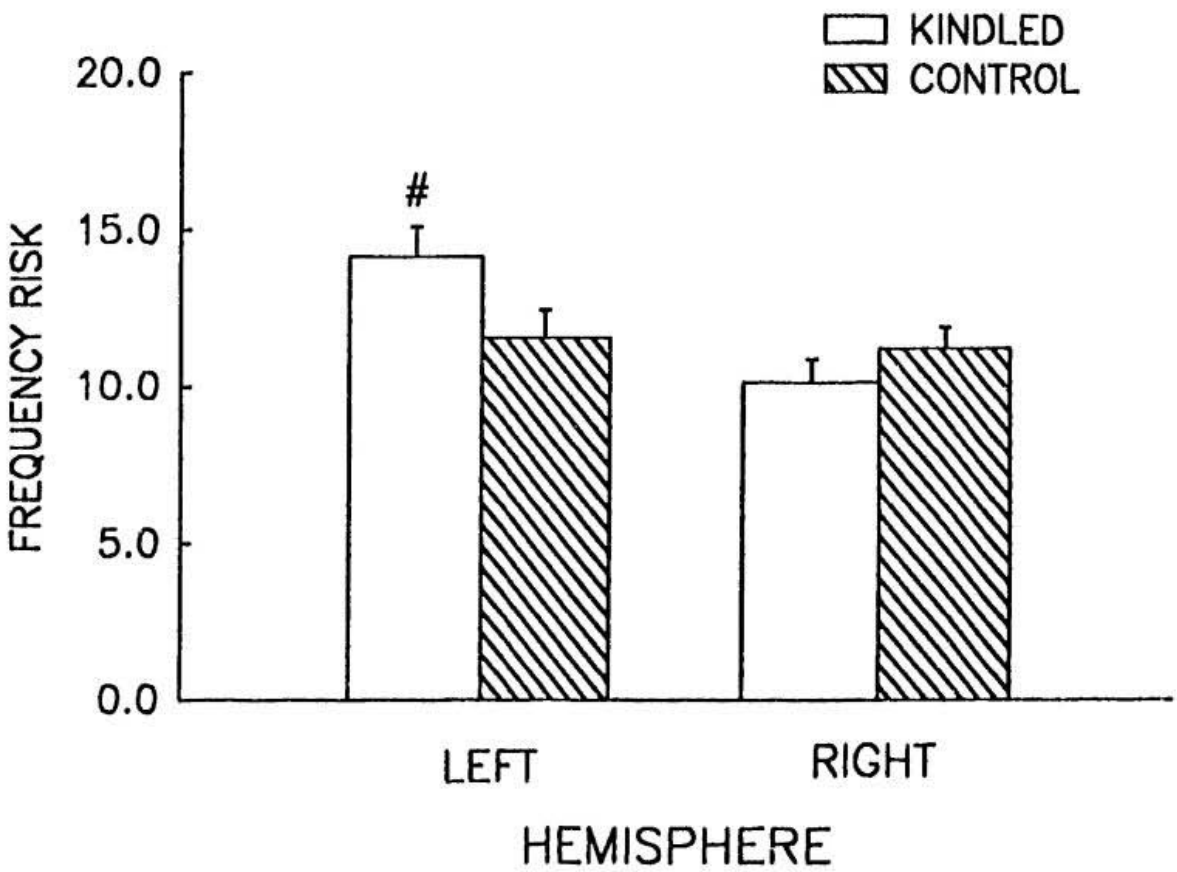


Figure 2. Plotted in the figure are mean ± SEM Frequency Risk observed in the elevated plus maze for rats with electrodes verified to be within the Medial or Lateral amygdala. Means are collapsed over amygdala nucleus and plotted separately for Kindled and Control animals with electrodes in the right and left hemisphere. The left Kindled mean marked with a # is larger than all the other groups, which do not differ from each other.

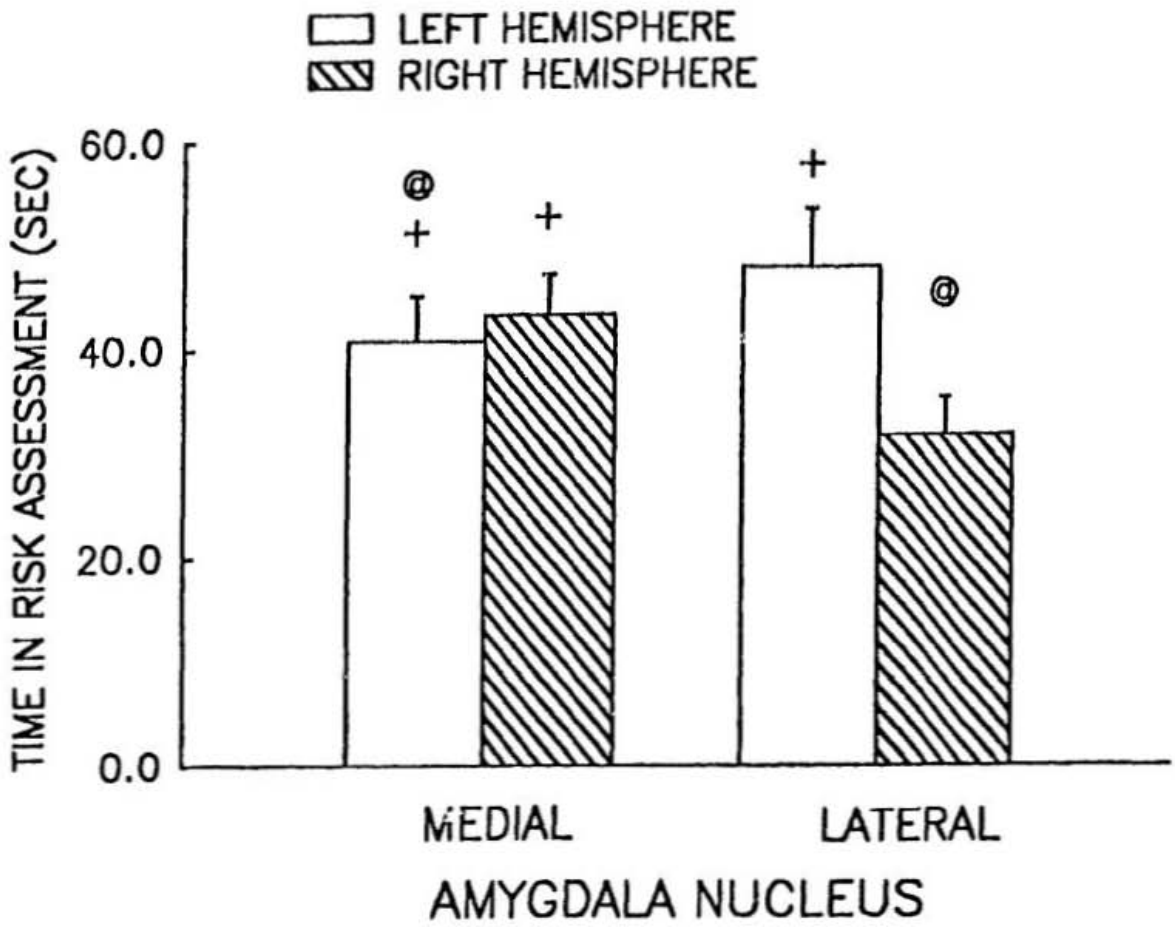


Figure 3. Plotted in the figure are mean ± SEM Time Risk observed in the elevated plus maze for rats with electrodes verified to be within the Medial or Lateral amygdala. Means are collapsed over kindling (Kindled/Not Kindled) and plotted separately for Medial and Lateral amygdala and left and right hemisphere. Means marked similarly do not differ, but differ from means marked with a different symbol.

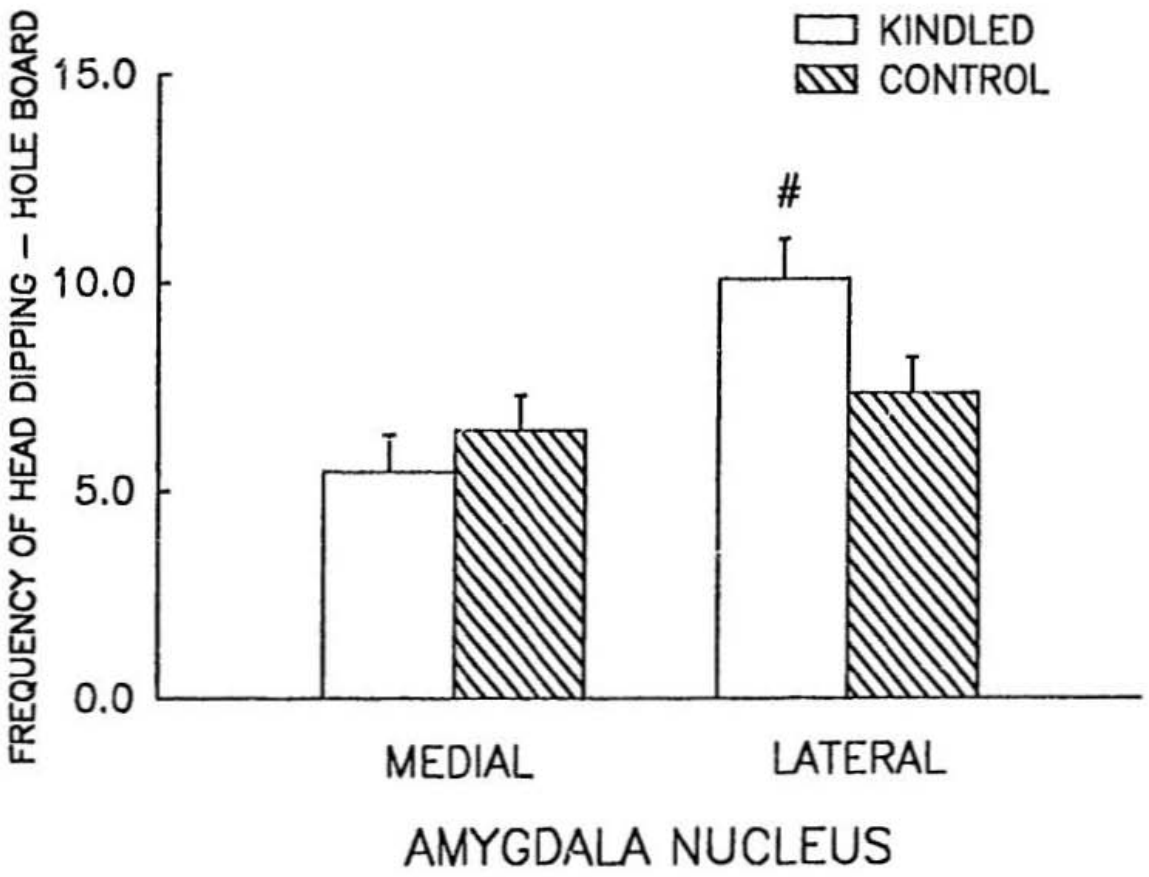


Figure 4. Plotted in the figure are mean ± SEM Frequency of Head Dipping observed in the hole board for rats with electrodes verified to be within the Medial or Lateral amygdala. Means are collapsed over hemisphere and plotted separately for Kindled and Control animals with electrodes in the Medial and Lateral amygdala. The Lateral Kindled mean marked with a # is larger than the all the other groups, which do not differ from each other.

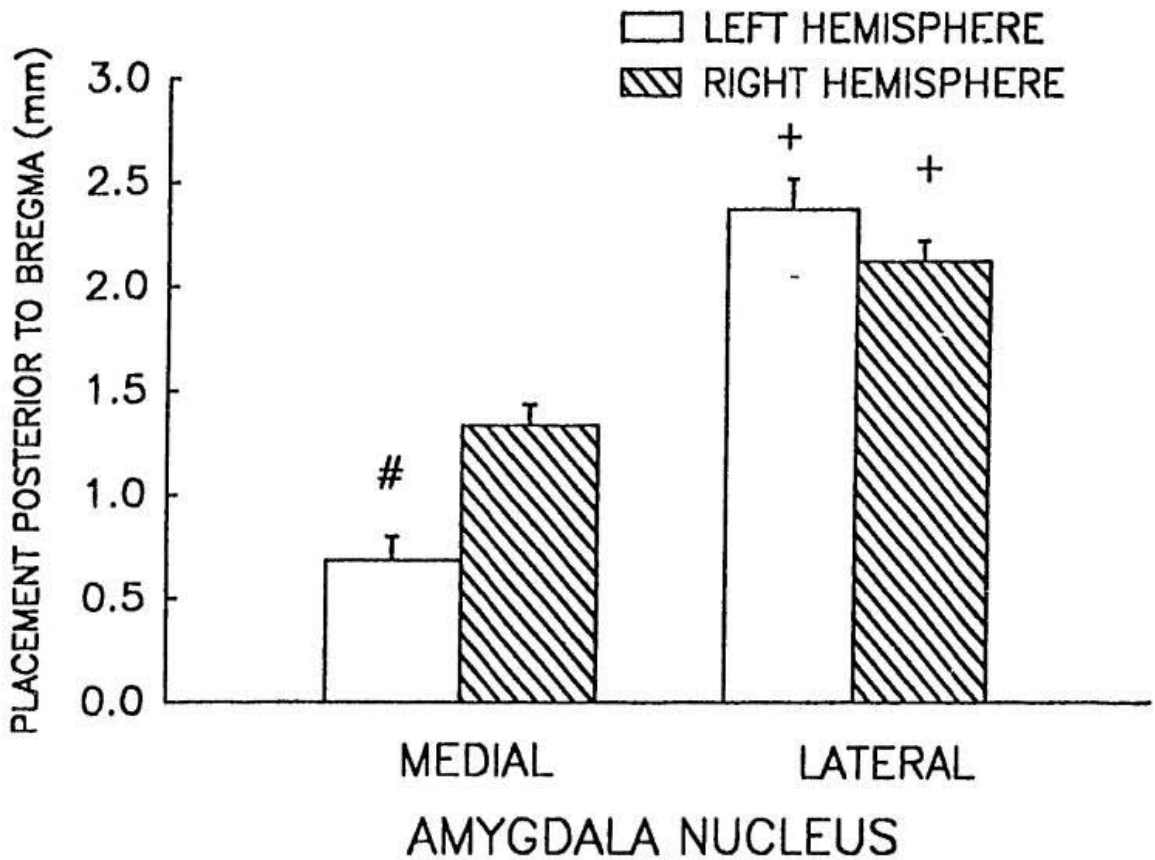


Figure 5. Plotted in the figure are mean ± SEM AP plane electrode coordinates in mm for rats with electrodes verified to be within the Medial or Lateral amygdala. Means are collapsed over kindling (Kindled/Not Kindled) and plotted separately for Medial and Lateral amygdala nuclei and for left and right hemispheres. Means marked similarly do not differ, but differ from means marked with a different symbol.

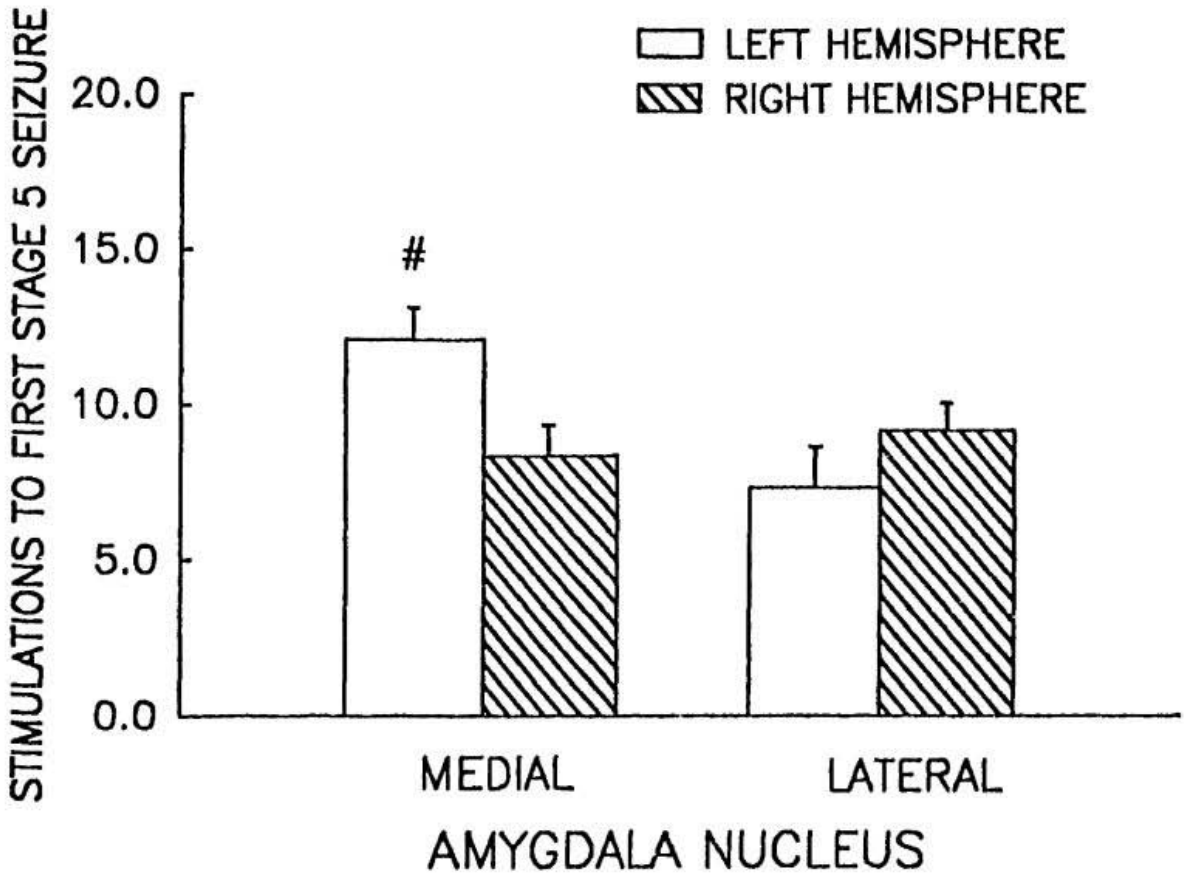


Figure 6. Plotted in the figure are mean ± SEM for number of stimulations to stage 5 seizure for rats with electrodes verified to be within the Medial or Lateral amygdala. Means are collapsed over kindling (Kindled/Not Kindled) and plotted separately for Medial and Lateral amygdala and for the left and right hemispheres. The left Medial mean marked with a # is larger than all other groups, which do not differ from each other.

