ANXIOGENIC AND ANXIOLYTIC EFFECTS IN THE ELEVATED PLUS MAZE PRODUCED BY KINDLING AND LOW FREQUENCY STIMULATION OF THE BASOLATERAL AMYGDALA

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Anxiogenic and Anxiolytic Effects in the Elevated Plus Maze Produced by Kindling and Low Frequency Stimulation of the Basolateral Amygdala

by

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A thesis submitted to the

School of Graduate Studies

in partial fulfillment of the

requirements for the degree of

Master of Science

Department of Psychology
Memorial University of Newfoundland

May 2001

St. John's Newfoundland

Abstract

Low frequency stimulation (LFS) has been shown to reverse long term potentiation (LTP) and block kindled seizures. Based on the premise that kindling-induced long term potentiation (LTP) in right basolateral amygdala (BLA) circuits increases anxiety, LFS should reverse kindling-induced anxious behavior. Ninety male Wistar rats were kindled in the right BLA to 4 stage 5 seizures, then administered right or bilateral BLA-LFS for 7 days. Kindling decreased open arm behavior and risk assessment in the elevated plus maze relative to control. Right LFS had no effect on behavior or seizures. Bilateral LFS increased open arm behavior and risk assessment to control levels on day 1, but did not block seizures. Risk assessment but not open arm behavior remained elevated 3 weeks later. Based on a study showing that amygdala LFS produces facilitation unless primed, it was concluded that the unprimed right BLA-LFS facilitated an already robust LTP, having a negligible effect on behavior and seizures. Unprimed left BLA-LFS, however, combined with seizure spread from kindling to produce a potentiation of the left BLA. As left BLA kindling is known to be anxiolytic, this would oppose the effect of right BLA kindling.

Acknowledgments

I would like to thank the Natural Sciences and Engineering Research Council of Canada for the research scholarship that made this thesis financially possible.

I would also like to thank my thesis supervisors, Dr. Robert Adamec and Dr. Charles Malsbury, and my thesis committee members, Dr. Virginia Grant and Dr. Carolyn Harley, for their advice and support in the planning and preparation of my thesis. As well, I acknowledge laboratory assistants Paul Burton and Tanya Shallow for their contribution to the laboratory work.

On a personal basis, I would like to thank my son, Robert Stanley. Without his unusual patience, understanding, and moral support, completion of this project would not have been possible.

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Epilepsy and Anxiety

Epilepsy, the most common neurological disease in humans next to stroke, affects 1 of every 100 people. Many people who have epilepsy develop increased interictal (between seizure) anxiety (Adamec, 1990b; Hermann & Whitman, 1984; Mittan & Locke, 1982; Strauss, 1989). Physiological characteristics of anxiety mimic fear, and can include increases in attention and arousal, startle reflex, heart rate, blood pressure, and respiration. Fear is a normal and automatic nervous system reaction to sensory information indicating an immediate threat. Under normal circumstances it is temporary, dissipating some time after the threatening situation has ended. It motivates rapid defensive behaviors necessary to overcome or avoid threat and is thus biologically important for survival. Anxiety, on the other hand, manifests itself in the absence of threat, or may continue long after the threat has ended. It maintains the animal in a prolonged and inappropriate vigilant state that can result in exhaustion of its physical and emotional resources. As anxiety does not appear to have an immediate biological value, it is considered maladaptive. Nevertheless, the physiological changes of anxiety are very similar to those of fear, suggesting that anxiety may be an abnormal excitation of fear pathways (Rosen & Schulkin, 1998). In support of this view, many people have reported feelings of fear as an after-effect of temporal lobe seizures (Gloor, 1978), suggesting that the fear pathway has, in fact, been activated.

A large amount of both human and animal evidence now implicates the amygdala and its connections in fear and fear conditioning (for review see Maren & Fanselow, 1996; Rogan & LeDoux, 1996; See also Davis, 1992; Davis, Rainie, & Cassell, 1994; Phillips & LeDoux, 1992). For example, electrical stimulation of the amygdala in animals has been shown to

produce fear-like changes, including increases in heart rate, blood pressure, respiration, and startle reflex (Davis, 1992; Davis, 1997), while amygdaloid lesions have been shown to abolish innate fear-related behavior such as that normally exhibited when rats are exposed to cats (Blanchard & Blanchard, 1972; Fox & Sorenson, 1994). In humans, amygdala damage has been shown to interfere with normal fear conditioning (Bechara et al., 1995; LaBar, LeDoux, Spencer & Phelps, 1995). If anxiety is an abnormal excitation of fear circuits, it follows then that the amygdala likely plays a significant role in its manifestation as well. Pitkanen, Tuunanen, Kalviainen, Partanen, & Salmenpera (1998) review amygdala complex damage in temporal lobe epilepsy in humans. They note that magnetic imaging studies have shown a 10% to 30% volume reduction of the amygdala in epileptic patients, and that neuronal loss and gliosis in the lateral and basal nuclei of the amygdala have been reported. Furthermore, data from human clinical studies indicate that epileptics with seizures involving the limbic system are more likely to experience psychiatric symptoms. In fact, Stark-Adamec and Adamec (1986) were able to correlate the progression of psychopathology in epileptics with the frequency and intensity of the illusory phenomena or 'auras' experienced by those epileptics. Stark-Adamec and Adamec suggested that these auras may be limbic system discharges related to the epileptic seizure activity.