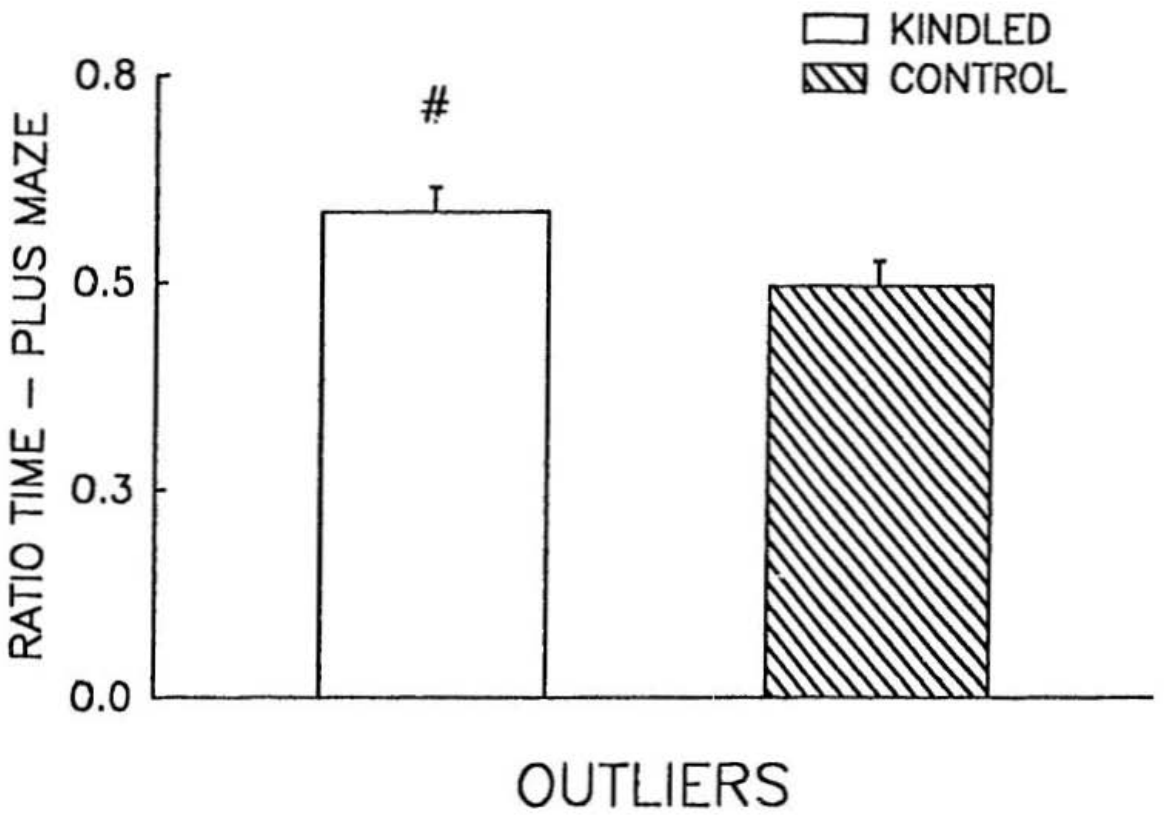


Figure 7. Plotted in the figure are mean ± SEM for Ratio Time observed in the plus maze for 'Outlier' rats collapsed over Medial/Lateral and Hemisphere. Means are for Kindled and Not Kindled (CONTROL) animals. The mean for Kindled animals marked with a # is larger than the mean for CONTROL animals.

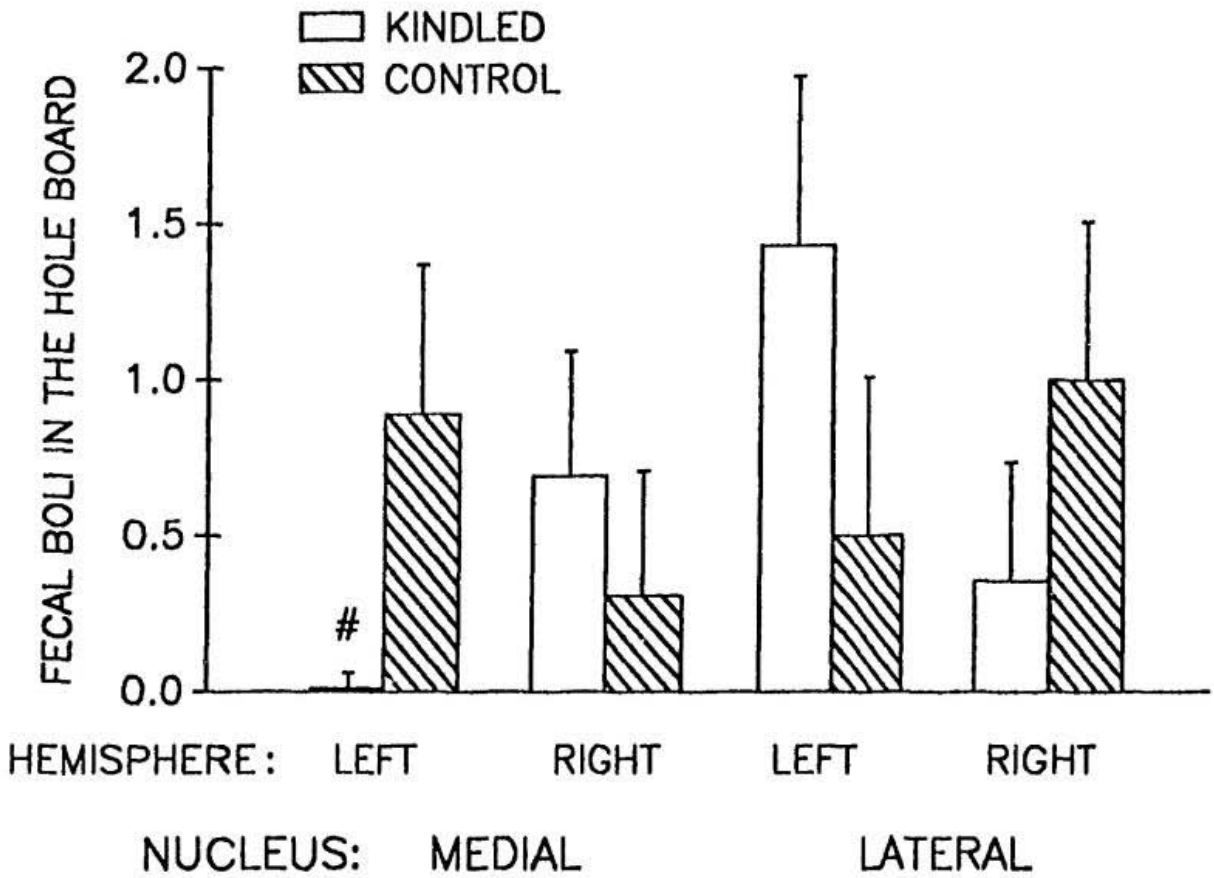


Figure 8. Plotted in the figure are mean ± SEM for Faecal Boli left in the Hole Board test for 'Outlier' rats. Means are plotted separately for Medial/Lateral, Left/Right hemisphere, and Kindled/Control groups. The mean marked with an "#" differs from all other groups, which do not differ from each other.

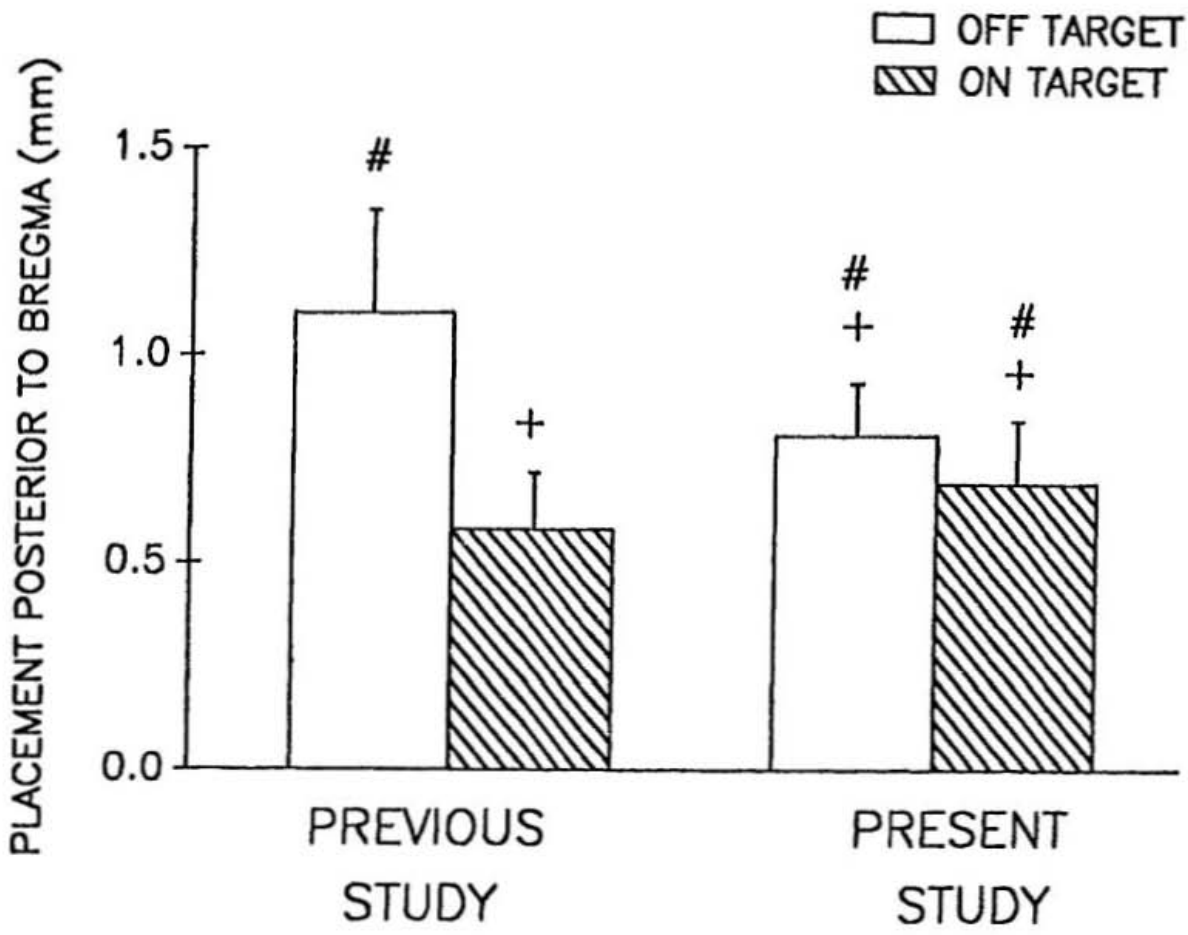


Figure 9. Plotted in the figure are mean ± SEM AP plane electrode coordinates in mm for rats with electrodes aimed at the right Medial amygdala in the Present study and in a Previous study (Adamec, 1990b). Means are collapsed over kindling and plotted separately for the Present and Previous studies and Off and On Targets. Means marked similarly do not differ, but differ from means marked with a different symbol.

APPENDIX A

RATS 1-121 (146-187)

Listed are all of the rats by nucleic electrode location (see Abbreviation Index). Groups are: Right Hemisphere Kindled (RK), Right Hemisphere Not Kindled (RNK), Left Hemisphere Kindled (LK) and Left Hemisphere Not Kindled (LNK) for Medial and Lateral target sites.

Lateral On Target Rats

BLA	BLV	BLA/BLV
RNK 5	RNK 23	RNK 70
RNK 64	RNK 30	RNK 97
RNK 79	RNK 180	
RNK 95	RNK 184	LNK 111
RNK 119		
RNK 175	RK 178	LK 22
RNK 176		LK 49
	LNK 18	
RK 66		
RK 75	LK 53	
RK 84		
RK 174		
RK 177		
RK 185		
RK 186		
LNK 54		
LNK 58		
LNK 78		
LK 11		
LK 90		
LK 104		
LK 121		

Lateral Off Target Rats

BLA/BLV VICINITY	BLA/ACE
RNK 86	RNK 48
RNK 70	RNK 179
RK 3	RK 47
RK 9	RK 77
RK 107	RK 118
RK 181	
	LK 60
LK 57	

Medial On Target Rats

AME	
RNK 14	LNK 8
RNK 76	LNK 29
RNK 96	LNK 52
RNK 152	LNK 59
RNK 164	LNK 74
	LNK 120
RK 10	LNK 149
RK 41	LNK 154
RK 43	LNK 158
RK 73	LNK 171
RK 88	
RK 151	LK 4
RK 160	LK 12
RK 163	LK 46
RK 168	LK 51
	LK 56
	LK 81
	LK 85
	LK 153
	LK 162
	LK 169
	LK 172

Medial Off Target Rats, continued

ACO/AME

RNK 106
RNK 159
RNK 173

RK 115
RK 155

LNK 146
LNK 161

LK 157

ACE

RNK 183

RK 147

LNK 50
LNK 68

LK 62
LK 92

ACO

RNK 24
RNK 35
RNK 100
RNK 102

RK 98

LNK 80
LNK 109

LK 28

PIR CTX

RNK 19
RNK 31
RNK 87
RNK 113
RNK 167

RK 99
RK 182

LNK 6
LNK 15
LNK 101

LK 109
LK 150*

IM

LK 7

RK 39

ABM

RK 55
RK 170*

LNK 45

BST1A

LNK 63
LNK 65

ZT

RK 21

RNK 31

BMP

LK 26

LNK 34

SUBICULUM

RNK 44

RK 33

LK 36

ABL

RNK 42

RK 112

AAA

LNK 91

CPU

187 RNK

Medial Off Target Rats

AME VICINITY

RNK 82
RNK 83
RNK 148

RK 2
RK 103

LNK 16
LNK 69
LNK 165

LK 20

RATS 122-144

Listed are all of the rats by nucleic electrode location. Groups are Kindled (K) and Not Kindled (NK) for Central amygdala target sites.

Central On Target Rats

CEM (PV)	CEL (CN)	CEL/BLA
NK 123	NK 125	NK 129
NK 138	NK 131	
NK 139	NK 142	K 126
NK 143		
	K 124	
K 122	K 128	
K 132	K 140	
K 135	K 141	