Based on an increasing variety of such human and animal evidence, it has been hypothesized that interictal anxiety experienced by many epileptics is due to physiological changes caused by seizure-induced repeated excessive activity within the limbic system (Adamec, 1990b; Hermann & Whitman, 1984; Strauss, 1989), with the amygdala playing a major role (for review, see Adamec, 1990a; Henke & Sullivan, 1985; Nieminen et al., 1992;

Rosen & Schulkin, 1998). Psychosocial confounds, however, make it problematic to study the neural mechanisms underlying seizure-induced anxiety in humans. It is difficult to differentiate between increased anxiety manifesting as a result of seizures, and increased anxiety arising from the perceived stigma of having epilepsy and the fear of having another seizure. There are also obvious technical limitations, although some progress is being made with non-invasive technologies such as magnetic resonance imaging (MRI). Kindling, an animal model of seizure spread, can be used to study seizure-induced changes in the limbic system. The amygdala is particularly sensitive to kindling stimulation (Goddard, McIntyre & Leech, 1969; Loscher, Ebert, Wahnschaffe & Rundfeldt, 1995). In addition, the rat amygdala appears to have both physical and functional similarities to the human amygdala (for review see Davis et al., 1994). Furthermore, there have been many studies of the physiological effects of kindling on the rat brain, and pharmacologically validated models of rat anxiety are available for investigation. The rat kindling model, therefore, has been proposed as a means by which the neural mechanisms underlying seizure-induced changes may be examined (Adamec, 1998).

Kindling - An Animal Model of Epileptic Seizure Spread

In epilepsy, abnormal cellular discharge interrupts normal brain activity, resulting in uncontrollable stereotypical behaviors. These behaviors can range from jerking movements to convulsions or seizures. Seizures may be partial, complex partial, or general, depending on the brain area in which they originate and the nature of their spread. Partial seizures begin in a discrete brain region and spread locally. The region involved in the local spread determines whether a loss of consciousness will occur. Complex partial seizures involve limbic system

structures within the temporal lobe and orbital frontal cortex. This type of seizure often results in illusory phenomena (called auras) where a person may experience a sensation of unrealness or of being outside the body. Generalized seizures, however, involve large brain areas and therefore almost always result in a loss of consciousness (Martin, 1991).

Partial epilepsy (also known as focal epilepsy) can be produced experimentally by kindling. In the kindling paradigm, administering weak electrical stimuli to discrete forebrain sites produces localized afterdischarges (electrographic seizures), with no motor involvement. On repeated stimulation, the threshold necessary to produce these afterdischarges is lowered (partial kindling). If repeated stimulation continues, the afterdischarges intensify and spread to other brain regions, eventually leading to convulsive motor seizures (full kindling). Motor seizures as defined by Racine (1978) begin with freezing behavior (stage 1). They progress to twitching and/or jaw movement (stage 2), unilateral forepaw clonus (stage 3), bilateral forepaw clonus (stage 4), and finally rearing and losing balance (stage 5). These motor seizures occur in response to the same weak stimuli that had originally produced only afterdischarges. This increased sensitivity has been shown to last for months, even if the animal is not further stimulated (Goddard et al., 1969; Martin, 1991). Electrical activity recorded during experimental seizures in animals shares many features of electrical activity occurring during human epileptic seizures (Prince, 1978). Furthermore, kindled animals display spontaneous (unprimed) interictal discharges (Pinel, 1981), and if repeated stimulation continues, spontaneous ictal discharges and seizures develop (Pinel, 1981; Pinel & Rovner, 1978). The nature of behavioral changes that occur as a result of kindling, and the responsiveness to anti-convulsant drugs is also similar to that in human epileptics (Martin,

1991). Due to such commonalities, kindling is considered to be an animal model of seizure spread in partial and complex partial seizure disorders (for review, see Adamec, 1990b).

Kindling and Long-Term Potentiation

The neural mechanisms underlying long-term changes resulting from kindling are not yet well understood. Neural plasticity in general, though, is believed to be induced by repetitive activation of excitatory afferents resulting in increased neurotransmitter release. Long term changes occur when altered neuronal synaptic transmission persists beyond the initial activating stimulus. Long term potentiation (LTP) is an activity-dependent enhancement of neuronal synaptic transmission which continues after the activity has been terminated. It is therefore a possible mechanism underlying neural plasticity.

LTP refers to a long-lasting (hours, days, or weeks) increase in the excitatory synaptic potential of a neuron, facilitated by a brief high-frequency (usually 100-400 Hz) train of stimuli to an afferent pathway. LTP occurs reliably in a number of brain regions that are involved in learning and memory, and has a variety of properties and induction paradigms (Kandel, 1991). One common mechanism appears to be increased NMDA-dependent calcium levels. Increased postsynaptic calcium levels catalyze calcium-dependent protein kinases (such as Ca^{2}+ calmodulin kinase and protein kinase C), second messanger systems that produce synaptic modification (Kandel). Most studies investigating LTP have concentrated on the hippocampus. However, a number of studies have successfully produced amygdala LTP, both *in vitro* (Chapman & Bellavance, 1992; Gean, Chang, Huang, Lin, & Way, 1993; Li, Weiss, Chuang, Post, & Rogawski, 1998; Shindou, Watanabe, Yamamoto, & Nakanishi, 1993;

Watanabe, Ikegaya, Saito, & Abe, 1996; Watanabe, Saito, & Abe, 1995), and *in vivo* (Clugnet & Ledoux, 1990; Maren & Fanselow, 1995). These studies have produced both NMDA-dependent and NMDA-independent LTP in the amygdala and its connections. For example, NMDA-dependent LTP has been produced *in vitro* in the projections from the endopiriform nucleus to the basolateral nucleus (Gean et al.) and *in vivo* in the hippocampal projections to the basolateral nucleus (Maren & Fanselow, 1995), while NMDA-independent LTP has been produced *in vitro* in the external capsule projections to the lateral (Watanabe et al., 1995) and basolateral nuclei (Chapman & Belavance). These findings suggest that NMDA dependence of LTP in the amygdala is not universal, possibly varying with pathway and/or nuclei being investigated (for a review of LTP in the amygdala, see Maren, 1996).

LTP in amygdala circuitry has also been produced by kindling paradigms (for review, see Adamec, 1990b). For example, Adamec (1993b) found that partial kindling of the left amygdalo-ventromedial hypothalamic pathway in the cat produced LTP of amygdala efferent transmission in both hemispheres. Full kindling has been found to induce LTP of amygdala efferent transmission in rodent amygdala efferents (Racine, Milgram, & Hafner, 1983). These findings demonstrate that amygdala LTP is produced by the kindling paradigm. As LTP is believed to be a mechanism for long-term neural change, it is possible that kindling-induced LTP results in the long-term changes in seizure susceptibility.

Kindling-Induced Anxiety

Like epilepsy in humans, limbic kindling in rats produces interictal changes in anxiety level (Adamec, 1990a; Adamec & McKay, 1993; Adamec & Morgan, 1994; Kalynchuk, Pinel & Treit, 1998, Nieminen et al., 1992). While there have been many general studies investigating kindling, only a small number have investigated kindling of particular amygdala nuclei, and results have been contradictory. For example, Neiminen et al. found left basolateral amygdala (BLA) kindling to be anxiogenic, while Adamec & Morgan found it to be anxiolytic. Both studies had used Wistar rats and measured behavior with the elevated plus maze. In the Adamec and Morgan study, the right medial/basolateral amygdala was also kindled, but results were inconclusive. The investigators did note, though, a correlation between the degree of anxiety exhibited following right hemisphere kindling and the anterior-posterior (AP) plane of the electrode location in the nuclei: the more anterior electrode placements were correlated with more anxiety, and the more posterior with less anxiety. Based on this finding and a similar correlation found by Adamec and McKay (1993), Adamec and Morgan hypothesized that anxiogenic and anxiolytic effects produced by their electrode placement may have canceled each other out, accounting for their inconclusive results and the difference between their results and those of Neiminen et al.

With the results of Adamec and Morgan (1994) and Adamec and McKay (1993) in mind, a meta analysis estimating AP plane of focus in previous studies was undertaken (Adamec, 1998). Adamec reviewed the data from a number of studies (Adamec, 1990a; Adamec & McKay, 1993; Adamec & Morgan, 1994; Helfer, Deransart, Marescaux, & Depaulis, 1996; Henke & Sullivan, 1985; Kalynchuck, Pinel, Treit, & Kippin, 1997; McIntyre,

1978; Neiminen et al., 1992; Witkin, Lee & Walczak, 1988) with respect to AP plane. A partial meta analysis was performed by plotting the electrode locations for each study on sections of the Paxinos and Watson atlas (Paxinos & Watson, 1982). Electrode locations were analyzed based on hemisphere, amygdala nuclei focus, and AP plane of focus within the nuclei. These electrode locations were then related to behavioral changes. After analyzing results from each study in this manner, the effect of kindling on anxious behavior was found to vary depending on nuclei and AP plane of focus, as well as hemisphere stimulated. In the case of the Neiminen et al. study, histology had not been reported. Adamec reviewed kindling rates in the Nieminen et al. study and compared them with other studies. He concluded that Nieminen et al. were kindling outside the left basolateral area. In the remaining studies with histologically confirmed left BLA focus, kindling appeared to be anxiolytic regardless of AP plane of focus (Adamec & Morgan; Kalynchuck et al., 1997; Witkin et al.). A complication appeared in the Kalynchuck et al. (1997) study, however. Kindling that was initially anxiolytic appeared to be anxiogenic when tested one month later. In addition, it took 60 - 100 stimulations to produce the anxiolytic behavior, compared to 15-20 in standard kindling. Adamec noted that hooded rats were used (as compared to Wistars in the Adamec & Morgan and Witkin et al. studies). He suggested that hoodeds are known to be more anxious in the plus maze and may therefore be more difficult to change behaviorally. In addition, he suggested that the effects of long-term kindling may differ from those in standard kindling paradigms, although interpretation is difficult without further studies. He concluded that, in general, kindling in the left hemisphere tended to be anxiolytic regardless of nuclei or AP plane of focus, although many areas remain uninvestigated.