Central Off Target Rats

BLA	IM	BST1A
K 137	NK 127	NK 134
	NK 136	

CPU

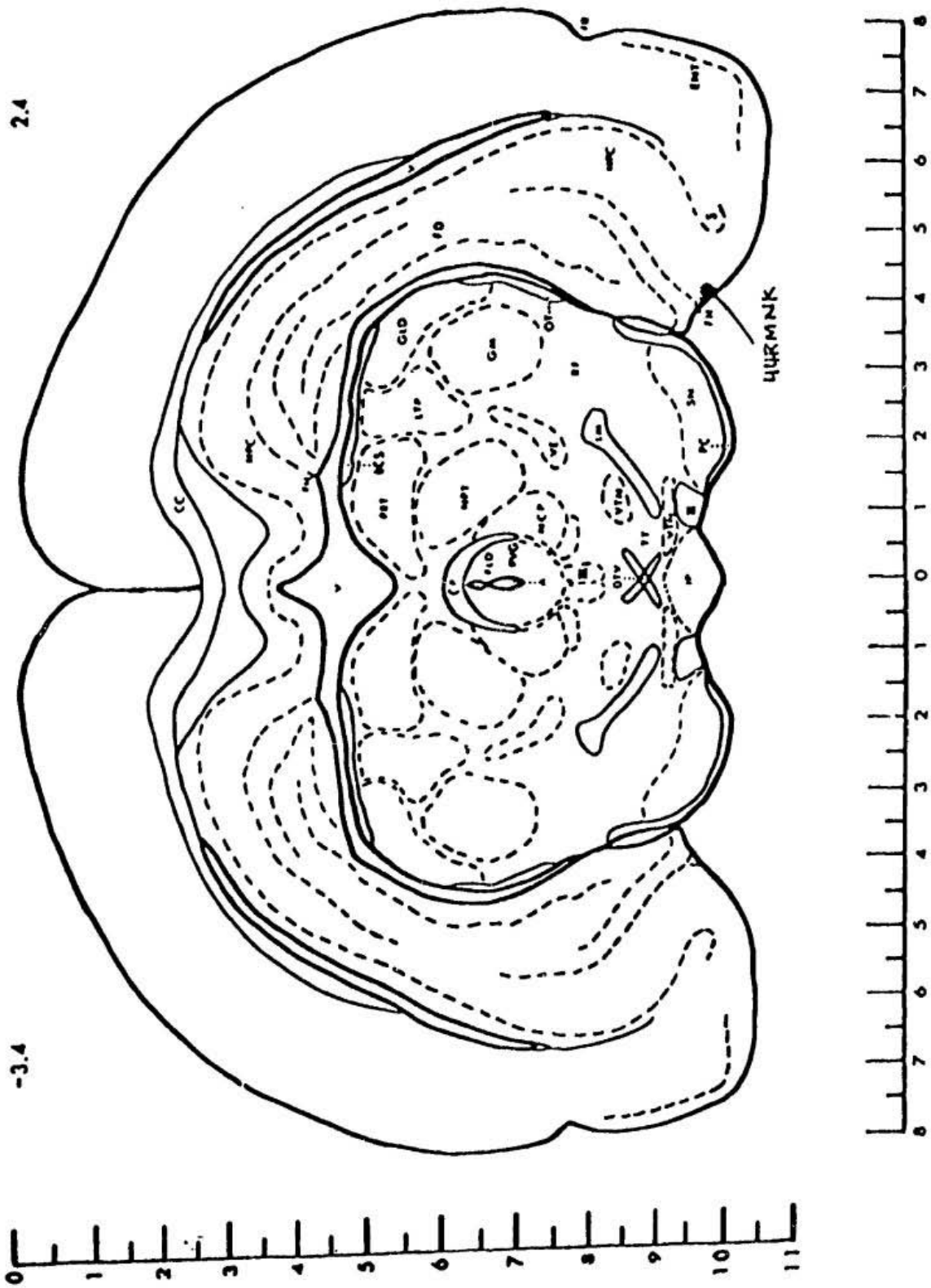
K 144

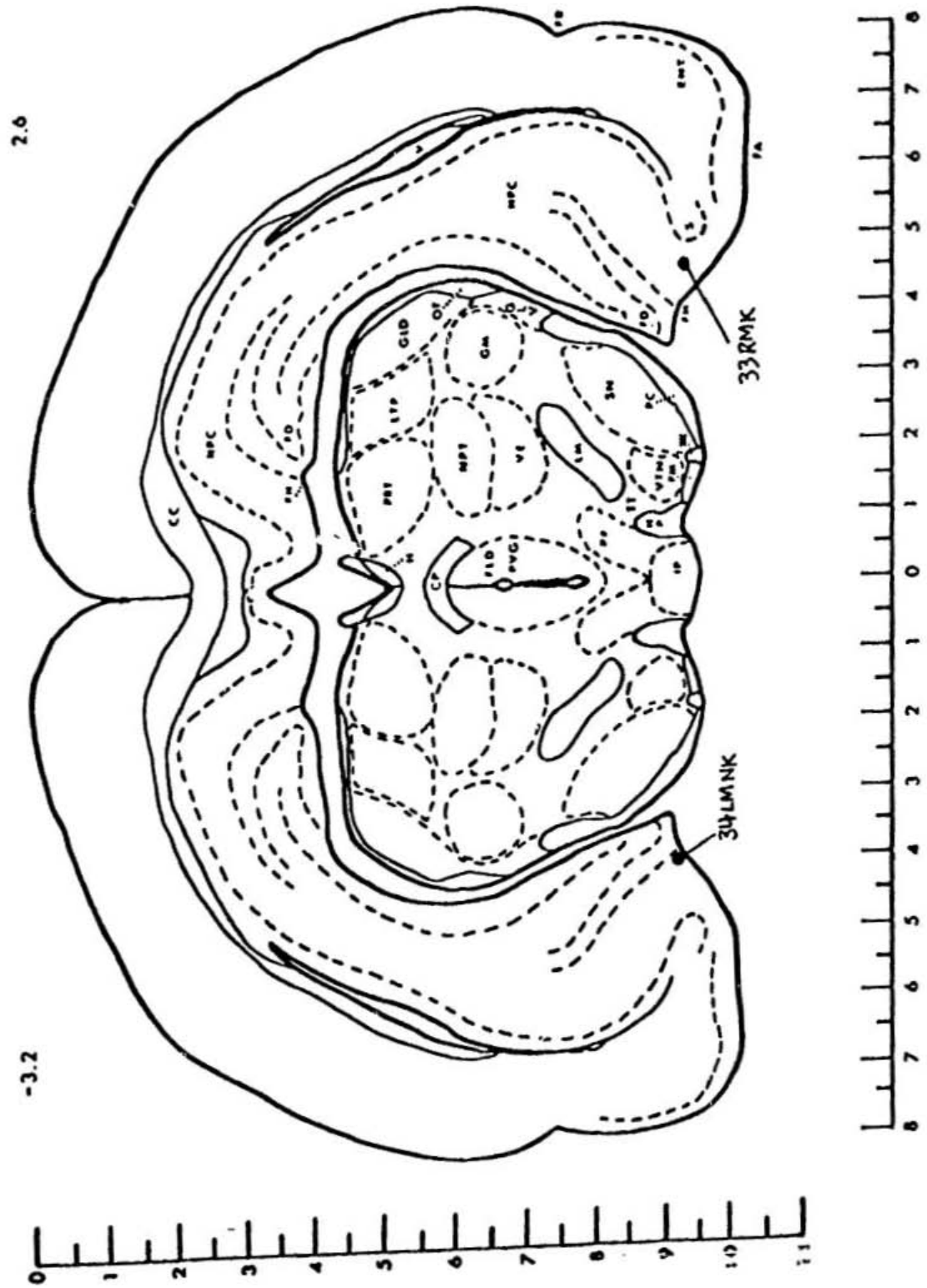
Index of Abbreviations

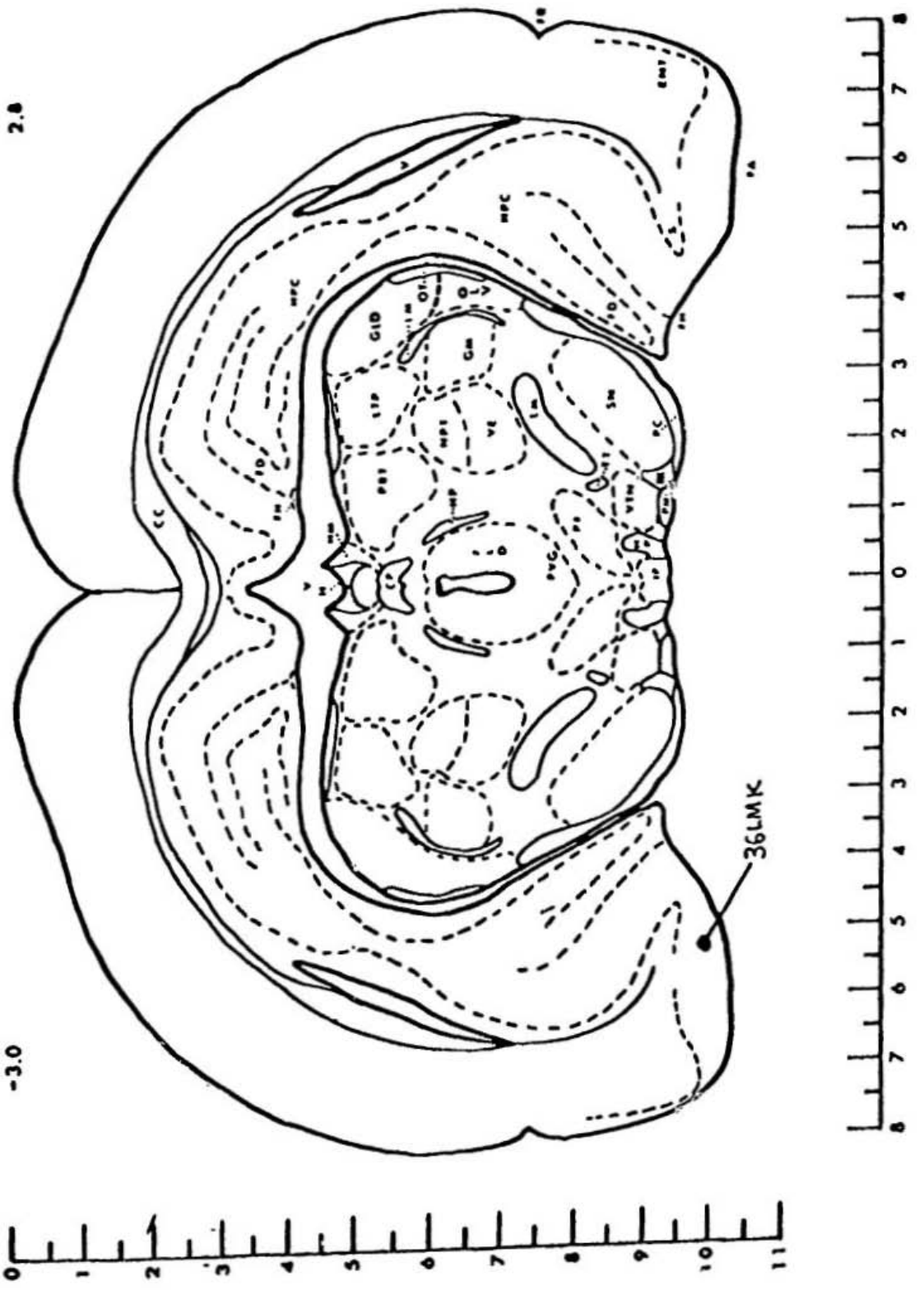
The following is a list of abbreviations used to identify the various nuclei of the amygdalar region in Appendices A and B.

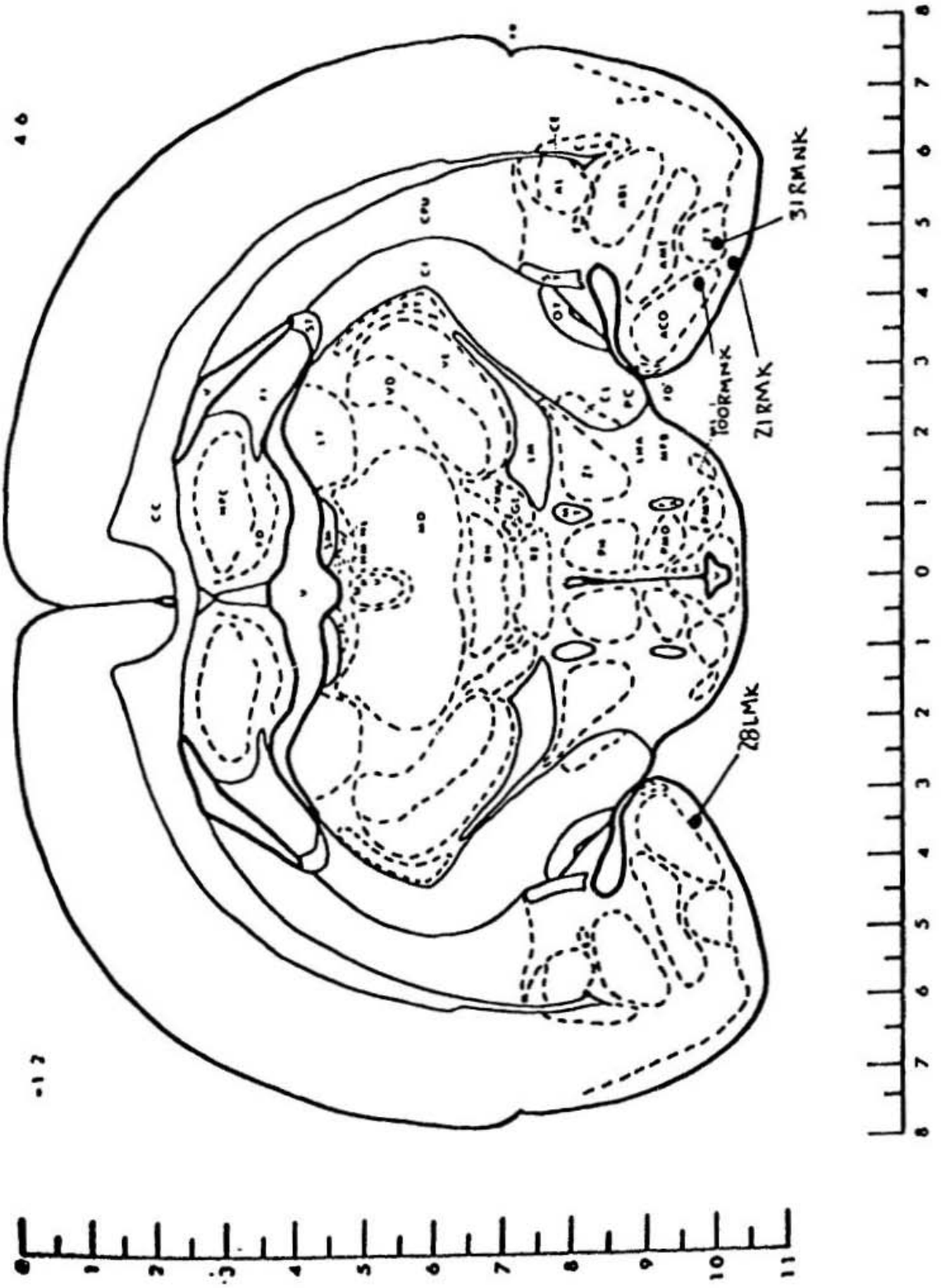
AAA	Anterior Amygdaloid area
ABL	Basal Amygdaloid Nucleus (Lateral part)
ABM	Basal Amygdaloid Nucleus (Medial part)
ACE	Central Amygdaloid Nucleus
ACO	Cortical Amygdaloid Nucleus
AL	Lateral Amygdaloid Nucleus
AME (ME)	Medial Amygdaloid Nucleus
BLA	Basolateral Amygdaloid Nucleus (Anterior)
BLP	Basolateral Amygdaloid Nucleus (Posterior)
BLV	Basolateral Amygdaloid Nucleus (Ventral)
BM	Basomedial Amygdaloid Nucleus
BST	Bed Nucleus of the Stria Terminalis
BST1A	Bed Nucleus of the Stria (Intra amygdaloid div)
PIR	Piriform Cortex
HPC	Hippocampus (Ammon's Horn)
ZT	Transitional Zone of the Amygdala
CLA	Claustrum
CE	External Capsule
CEL	Central Amygdaloid Nucleus (Lateral)
CELCN	Central Amygdaloid Nucleus (Lateral) Central part
CEM	Central Amygdaloid Nucleus (Medial)
CEMPV	Central Amygdaloid Nucleus (Medial) Posteroventral
CPU	Caudate Putamen
IM	Intercalated Amygdaloid Nucleus
MEPD	Medial Amygdala (Posterodorsal)
MEPV	Medial Amygdala (Posteroventral)_