Behavioral results of kindling in the right hemisphere varied with the nuclei stimulated as well as AP plane of focus. For example, behavioral effects of kindling in the anterior central nucleus and the nucleus basalis paralleled each other: rats with a more anterior focus tended to have less anxious behavior, rats with a more posterior focus tended to have more anxious behavior, and those that had a midway orientation of focus tended to be like controls (i.e., no effect on anxious behavior). However, kindling of the BLA was anxiogenic, regardless of AP plane of focus.

Based on the results of this meta analysis, Adamec concluded that there is a highly organized functional differentiation within the rat amygdala, including AP plane differences. He suggested that previous studies failed to relate kindling-induced changes in anxious behavior to affective changes in epilepsy because investigators did not confirm kindling focus, and had not considered electrode location within the AP plane of the nuclei. Consistent with Adamec's idea that there are AP plane differences in the limbic system of the right hemisphere, Lehmann, Ebert and Loscher (1998) found right BLA kindling reduced GABA immunoreactive cells in the zone between the anterior and posterior piriform cortex (an area believed to be key in the spread of kindled seizures to other brain areas). Similar to Adamec, they conclude that neurochemical and therefore likely functional differentiation exists along the AP axis.

The Elevated Plus Maze

In the kindling studies reviewed by Adamec (1998) for his partial meta-analysis, a number of methods had been used to measure behavioral change. They included susceptibility to stomach ulcers (Henke & Sullivan, 1985), punished responding (Witkin et al.1988), latency to attack mice (McIntyre, 1978), the social interaction test (Helfer et al., 1996), and elevated plus maze behavior (Adamec & McKay, 1993; Adamec & Morgan, 1994; Helfer et al., 1996; Kalynchuck et al., 1997; Nieminen et al., 1992). The elevated plus maze, most common to these studies, is an ethologically sound method that has been behaviorally and pharmacologically shown to be a valid and reliable measure of anxiety in rodents (Lister, 1987; Pellow, Chopin, File & Briley, 1985), and is widely used in studying both anxiolytic drugs and anxiety.

The elevated plus maze was developed on the basis that rats are normally motivated to explore novel environments, but have a natural aversion to open spaces. When placed in exposed novel environments, they have two conflicting motivations: to explore the novel area, and to avoid the exposed area. While rats in general tend to avoid open areas, anxious rats will show a greater degree of avoidance than less anxious rats. The maze is constructed to provide both a novel exposed area (open arms) and a safe enclosed area (closed arms), with the rat freely able to choose between them. Anxious rats will spend less time exploring the exposed novel arms than will rats that are less anxious. Administration of anxiolytic drugs increases the amount of time spent in the exposed arms, while anxiogenics decrease the amount of time. Factor analysis has confirmed that percentage scores of open arm entries and

open arm time are good measures of anxiolytic behavior (Cruz, Frei & Graeff, 1994; File, 1991).

The elevated plus maze can also be used to obtain a different measure of anxiety by measuring risk assessment behavior. Blanchard and Blanchard (1989) described behavior of rats coming from a closed area out into an open area in a visible burrow system. They found that rats extended their heads into the open area while keeping their bodies in the closed area. While in this position, they made scanning head movements, as if assessing the 'risk' associated with moving into the open space. Blanchard and Blanchard found that on exposure to a cat, the number and duration of risk assessments performed increased. The benzodiazepine agonist diazepam, an anxiolytic, further increased risk assessment in rats that had been recently exposed to a cat (Blanchard, Blanchard, Tom, & Rodgers, 1990), suggesting that anxiety reduces the amount of risk assessment that would normally be exhibited. This finding makes sense, as anxious rats will avoid open spaces more than less anxious rats. In order to perform risk assessment, the rat has to stick its head out into the open space. Although the natural behavior when faced with uncertainty is to assess risk, more anxious rats will perform less risk assessment in their attempt to avoid the open space than will less anxious rats. A similar measure of risk assessment can be obtained in the elevated plus maze when a rat in the closed arm peaks its head into the open by stretching out its back, thereby keeping most of its body in the closed arm. Factor analysis has shown that risk assessment measured in the elevated plus maze loads negatively on the same factor as open arm avoidance (Cruz, Frei & Graeff, 1994), suggesting that it is also a good measure of anxiety.

In addition to measures of anxiety, the elevated plus maze can also measure general

activity and exploration tendency. These measures are useful in arguing that changes in anxious behavior are not due to changes in general activity level. Based on factor analysis, closed arm entries are the best measure of activity level (Cruz, Frei & Graff, 1994).

The Holeboard Test

The holeboard is a test of activity and exploration tendency. Exposing rats to the holeboard has been found to increase open arm entries on the elevated plus maze without affecting the response to anxiolytics (Lister, 1987). This test is therefore used in conjunction with the elevated plus maze for anxiety testing.

Holeboard performance also provides a valid measure of activity and exploration tendency (File & Wardill, 1975). The holeboard consists of a large square box with high walls and a slightly elevated floor containing a number of holes big enough for a rat to poke its head through. Rats will poke (i.e., dip) their heads or noses into the holes to see what lies below and will rise up on two hind legs (i.e., rear) to try and see over the walls. Number of head dips and number of rears are used as measures of activity and exploration tendency.

The Current Study

As discussed above, kindling has been shown to produce LTP. Also suggested is that kindling of particular areas of the rat amygdala results in increased interictal anxiety believed due to repeated activation of the limbic system. Furthermore, fear conditioning produces LTP in the amygdala (McKernan & Shinnick-Gallagher, 1997; Rogan, Staubli, and LeDoux, 1997). It seems likely, then, that excitation of the fear pathway produced by kindling may induce LTP, which in turn catalyzes physiological changes that result in increased anxiety. A number of studies have shown LTP induced by partial kindling in the cat amygdala (in the amygdalo-ventromedial hypothalamus pathway) to be accompanied by increases in defensive behavior (Adamec & Stark-Adamec, 1983; Adamec, 1991; Adamec 1992; Adamec, 1993a). Adamec (1991) also found a close correlation between the degree of defensive behavior and LTP in this pathway. In support of the idea that the LTP may contribute to the development of behavioral change, Adamec (1993a) found that LTP was no longer evident when the defensive behavior had reversed.

Studies mentioned previously illustrate that LTP is produced by the kindling paradigm. LTP has also been produced in the amygdala. Furthermore, results of behavioral studies suggest that LTP produced by kindling contributes in some way to behavioral change and may play a role in its maintenance. If so, then reversing LTP may also result in a reversal of this contribution and therefore of behavioral change. In this regard, researchers are becoming interested in the ability of low frequency stimulation (LFS) to produce a lasting depression of neuronal synaptic transmission known as long term depression (LTD). LTD, the opposite of LTP, is an activity-dependent depression of neural transmission (as opposed to a facilitation

of neural transmission with LTP) that continues after the activity has been terminated. It is induced by administering LFS (usually 1 - 5 Hz) for a long duration (as opposed to administering high frequency stimulation for a short duration in LTP). Although not as reliable as LTP, LTD has been observed with various properties and induction paradigms in many nervous system regions involved in learning and memory (for example, see Christie & Abraham, 1992; for review, see Abraham & Bear, 1996; Christie, Kerr & Abraham, 1994). Similar to LTP, events initiated by the various induction paradigms may lead to increased calcium levels. LTD is not necessarily NMDA dependent, and the means by which it leads to long term modifications of synaptic transmission are not yet clear (for review, see Christie, Kerr & Abraham).

As with LTP, the majority of LTD studies have investigated and characterized LTD in the hippocampus. However, in a recent *in vitro* study, LFS stimulation (1 Hz for 15 mins) was administered to the external capsule of the rat amygdala (Li et al., 1998). Contrary to the expectation based on hippocampal studies, synaptic activity recorded in the BLA was mildly facilitated rather than depressed following LFS. However, when the researchers administered high frequency stimulation *immediately prior* to the LFS (i.e., the pathway was primed), LTD (i.e., depression *below baseline*) was reliably obtained. This finding is not unlike that of Wagner and Alger (1995) with regard to LFS of the hippocampus: LTD could be reliably produced in hippocampal slices of both young (16-22 days) and naive adult (5 - 10 weeks) rats, but a priming stimulation was necessary in the slices from the adults. The authors suggest that developmental factors may be involved in the production of LTD, explaining contradictory results in previous studies. In a related study, Wetzlar and Stanton (1993) found that LFS

elicited only a small amount of LTD. However, when potentiation was induced just prior to the LFS, the amount of LTD elicited more than doubled. Although the age of the rats from which the slices had been taken is not provided, it was noted that they were Sprague-Dawley rats weighing from 125-175 gm. This weight indicates that they were likely greater than 22 days old, supporting the theory of Wagner and Alger. In fact, several studies investigating the age-dependence of LTD induction have found that LTD expression decreases with age (Dudek & Bear, 1993; Kamal, Biessels, Gispen, & Urban, 1998). Although the age of the rats was not given in the Li et al. study, the slices were taken from male Sprague Dawley rats weighing between 75 and 150 gm. Once again, it is not likely that rats of this weight range were 16-22 days old. Had younger rats been used, LTD may have been produced in the unprimed amygdala slices.