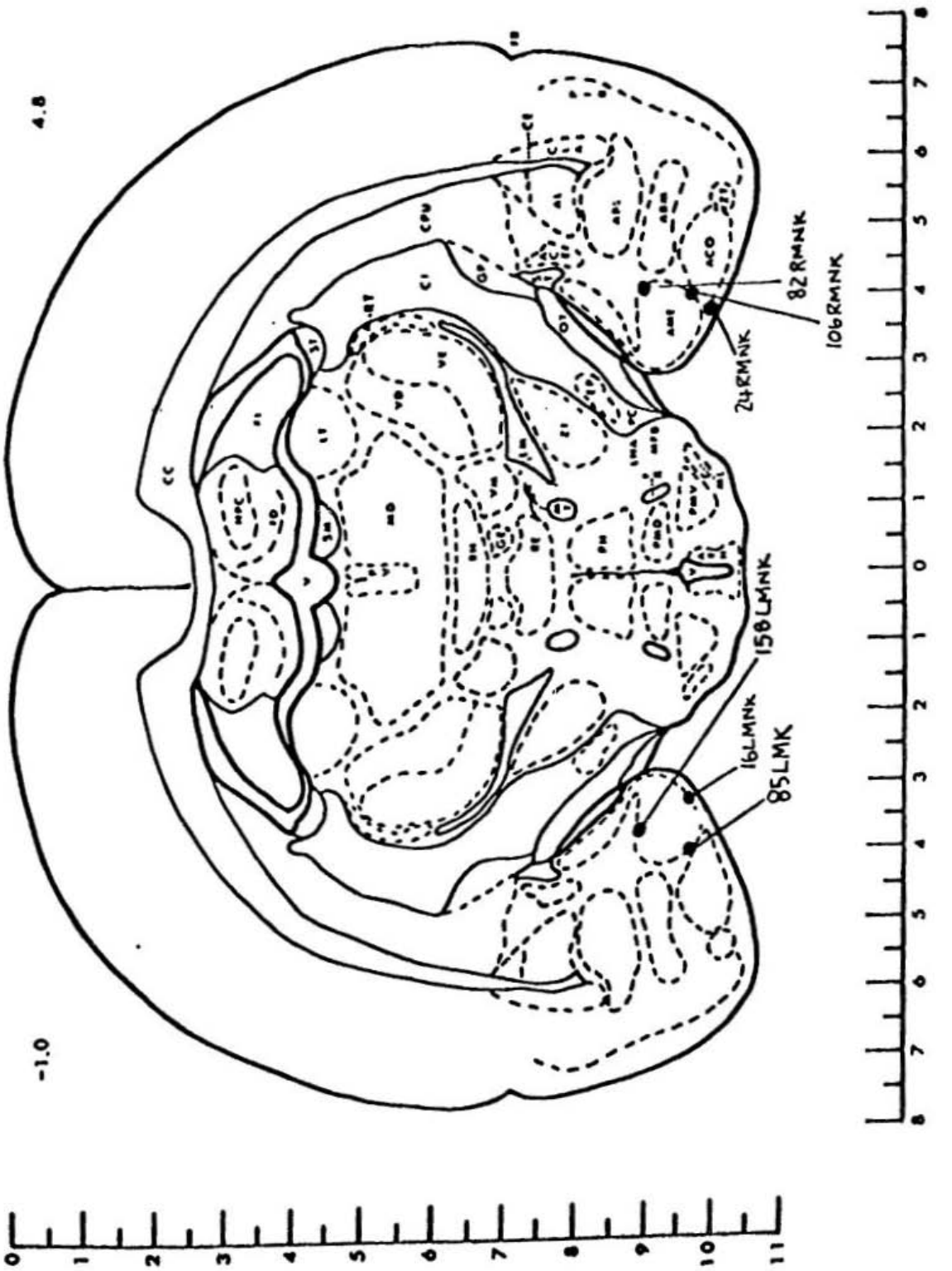
APPENDIX B

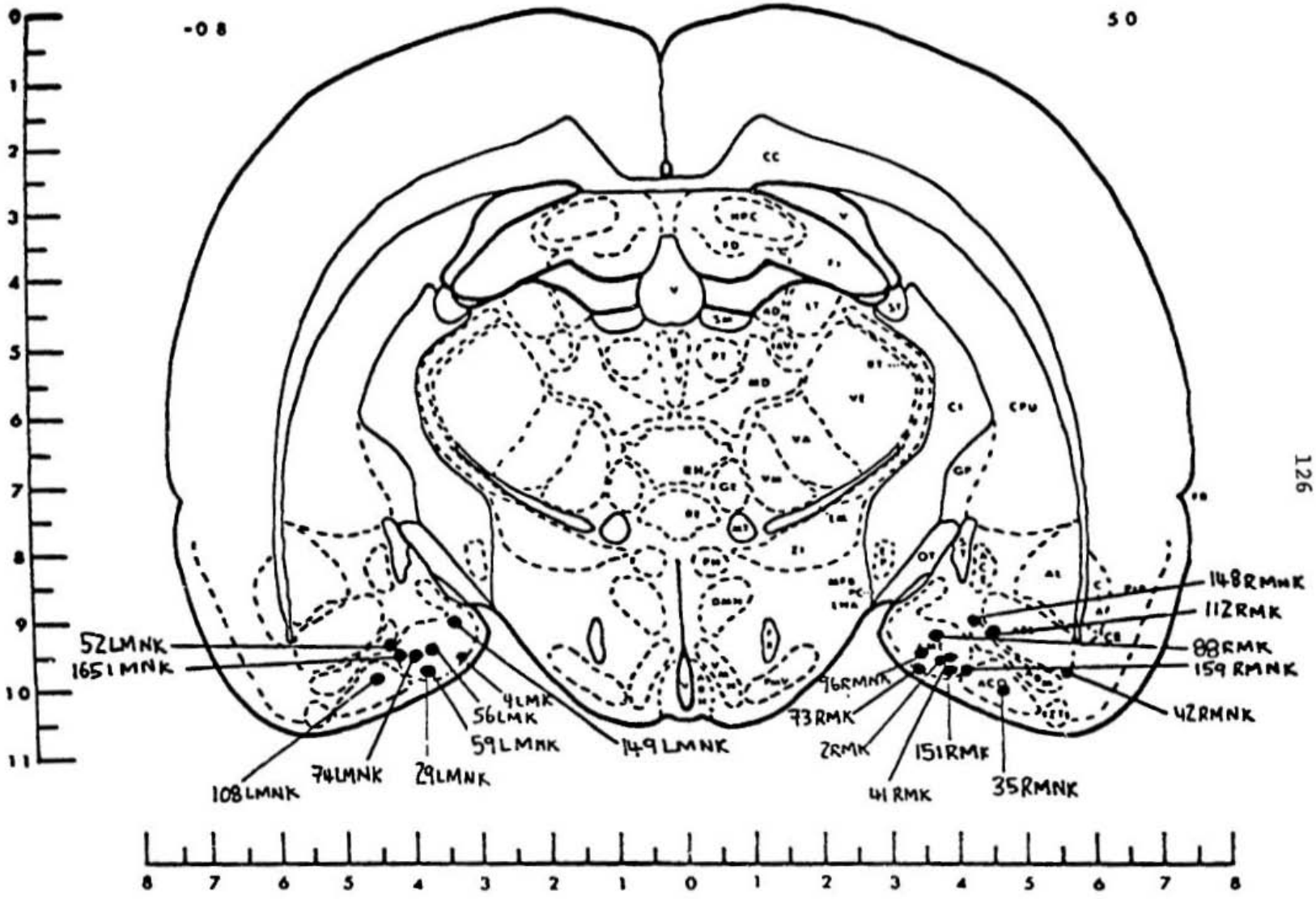

2.4
-3.4
0
1
2
3
4
5
6
7
8
9
10
11
8
7
6
5
4
3
2
1
0
1
2
3
4
5
6
7
8

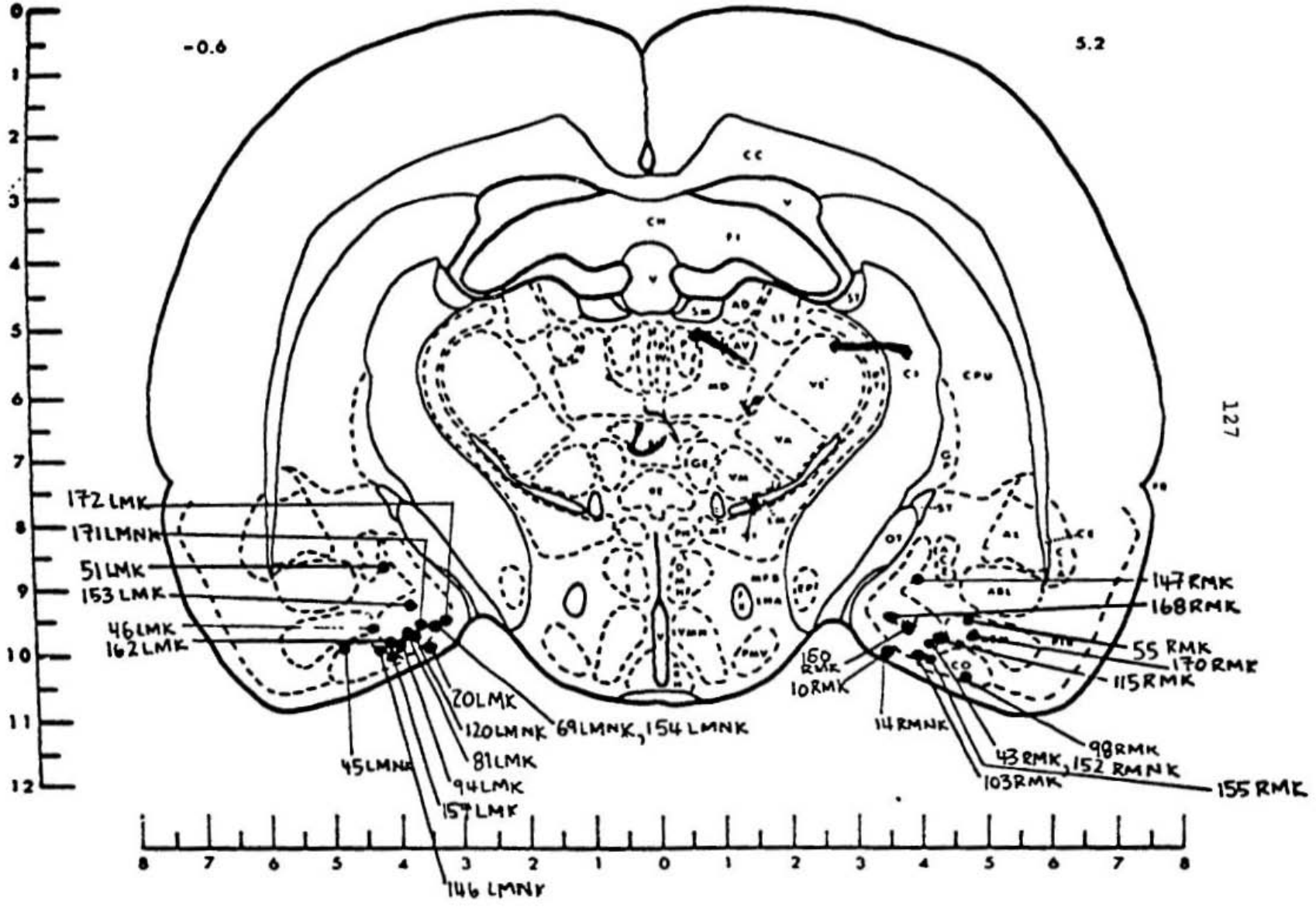

2.0
-3.2
33RMK
34LMNK

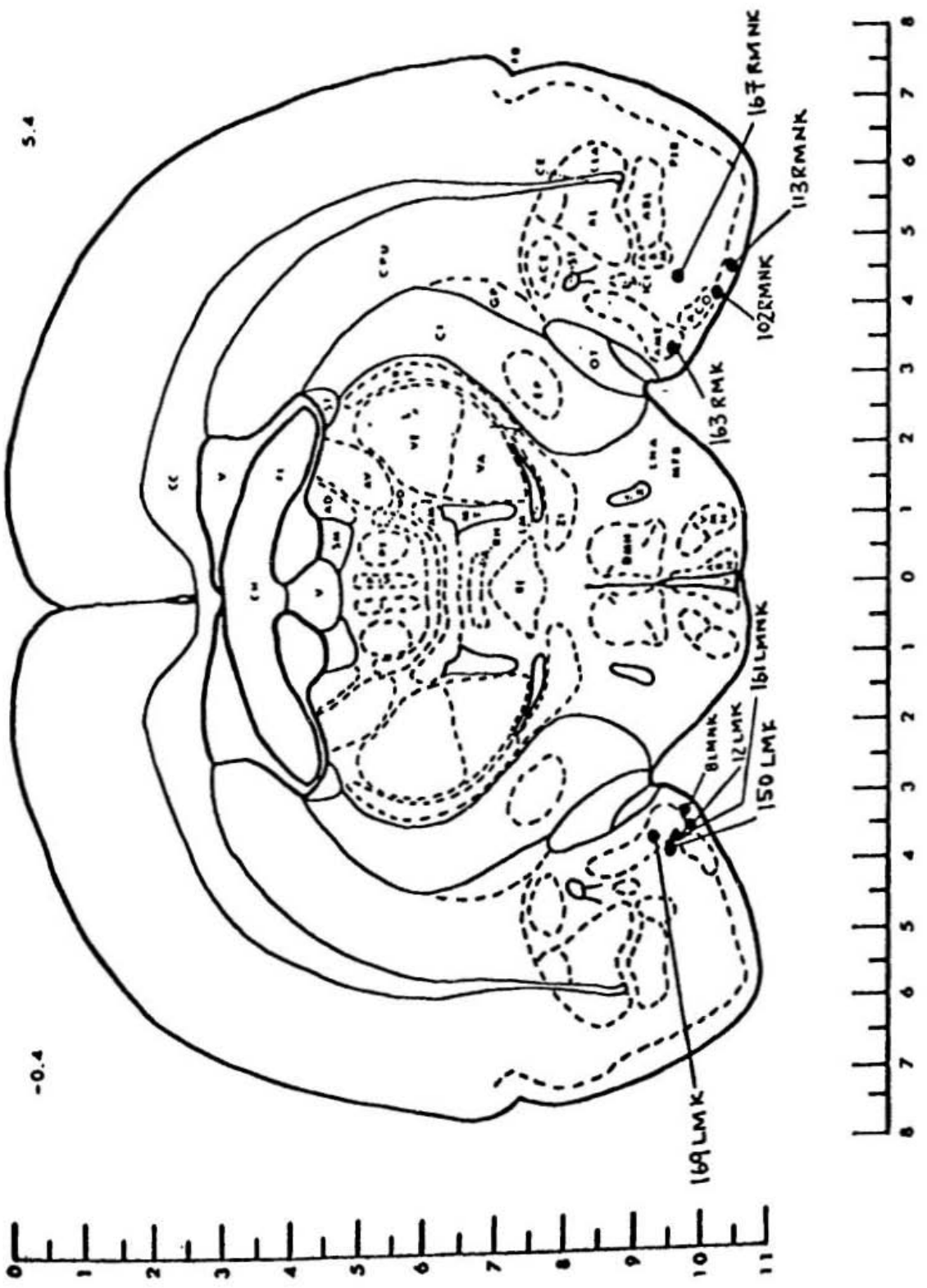

2.8
-3.0
36LMK

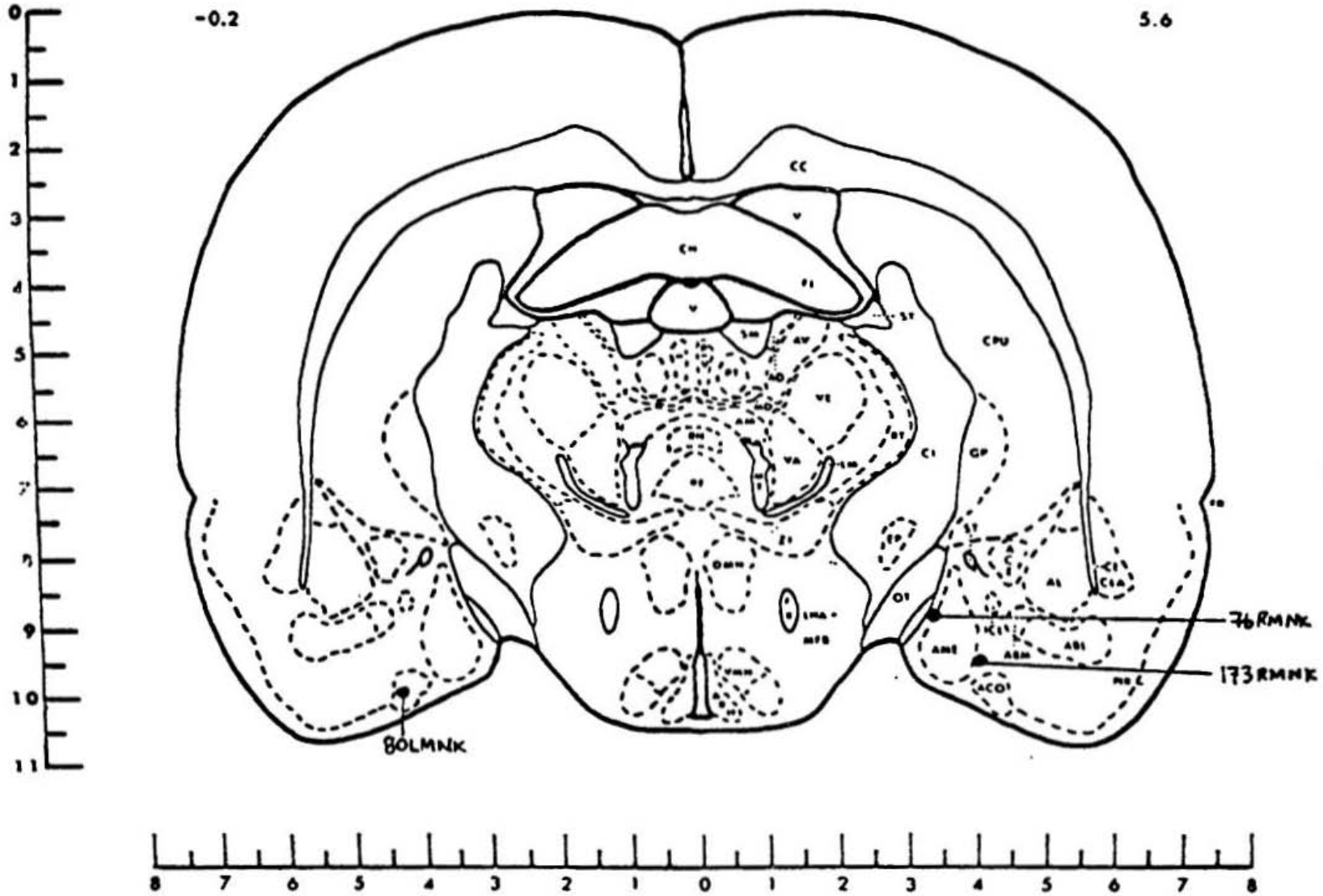

4.0
-1.2
31RMNK
21RMK
10ORMNK
28LMK

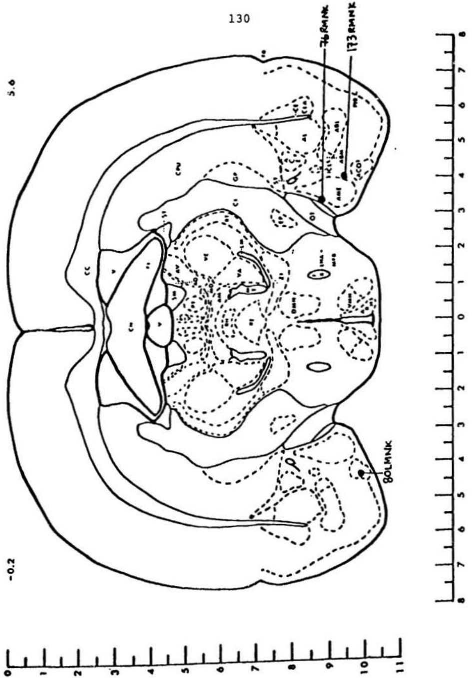

4.8
-1.0
82RMNK
106RMNK
24RMNK
158LMNK
16LMNK
85LMK

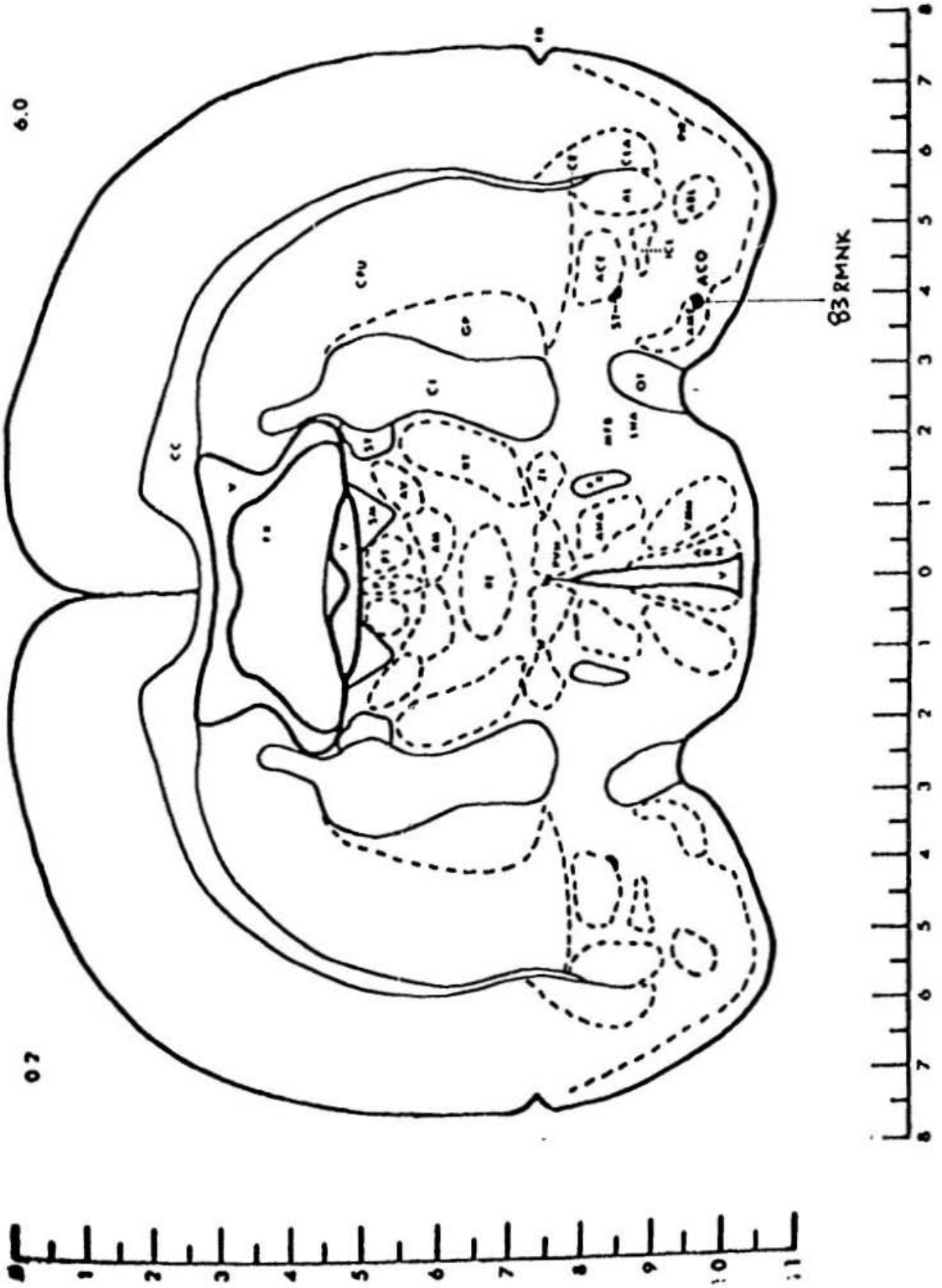

-0.8
5.0
52LMNK
165LMNK
108LMNK
74LMNK
29LMNK
41LMK
56LMK
59LMNK
149LMNK
148RMNK
112RMK
88RMK
159RMNK
42RMNK
96RMNK
73RMK
2RMK
151RMF
41RMK
35RMNK