In the Wagner and Alger (1995) study, priming was required to elicit LTD in hippocampal slices from mature naive rats. However, depotentiation (the reduction of a potentiated response towards baseline as opposed to the reduction of a response below baseline in standard LTD) was readily obtained. Priming stimulation was not required to produce depotentiation, regardless of the age of the rats from which the slices had been obtained. Likewise, in the Li et al. (1998) study, potentiated responses were readily depotentiated by the administration of LFS. Depotentiation of amygdala LTP has also been obtained *in vivo* in felines. Adamec (1999) found that LFS applied following partial kindling depotentiated right amygdala efferent LTP.

The finding that LFS can reverse or reduce amygdala LTP (i.e., return a potentiated response toward baseline) suggests that it may also be able to reverse or reduce seizures

catalyzed by amygdala LTP. Weiss et al. (1995) investigated this possibility by examining seizure parameters of rats following administration of kindling stimulation. LFS of 1 Hz for 15 min was applied daily for 1 week to the amygdala of rats that had already developed stage 5 seizures (as per Racine, 1978). This reduced the ability to stimulate further seizures. In addition, when LFS was administered to rats during the kindling paradigm, seizures did not develop as would normally have been expected. In fact, marked increases in afterdischarge threshholds were found.

As LTP produced by seizure spread in the kindling paradigm is believed to catalyze events leading to the interictal changes seen in anxious behavior, the finding that LFS can reverse kindling-induced decreases in afterdischarge and seizure threshholds suggests that it may also be able to reverse increases in kindling-induced anxiety. Adamec (1999) investigated this possibility in felines. He found that depotentiation of right amygdala efferent LTP was accompanied by a reversal of changes in defensive behavior induced by partial kindling, strongly suggesting that this may be the case. The current study was undertaken in an effort to determine whether LFS could reduce or reverse kindling-induced affective change in rats as partial kindling does in felines.

Kindling and Low Frequency Stimulation

As much evidence implicates the BLA in fear-conditioned behavior (for review see Davis et al., 1994), and right BLA kindling has been found to be reliably anxiogenic (Adamec, 1998), rats were kindled in the right BLA. A standard kindling procedure used for previous kindling studies in this laboratory was applied until rats had attained 4 stage 5 seizures as per

Racine (1978), with the duration of the fourth seizure being recorded. To assess the effects of kindling on anxious behavior, open arm exploration (Lister, 1987; Pellow, Chopin, File & Briley, 1985) and risk assessment (Blanchard & Blanchard, 1989) in the elevated plus maze were measured one week following cessation of kindling. It was expected that the group of rats receiving right BLA kindling stimulation would show less open arm exploration and less risk assessment than operated controls.

For the LFS rats, the LFS protocol was begun on the day following kindling cessation. Stimulation was set at 1 Hz for 15 mins as used in both the *in vitro* (Li et al., 1998) and *in vivo* (Weiss et al., 1995) studies. As in Weiss et al. (1995), LFS was administered on 7 consecutive days via the kindling electrode. The Weiss et al. (1995) study, however, did not investigate the effect of LFS on behavior. As kindling is known to result in seizure spread to the contralateral hemisphere, it is possible that both hemispheres may be involved in interictal behavioral change. To account for the possibility that behavioral change may arise from potentiation of both hemispheres due to seizure spread, an additional group of rats received bilateral LFS.

To assess the effects of LFS on the kindling-induced anxious behavior, open arm exploration (Lister, 1987; Pellow, Chopin, File & Briley, 1985) and risk assessment (Blanchard & Blanchard, 1989) in the elevated plus maze were measured on the day following cessation of the LFS protocol. It was expected that right LFS would depotentiate kindling-induced LTP in the right hemisphere, therefore reducing the behavioral change catalyzed by kindling in that hemisphere. This was expected to manifest as an increase in both open arm exploration (open arm time and open arm entries) and risk assessment compared to rats that were kindled only

(i.e., a reversal or partial reversal of the kindling-induced changes). Bilateral LFS was expected to depotentiate left LTP caused by seizure spread, in addition to depotentiation of right LTP. If both hemispheres contribute to behavioral manifestations, then bilateral depotentiation should result in a more robust reversal of kindling-induced behavioral change than unilateral LFS (although there may be some spread of unilateral LFS to the contralateral hemisphere, it would not be nearly as robust as direct stimulation). Rats in the bilateral LFS group, therefore, would show more open arm exploration and risk assessment than both the kindled-only group and the kindled and right LFS group.