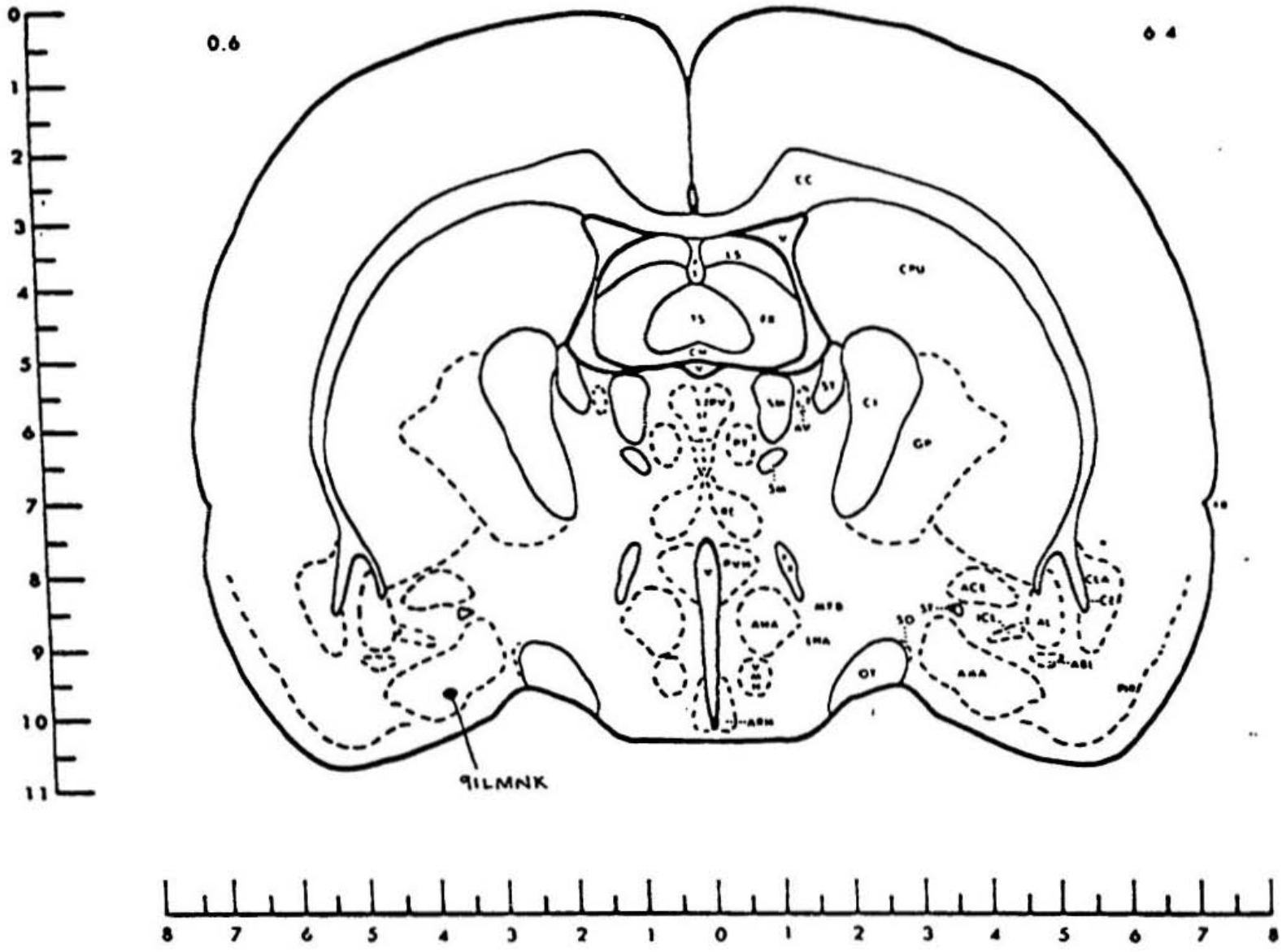

-0.6
5.2
172 LMK
171 LMNK
51 LMK
153 LMK
46 LMK
162 LMK
20 LMK
120 LMNK
69 LMNK, 154 LMNK
81 LMK
45 LMNK
94 LMK
157 LMK
146 LMNK
147 RMK
168 RMK
55 RMK
170 RMK
115 RMK
160 RMK
10 RMK
14 RMNK
98 RMK
43 RMK, 152 RMNK
103 RMK
155 RMK
127

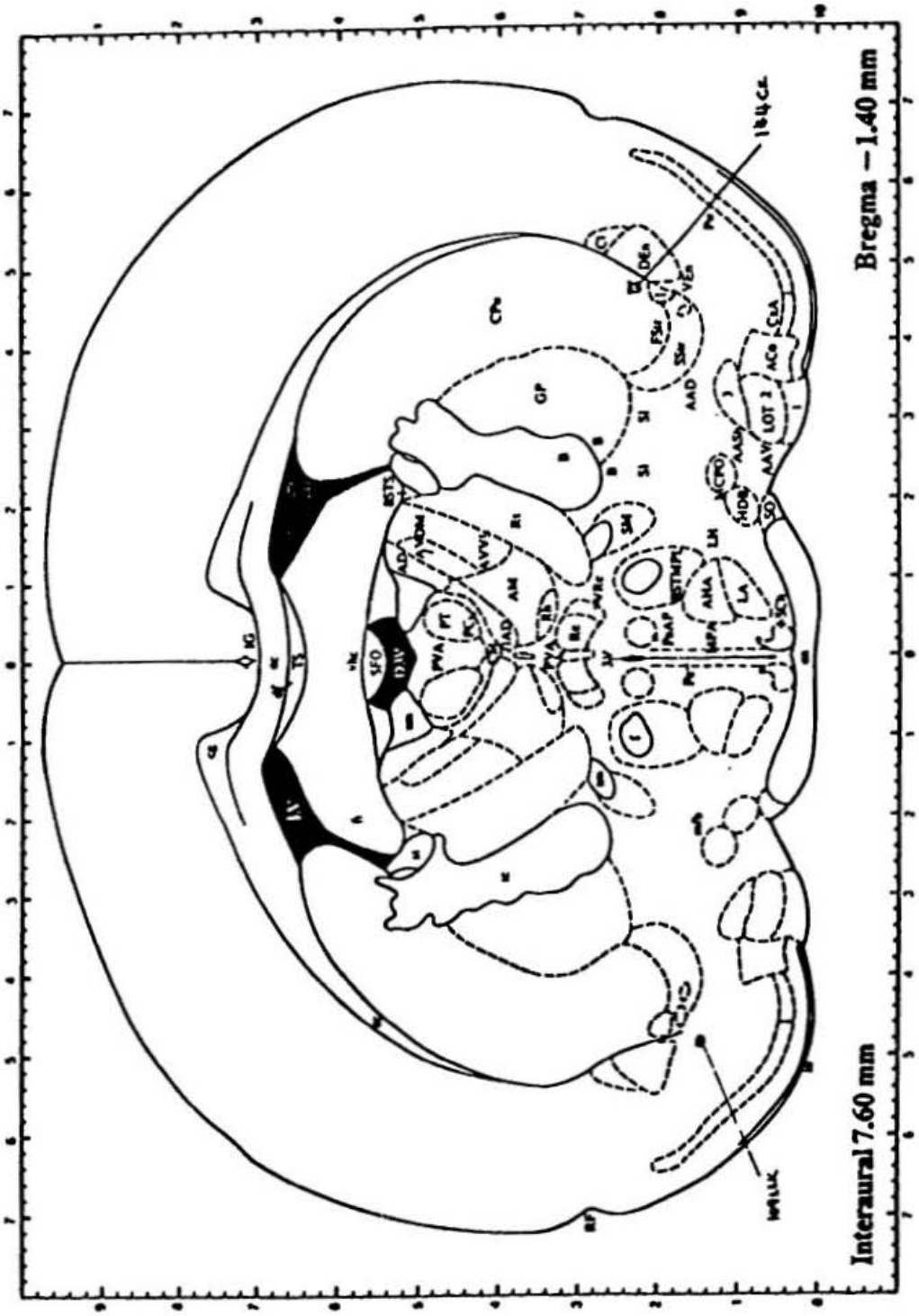

5.4
-0.4
167RMNK
113RMNK
102RMNK
163RMK
161LMNK
150LMK
81MNK
12LMK
169LMK

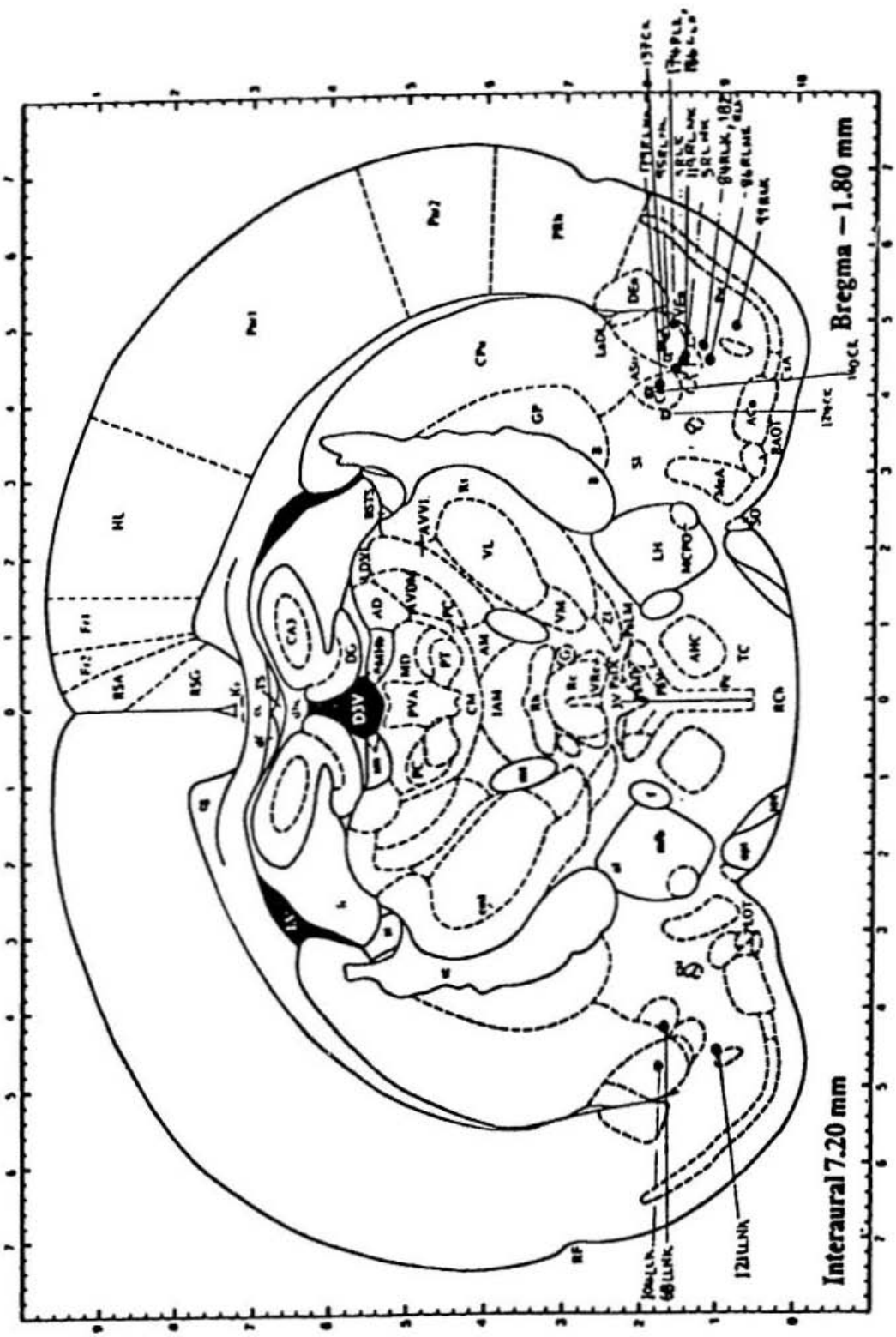

-0.2
5.6
CC
CPU
GP
AI
AM
ABI
ABM
ACO
76RMNK
173RMNK
80LMNK

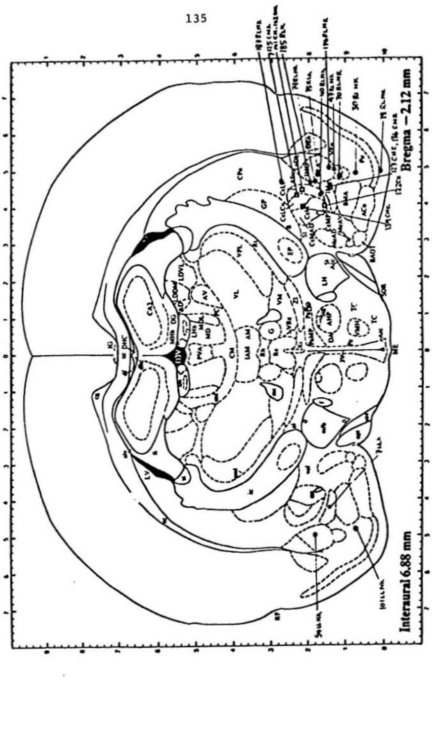

5.0
-0.2
76RMNK
173RMNK
80LMNK

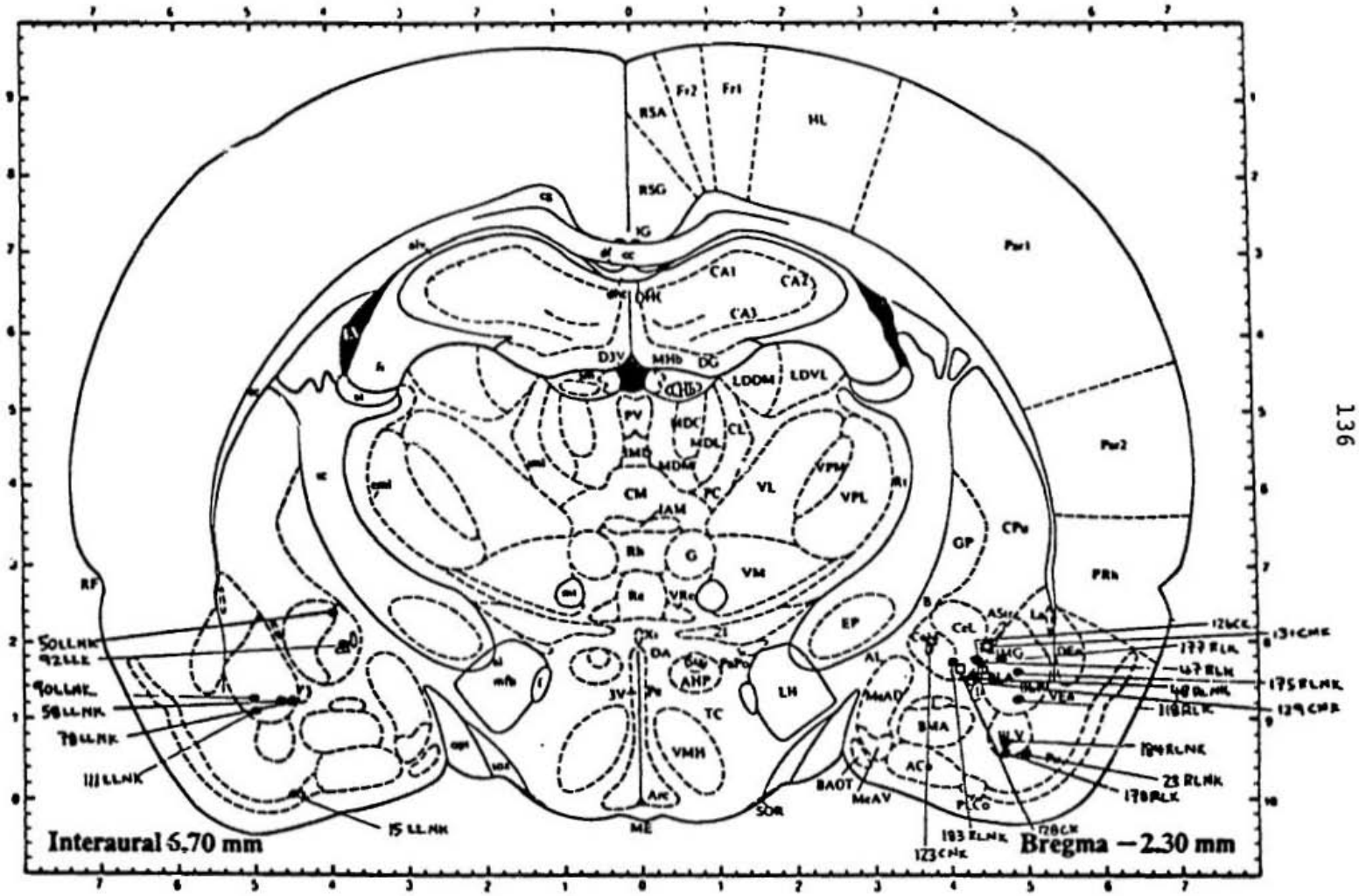

6.0
83 RMNK
ACO
0.7

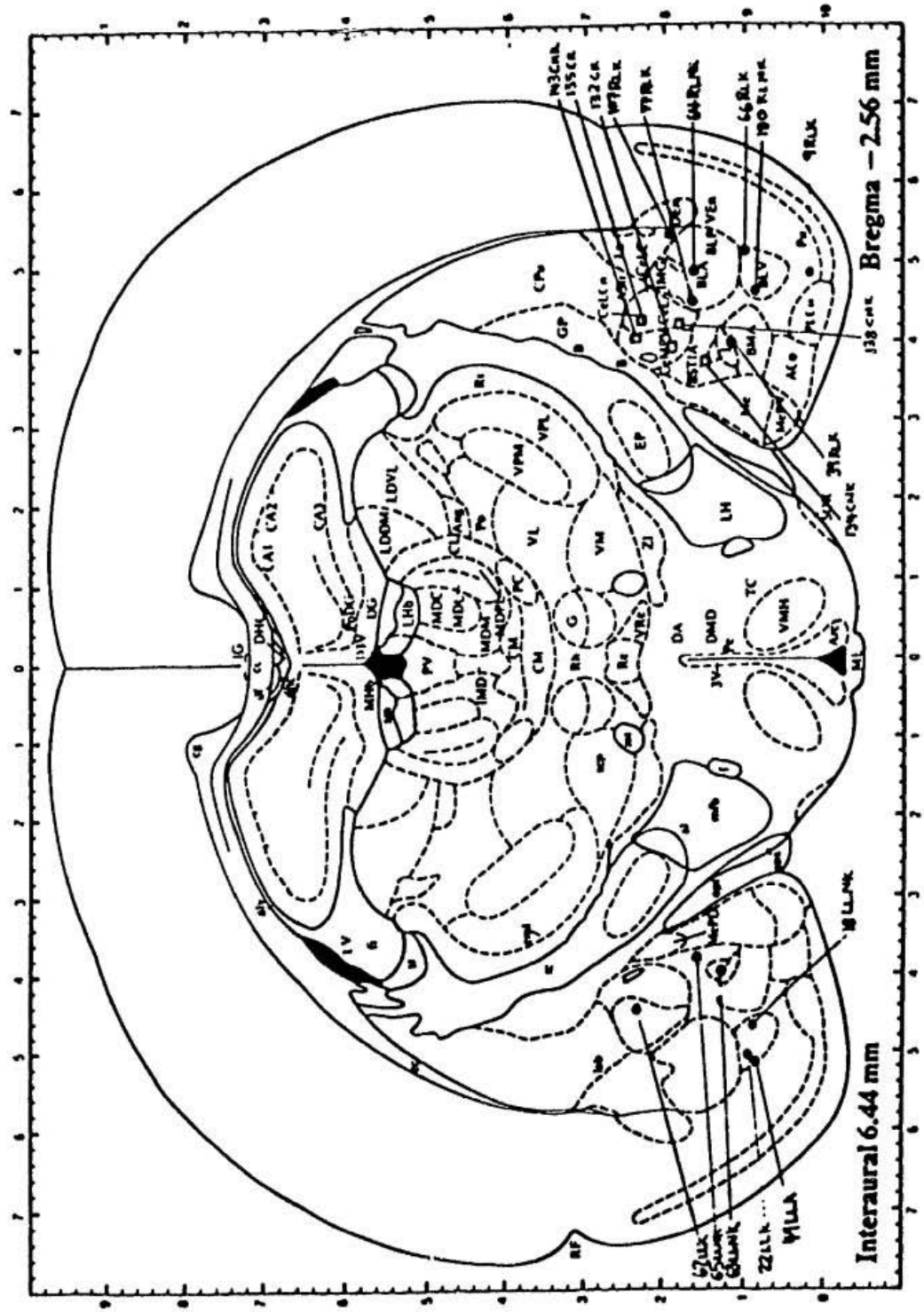

0.6
6.4
CC
CPU
CI
GP
MTD
AHA
LHA
OT
ACE
ACI
AI
ABI
AAA
PIR
9ILMNK
0
1
2
3
4
5
6
7
8
9
10
11
8 7 6 5 4 3 2 1 0 1 2 3 4 5 6 7 8