Testing for Longevity of Behavioral Effects

The elevated plus maze and holeboard were also used to assess the longevity of behavioral effects. While a 5-minute plus maze trial has been determined to be reliable and valid for initial tests of anxiety, its validity for repeated testing has been in question. A number of researchers have found that rats spend less time in the open arms on retesting within one or two days. Because this change is resistant to the application of anxiolytics (File, 1990; File, Zangrossi, Viana, & Graeff, 1993; Lister, 1987; Rodgers et al, 1992; Triet, Menard, & Royan, 1993), it is hypothesized that this effect is due to experience in the open arms (File, Mabbutt, & Hitchcott, 1990; for review, see File, 1993). File et al. (1993) found that two 10-minute trials could overcome this problem in male hooded Lister rats. Referring to Marks (1987), File et al. suggests that the results from the second 5-minute test may represent a phobic state which is generally known to be resistant to anxiolytic administration, but which diminishes with repeated exposure to the phobic situation, thereby accounting for the 10-minute trials

overcoming the problem. Treit et al. (1993) explored this idea by repeatedly exposing rats to the plus maze in an attempt to habituate the hypothesized fear. However, avoidance increased after repeated exposure, and did not habituate by trial 18. A "flooding" treatment (confining the rats to the open arms for three 30-minute sessions) likewise did not result in habituation, with open arm avoidance once again increasing, and with no indication of habituation by trial 18. In light of these results, the idea that the second test is measuring a phobic state that readily habituates seems unlikely.

Dawson, Crawford, Stanhope, Iversen & Tricklebank (1994) investigated the test 2 change in behavior by measuring distance traveled as an indication of exploratory behavior. Similar to the results of others, they found that pre-exposure to the elevated plus maze resulted in a significant reduction of distance traveled. However, exposure with an amnestic dose of chlordiazepoxide significantly increased open arm travel on the second test, suggesting that test 2 changes are due to the habituation of exploratory behavior. Rodgers, Johnson, Carr and Hodgson (1997) further investigated this possibility by re-orienting the plus maze and using a different laboratory for the second test. They found that behavior on test 2 was not affected by this treatment. However, in their discussion they note that they had used dim red lighting, which may have made distal cues less salient than they would have been under normal lighting. In addition, they investigated the behavior of mice, which are known to differ from rats on behavioral testing. In a similar study, Adamec, Burton, Shallow and Budgell (1999) investigated rat behavior on test 2 with the plus maze located in a different room (i.e., the rat is once again placed in a novel environment) under normal laboratory lighting. Exploration levels were found to be similar to those seen on the first test. The results of Adamec et al.

support the suggestion of Dawson et al. that exploratory behavior habituates on the second test if the test environment remains the same. Based on the foregoing, the current study tested longevity of LFS effects on kindling-induced anxious behavior using the holeboard and elevated plus maze following the Adamec et al. protocol (i.e., both the holeboard and the elevated plus maze were situated in a different room from that used for the first test under standard fluorescent lighting).

In testing longevity of LFS on seizure parameters, Weiss et al. (1995) had found seizure thresholds remained elevated as long as ten weeks after the LFS protocol had been terminated. Further, LFS-induced blockade of seizure response to the kindling stimulus lasted for an average of 17 ± 7 days after LFS was discontinued, and more than six weeks in some animals. To test for longevity of behavioral effects in the current study, rats were tested 21 days following the first test. Based on the results of Weiss et al.(1995), it was expected that LFS effects on behavior would still be evident but possibly declining by three weeks, resulting in rates of open arm exploration and risk assessment lying somewhere between control group performance on the second test and LFS group performance on the first test.

Summary

Rats were kindled in the right BLA until 4 stage 5 seizures had been produced. The kindling paradigm should increase anxiety levels, manifesting as a decrease in both open arm behavior and risk assessment in the elevated plus maze. Administering LFS (1 Hz for 15 mins daily for 7 days) to the BLA was expected to increase open arm behavior and risk assessment toward control levels, with bilateral LFS producing a more robust effect than that of right LFS.

Longevity of these changes was tested by measuring open arm behavior and risk assessment with the elevated plus maze in a different room three weeks following the first test. It was expected that the counteractive effect of LFS on the kindling-induced anxious behavior would still be evident three weeks following the cessation of the LFS, but less robust than on initial testing.

The results of this thesis have appeared as part of a larger study of kindling and anxiety (Adamec & Young, 2000).

Method

Subjects

Ninety male Wistar rats were obtained from Charles River Canada. Male Wistars were chosen for this study to be consistent with the previous kindling and behavioral studies in this laboratory. Rats weighed 150 - 170 grams at the time of delivery, and were housed individually in 47 cm x 24.5 cm x 21 cm clear polycarbonate cages with commercial wood chip bedding. The lids were flat wire grates with a downward v-shaped section in the middle to accommodate chow pellets and a water bottle. Commercial rat chow pellets and water were available ad libitum in the home cages. The cages were housed in a ventilated windowless room and maintained on a 12-hr light/dark cycle with lights automatically on at 0700 and off at 1900 hr.