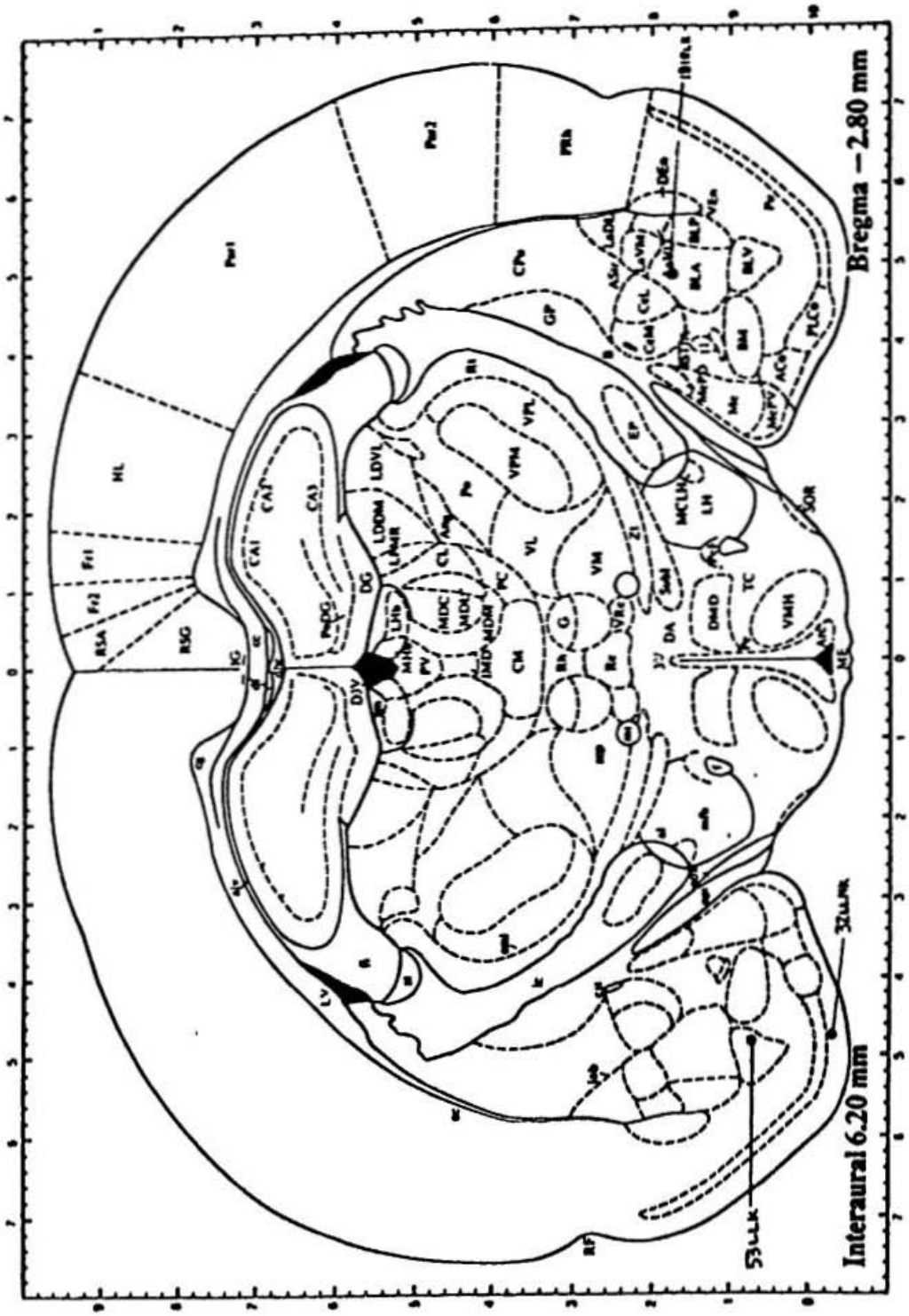

Bregma −1.40 mm
Interaural 7.60 mm

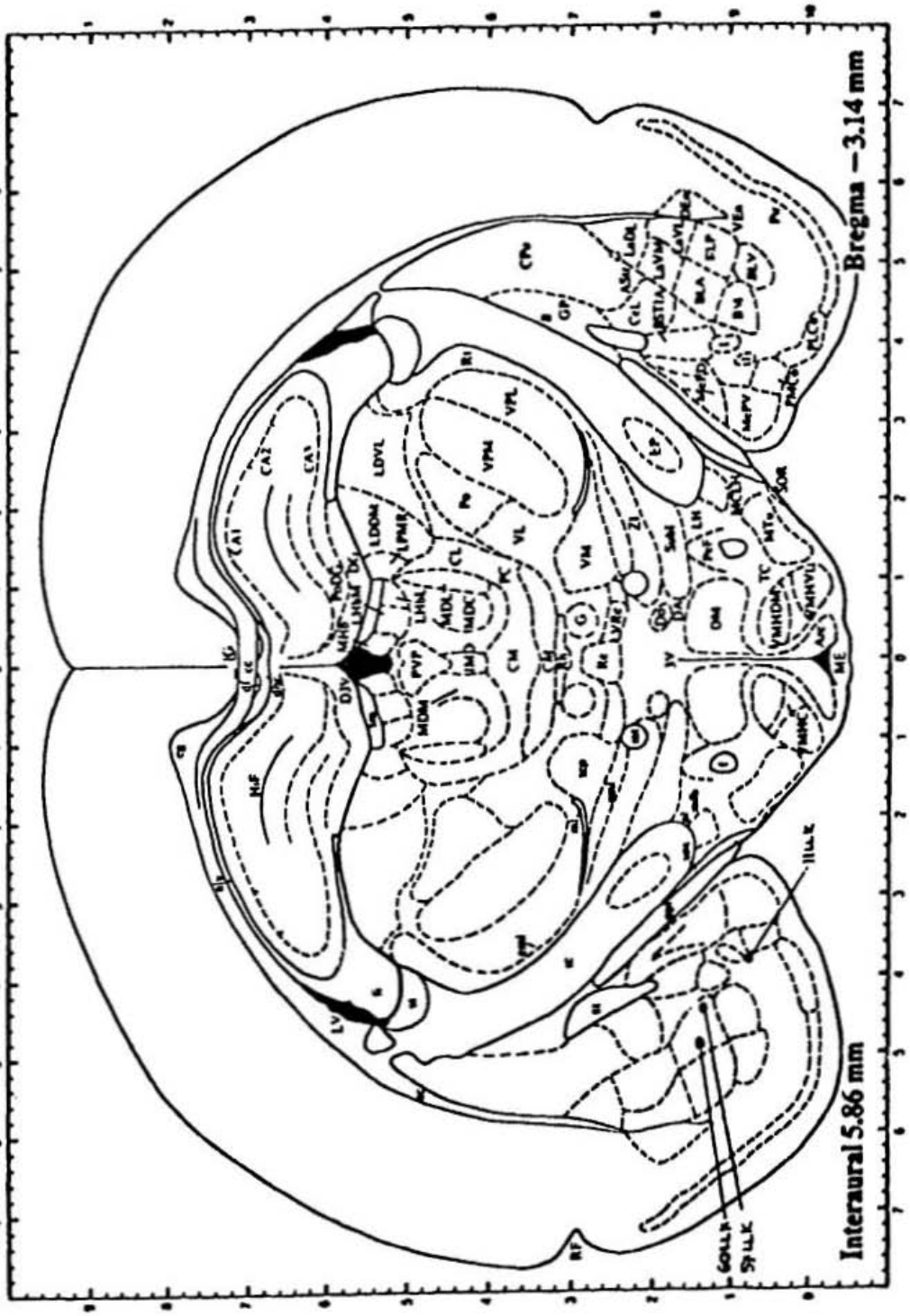

Bregma −1.80 mm
Interaural 7.20 mm

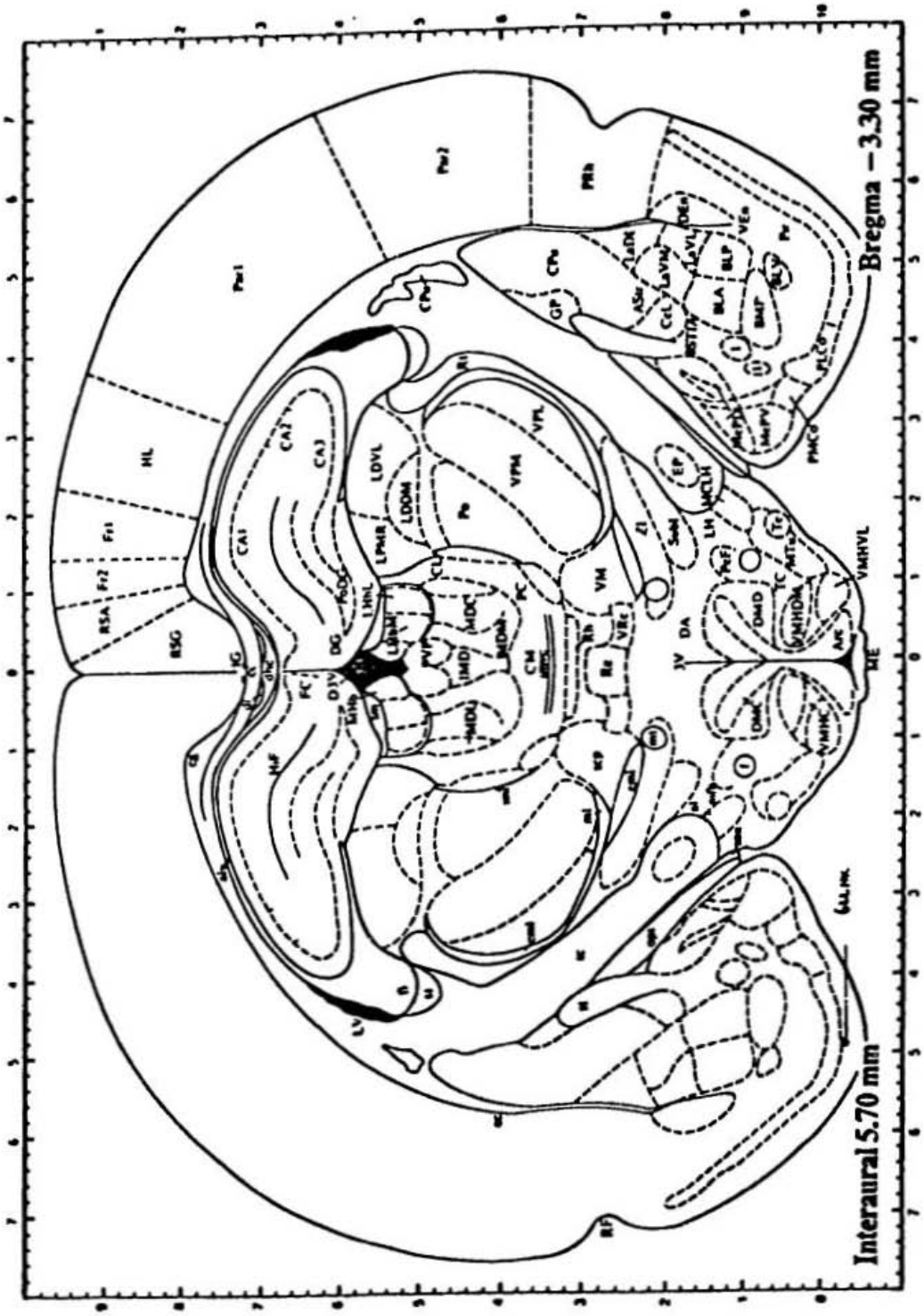

Bregma −2.12 mm
Interaural 6.88 mm

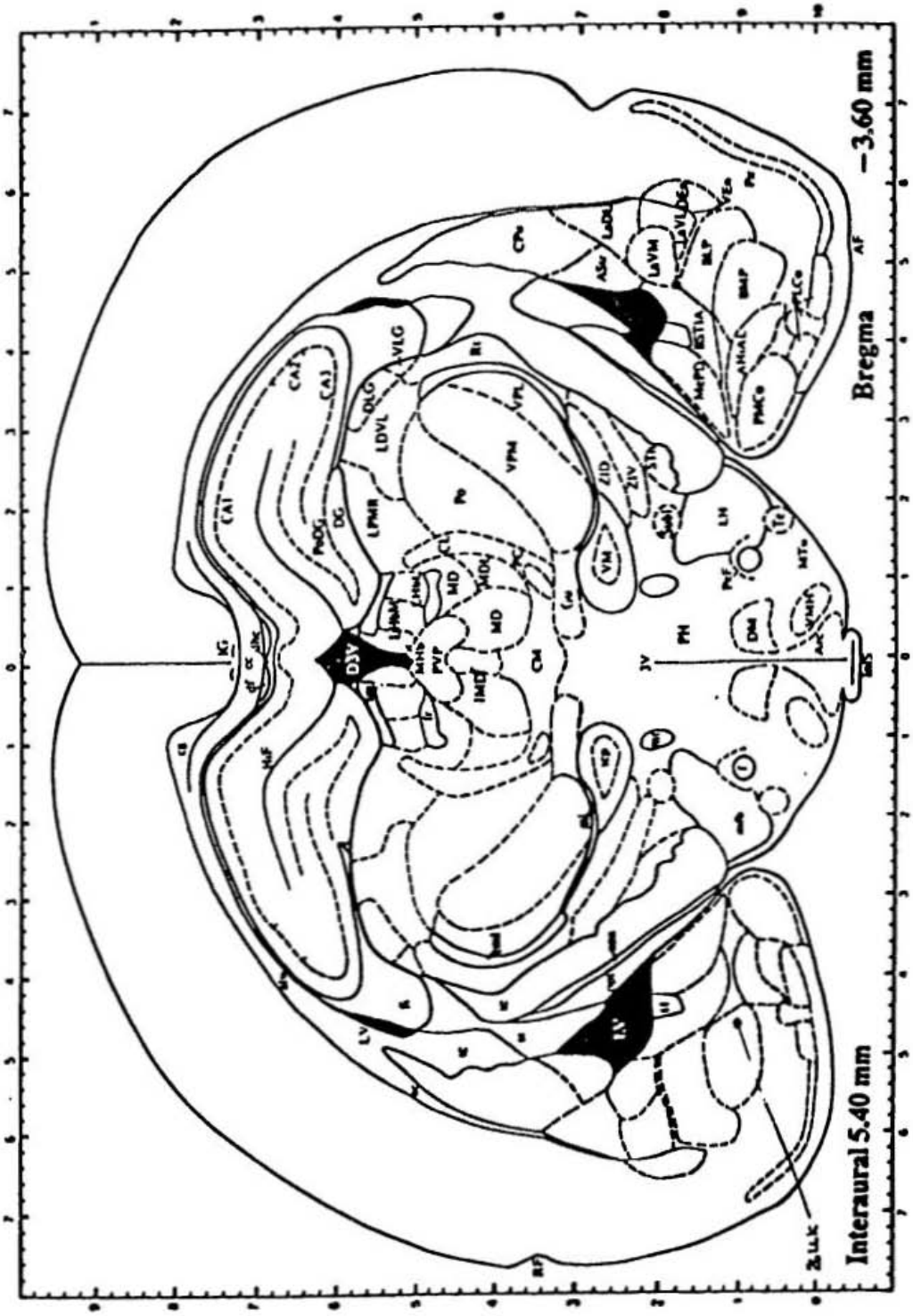

RSA
Fr2
Fr1
HL
RSG
IG
Par1
CA1
CA2
CA3
DJV
MHb
DG
LDDM
LDVL
PV
MDC
CL
IMD
MDM
MDL
CM
PC
VL
VPM
VPL
IAM
Rh
G
VM
Re
VRe
RF
EP
GP
CPu
Par2
PRh
DA
AMP
JV
TC
LH
AL
AMAD
VMH
APE
ME
SOR
BAOT
MeAV
BMA
AC
PLCO
Interaural 6.70 mm
Bregma −2.30 mm

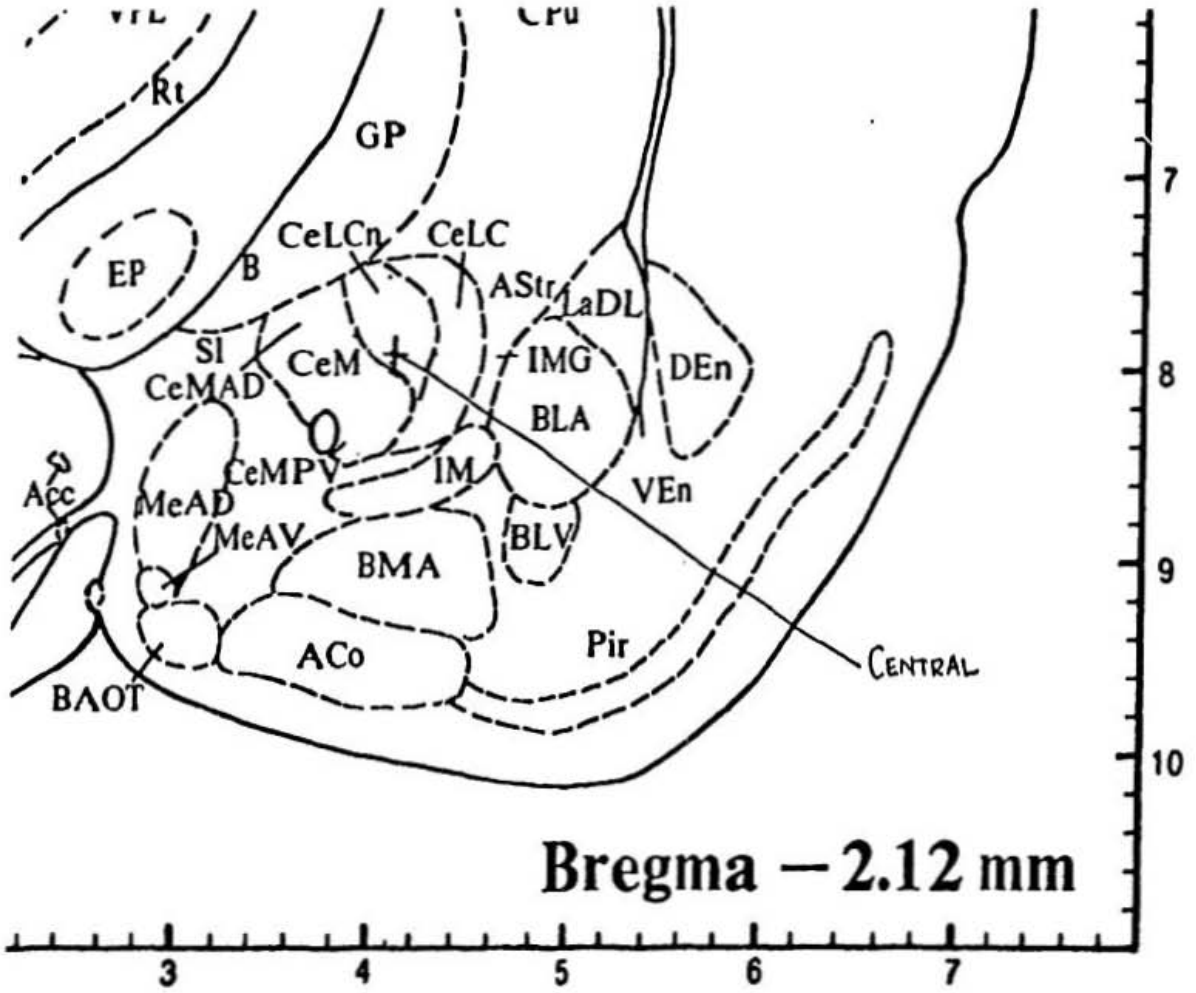

Bregma −2.56 mm
Interaural 6.44 mm


Bregma −2.80 mm
Interaural 6.20 mm

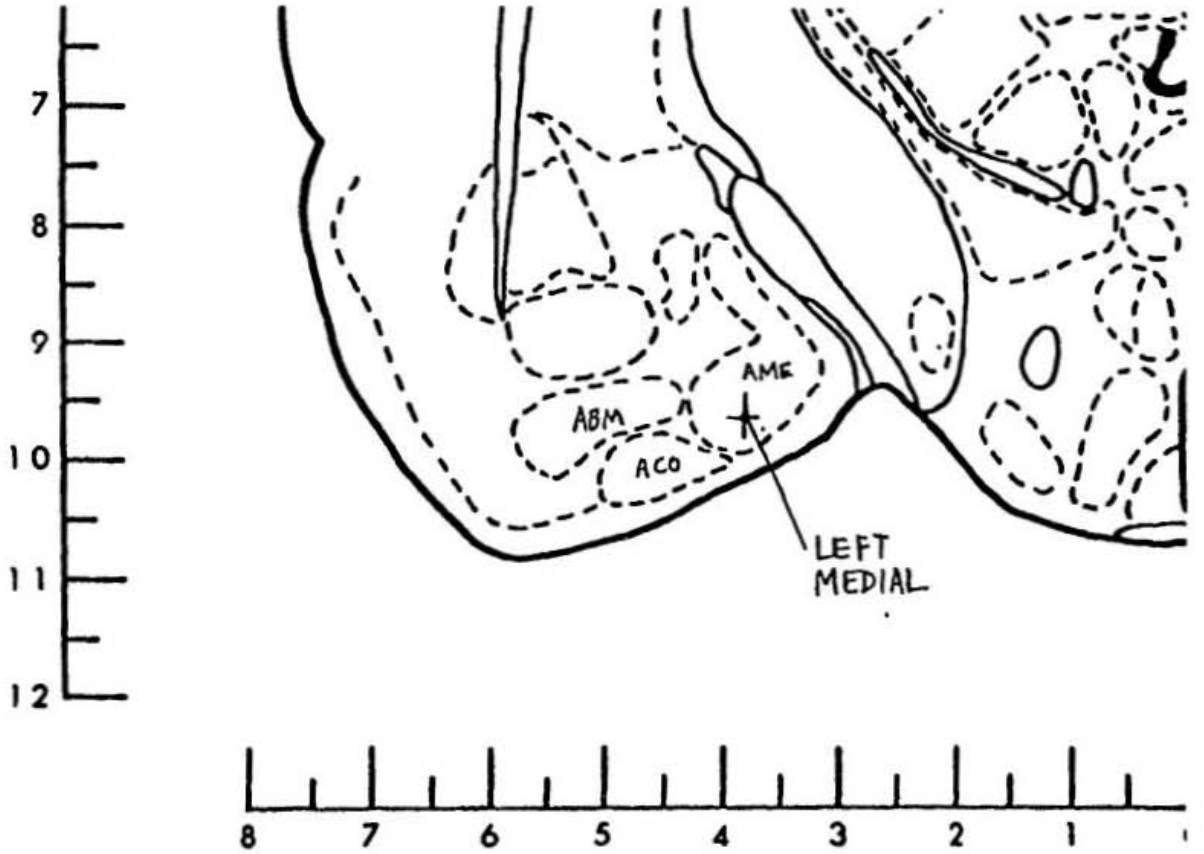

Bregma −3.14 mm
Interaural 5.86 mm


Bregma −3.30 mm
Interaural 5.70 mm

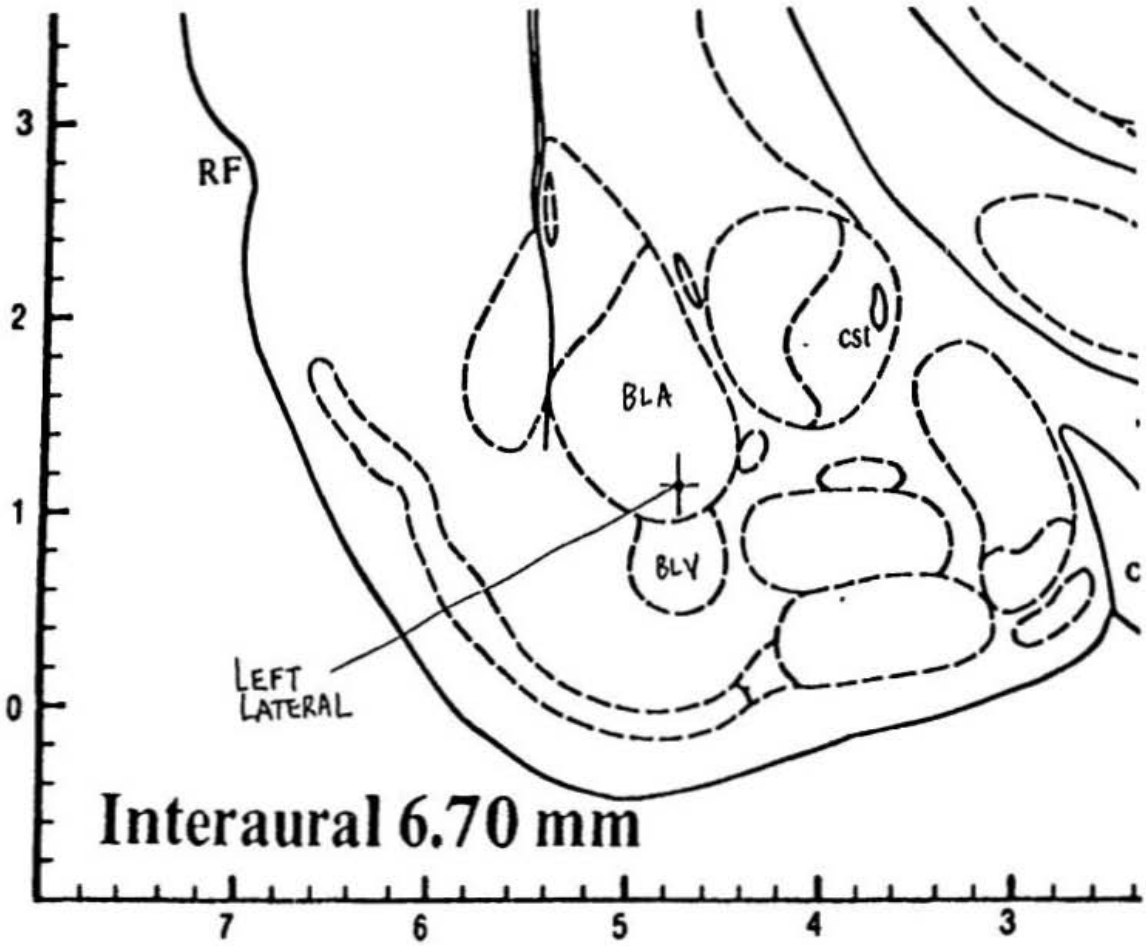

Interaural 5.40 mm
Bregma −3.60 mm



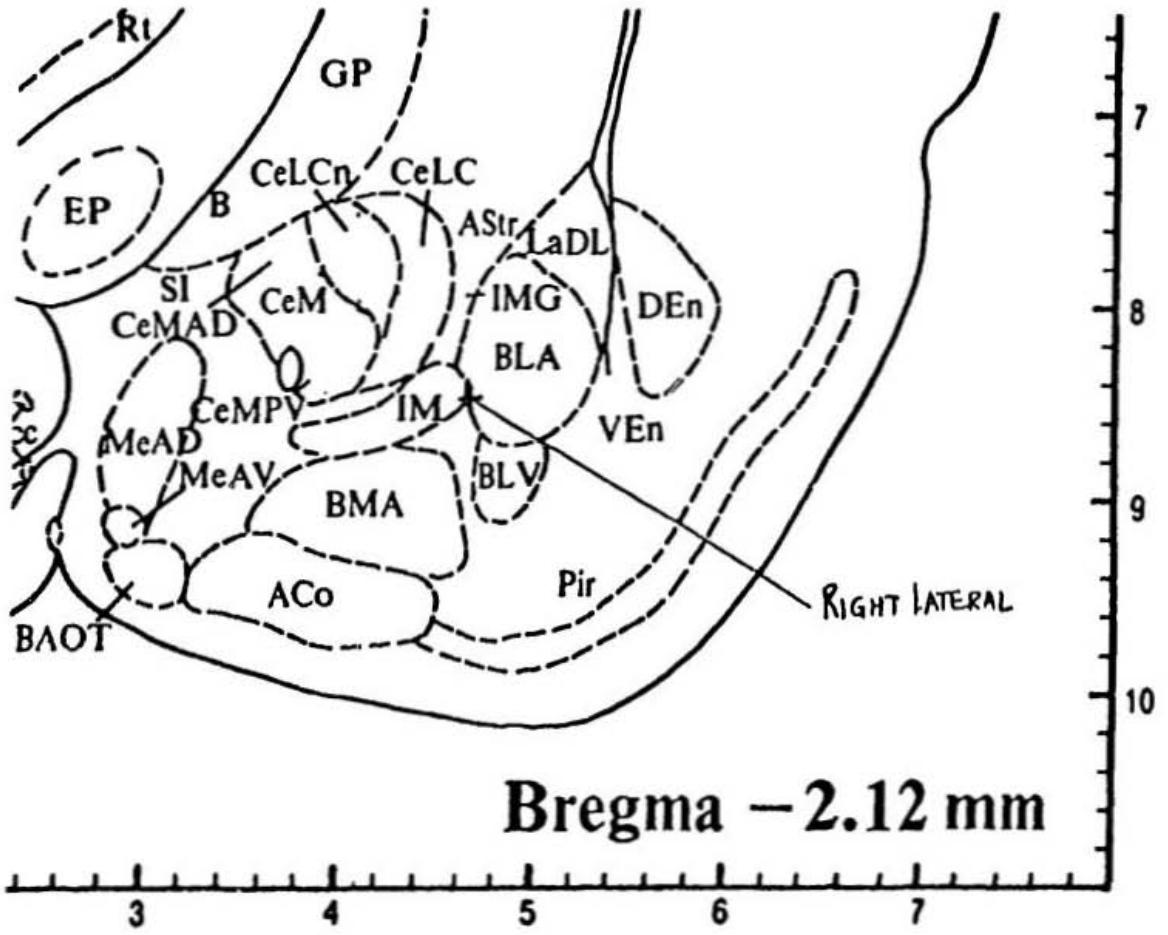
Mean electrode placements for Central amygdala (right hemisphere) rats +/- SEM (standard error of the mean) (-2.19+/- .06 AP plane, 4.06+/- .068 in the lateral plane, -7.97+/-.09 in the ventral plane). The SEM is indicated on the plate by bars.



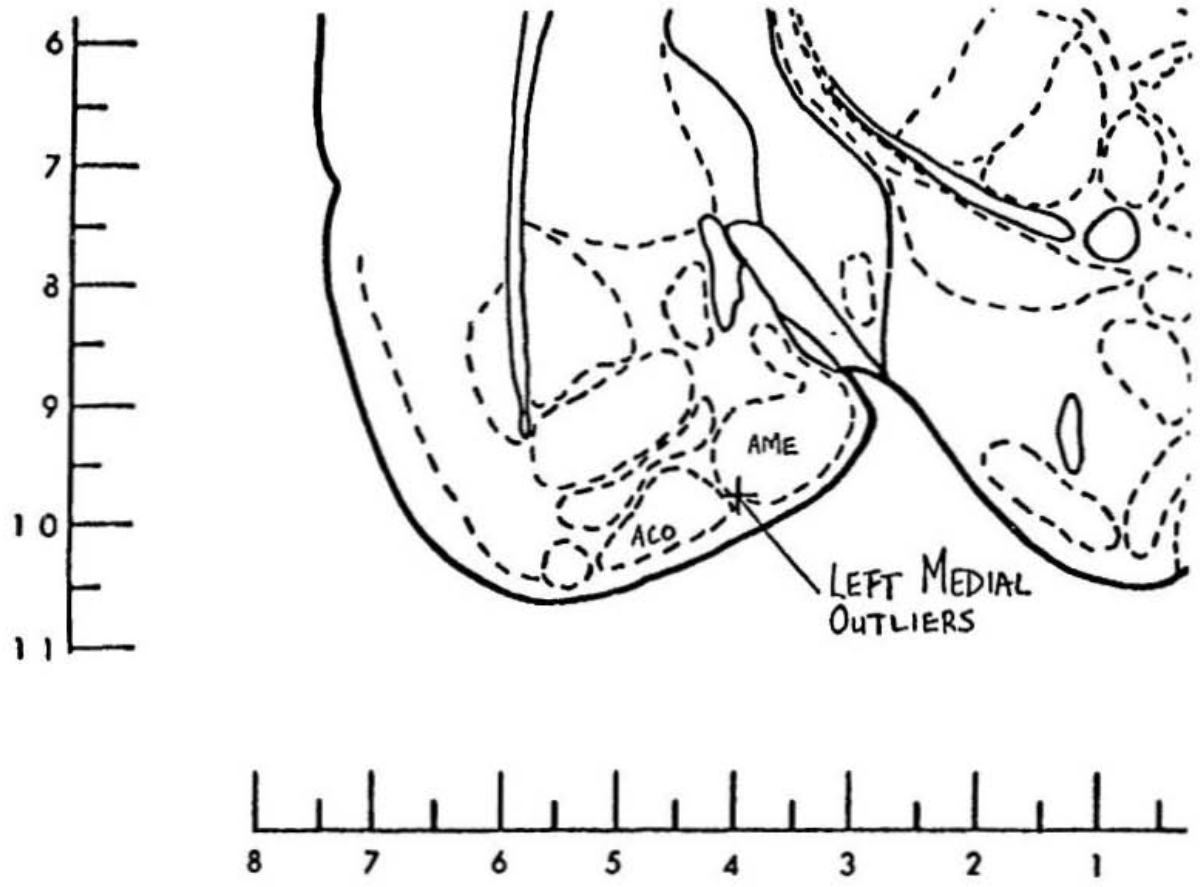
Mean electrode placements for left hemisphere Medial amygdala rats +/- SEM (-.682+/-.11 AP plane, 3.86+/-.07 lateral plane, -9.57+/-/14 ventral plane). The SEM is indicated on the plate by bars.



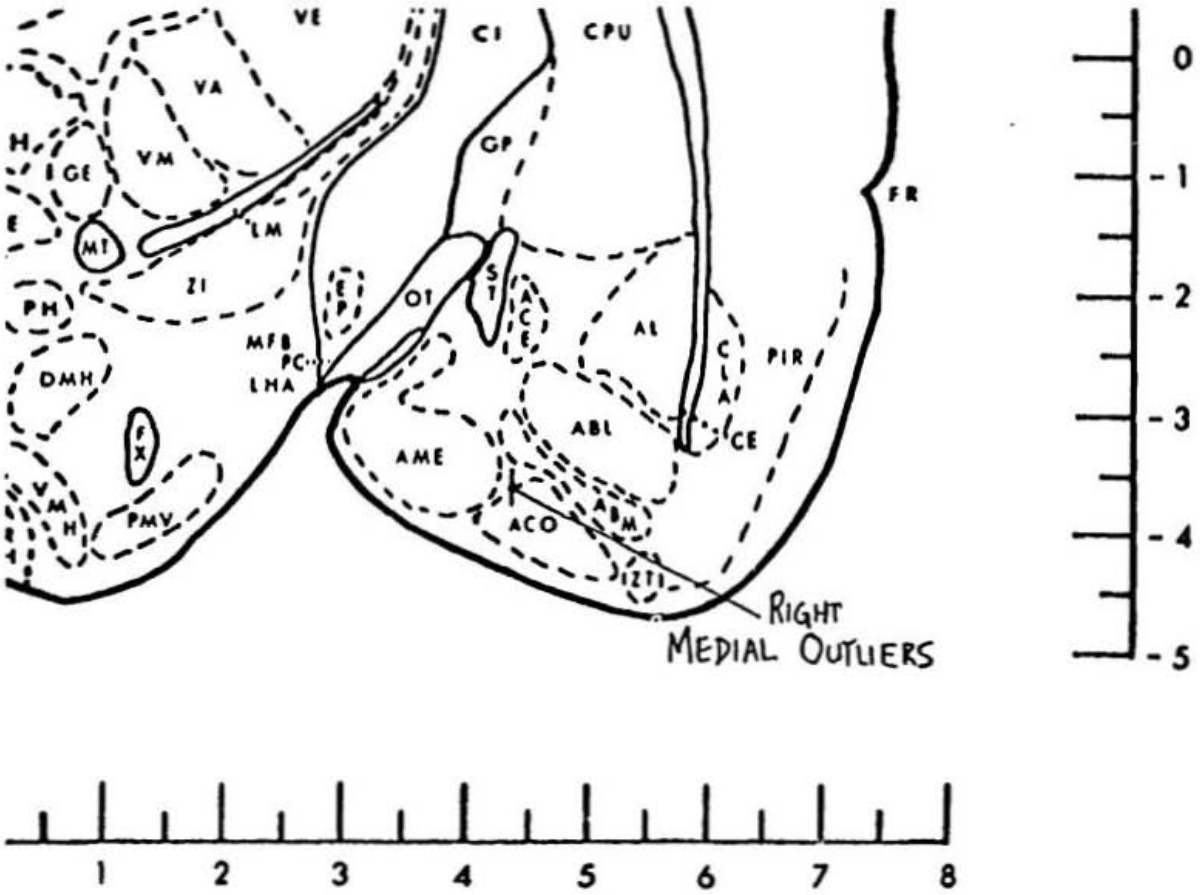
Mean electrode placements for left hemisphere Lateral amygdala rats +/- SEM (-2.37+/-.14 AP plane, 4.86+/-.10 lateral plane, -8.90+/-.71 ventral plane). The SEM is indicated on the plate by bars.



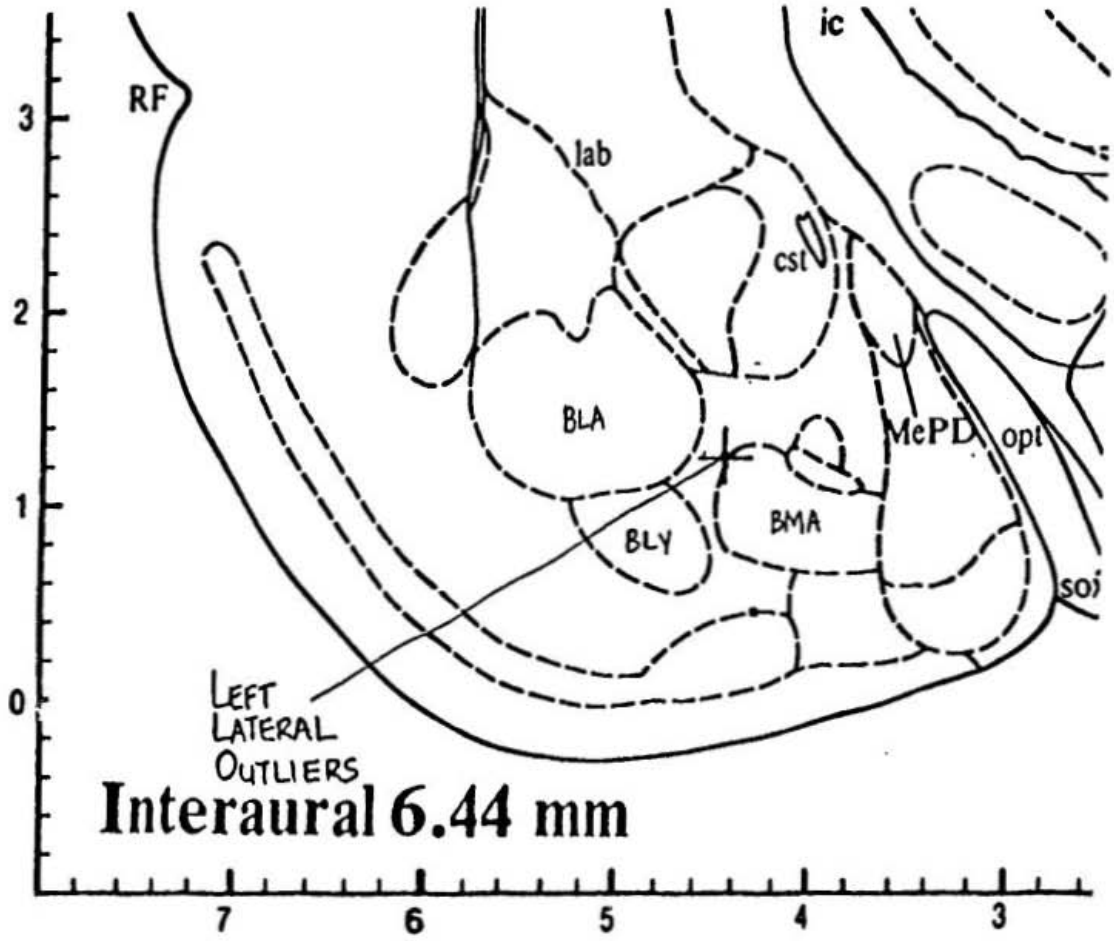
Mean electrode placements for right hemisphere Lateral amygdala rats +/- SEM (-2.12+/-.10 AP plane, 4.62+/-.06 lateral plane, -8.47+/-.11 ventral plane). The SEM is indicated on the plate by bars.



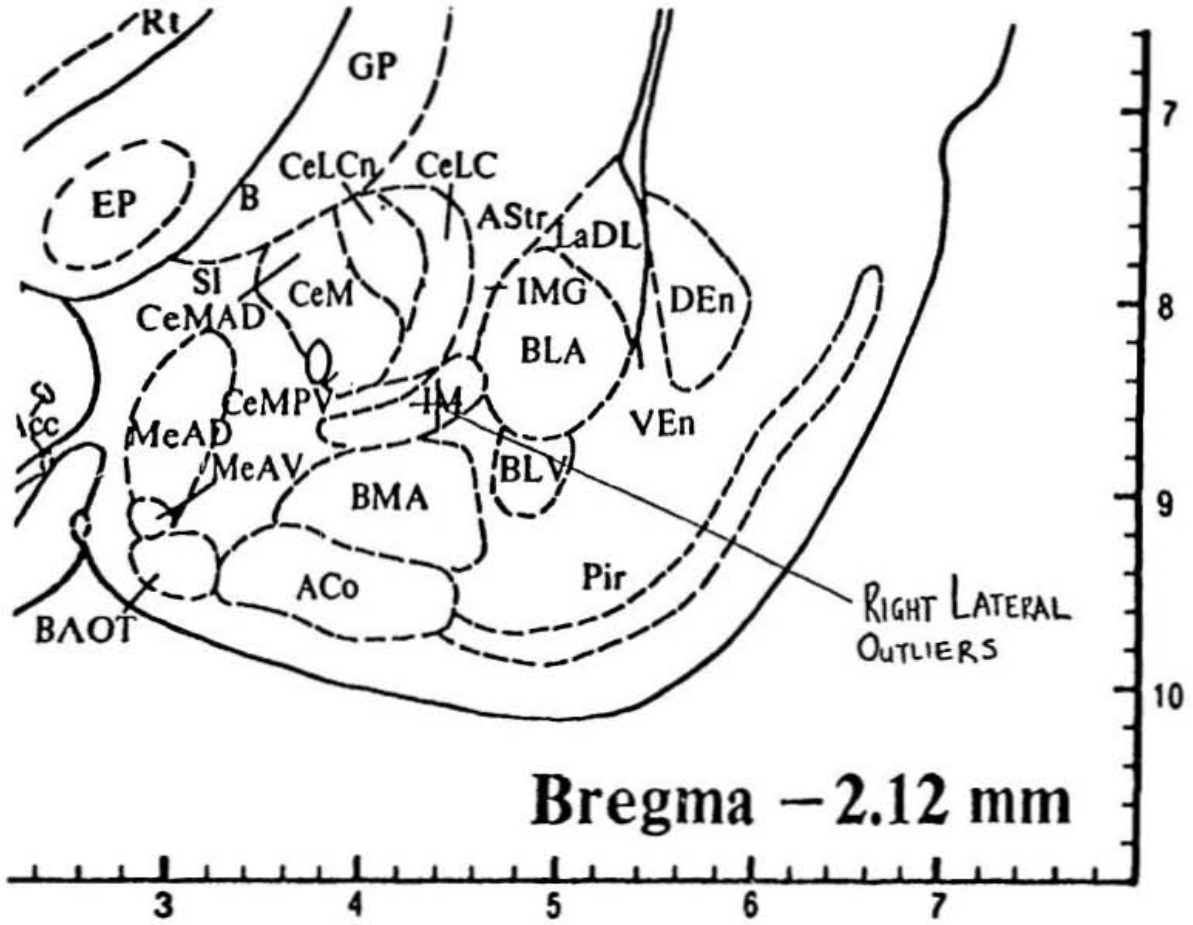
Mean electrode placements aimed at the Medial amygdala for left hemisphere Outlier rats +/- SEM (-.852+/-.2 AP plane, 3.95+/-.13 lateral plane, -9.75+/-.14 ventral plane). The SEM is indicated on the plate by bars.



Mean electrode placements aimed at the Medial amygdala for right hemisphere Outlier rats +/- SEM (-.847+/-.15 AP plane, 4.29+/-.01 lateral plane, -9.62+/-.12 ventral plane). The SEM is indicated on the plate by bars.



Mean electrode placements aimed at the Lateral amygdala for left hemisphere Outliers +/- SEM (-2.58+/-.15 AP plane, 4.41+/-.13 lateral plane, -8.79+/-.16 ventral plane). The SEM is indicated on the plate by bars.



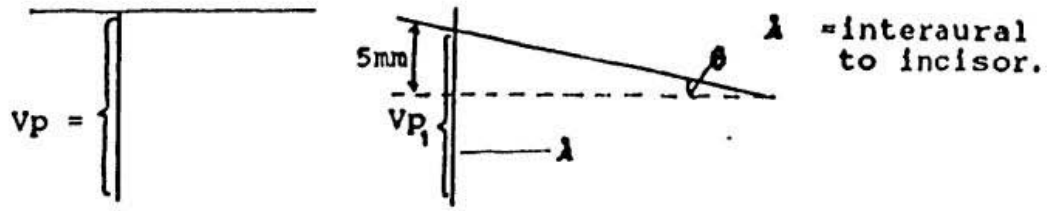
Mean electrode locations aimed at the Lateral amygdala for right hemisphere Outliers +/- SEM (-2.13+/-.17 AP plane, 4.41+/-.13 lateral plane, -8.49+/-.14 ventral plane). The SEM is indicated on the plate by bars.

APPENDIX C

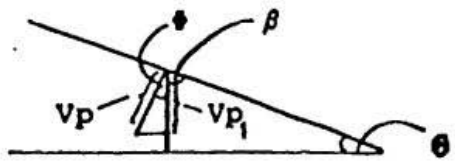
Vertical Adjustment

To convert Paxinos and Watson (1986) stereotaxic coordinates to Pellegrino et al. (1981) coordinates - to 5mm incisor bar elevation (in the vertical plane):

Paxinos Pellegrino



Assume we carry Vp upward in the conversion:



The equivalent can be found if ϕ is known.

It can be shown that $\phi = \theta$

Since there are 180° in a triangle:

a) $\theta = 180 - 90 - \beta = 90 - \beta$
b) since $\beta + \phi = 90°$

$\phi = 90 - \beta$

Therefore: $\theta = \phi$

Given the above:

$\cos(\theta) = Vp_1/Vp$

$Vp_1 = \cos(\theta)Vp$

$\sin(\theta) = 5/15.7 = .318; \quad \theta = 18.57°$

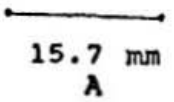
$\cos(\theta) = .9479$

$Vp_1 = .9479(Vp)$ for lateral animals

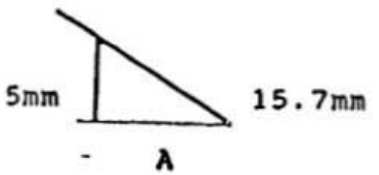
AP Adjustment

To convert Paxinos and Watson (1986) stereotaxic coordinates to Pellegrino et al. (1981) coordinates - to 5mm incisor bar elevation (in the AP plane):

Paxinos: head flat - distance from aural zero to front = 15.7mm.



Pellegrino: head elevated 5mm



Solve for A:

$$15.7^2 = A^2 + 25$$

$$A^2 = 246.49 - 25$$

$$A^2 = 221.49$$

$$A^2 = 14.88\text{mm}$$

Correction = 15.7 - A = .817

AP correction = .82mm