Apparatus

Electrical stimulation cart. The electrical stimulation cart was a large, unpainted plywood box on wheels, measuring 40.6 cm wide, 81.3 cm long, and 91.4 cm high. The top of the box was divided into two rows of four compartments measuring 17.8 x 17.8 x 33.7 cm deep each. Each compartment consisted of three wooden walls with an open wall facing outward (i.e., the compartments opened to the room but not each other) and an open ceiling. Every compartment contained a wire mesh cage measuring 17.8 x 17.8 x 24.8 cm (the cages slid in like drawers for easy removal). The cages each had an open top with an unpainted plywood cover laid over it. Metal litter trays mounted under the wire cages could slide out

for cleaning. Electrical leads were suspended from the ceiling on rubber bands to allow for ease of movement. Leads entering each compartment were accommodated by leaving the cover ajar. All stimulations and sham stimulations took place in this stimulation cart, with one rat per compartment/wire mesh cage. The rats were unable to see each other, but could see out into the room through the mesh on the open side of the box.

Holeboard. The holeboard was a locally made square wooden box painted a flat gray. The sides of the box were 60 cm wide and 47 cm high. The floor was 12 cm above the bottom of the walls and had four evenly spaced round holes. Each hole was 2.54 cm in diameter and 14 cm out from the wall.

Elevated Plus maze. The plus maze was a locally made wooden object painted a flat gray and built in accordance with Pellow, Chopin, File and Briley (1985). It consisted of four arms in the shape of a plus sign raised 50 cm off the floor. Each arm measured 50 x 10 cm, and was connected to the other arms by a 10 cm square central area in common. Two of the arms opposite each other were completely open, except for a 3 cm quarter round ledge on each side and the far end. This ledge was added to increase baseline exploration of the arms and prevent the rats from falling off the arm (Treit, Menard & Royan, 1993). The two remaining opposed arms were closed in on both sides and the far end by 40 cm high walls, but had no ceiling (to facilitate rat removal).

Procedure

Ethics. The study was approved by the Institutional Animal Care Committee, Memorial University of Newfoundland, Protocol Number 97-64-RA.

Groups. Based on previous kindling and behavior studies in this laboratory, fifteen rats were randomly assigned to each of the following groups:

1. Bilateral BLA electrodes, right kindling, no LFS.
2. Bilateral BLA electrodes, right kindling, sham LFS.
3. Bilateral BLA electrodes, right kindling, right LFS.
4. Bilateral BLA electrodes, right kindling, bilateral LFS.
5. Operated control (bilateral BLA electrodes, sham kindling and sham LFS).
6. Unoperated control (no electrodes, no kindling and no LFS).

Batching. Due to the large number of subjects, rats were handled, operated, stimulated, and tested in batches on different days. Every batch contained one rat from each group to control for any day effects.

Handling. Rats were handled on three separate days prior to testing. For the first handling, each rat was picked up by a gloved hand and held securely on the forearm for 1 min while being gently rubbed behind the ears. When this was completed, the rat was placed back in the home cage, then immediately picked up in succession six times before the cage was closed in order to accustom it to pick-up from above. The second and third handlings proceeded as for the first handling, minus the successive pick-ups.

Surgery. Coordinates for implantation of bilateral electrodes to the BLA nucleus were determined in accordance with Paxinos and Watson (1986). They were calculated to be -2.56 mm posterior to bregma, +4.6 and -4.6 mm lateral to the midline, and -8.5 mm ventral to the skull surface.

Once the rats had received their three handlings, the rats in the test groups and the operated control group were prepared for surgery. Each rat was anaesthetized with sodium pentobarbital 60 mg/kg and atropine 0.05 mg/kg ip, then placed in a stereotaxic instrument. The incisor bars were adjusted so that the height of bregma and lambda were equal. A local anaesthetic (Marcaine) was injected under the scalp. A small incision was then made with a scalpel through the skin at the top of the skull, and the cut edges clamped to the side. Two skull holes were drilled, -2.56 mm posterior to bregma, and +4.6 and -4.6 mm lateral to the midline. Four stainless steel screws were inserted into the skull surface surrounding the drilled holes. Twisted 0.125 mm bipolar stainless steel electrodes were lowered through the drilled holes to -8.5 mm ventral to the skull surface. Acrylic dental cement was then mixed and applied directly to the electrodes and the stainless steel screws. Once the cement was dry, dust caps were placed on the protruding ends of the electrodes and the rats were given 10 mg of Chloramphenicol subcutaneously to prevent infection. Following surgery, each rat was returned to its home cage and allowed a one-week recovery period.

Adaptation. Following recovery from surgery, the rats were adapted to the stimulation cart for two days. On the first day, rats were placed individually in the mesh cages on the cart and lids placed loosely on top. They remained there for 20 minutes, then were returned to their home cages. On the second day, rats were again placed in the mesh cages. All operated rats were hooked up to a lead. Lids were then placed loosely on top, taking care to leave room for the leads. The rats were again left for 20 minutes, then returned to their home cages.

Kindling stimulation. Kindling commenced on the day following adaptation to the

stimulation cart. During the procedure, unoperated controls were placed in cart without leads. Leads were attached to the right electrode of operated controls, but they were not stimulated.

On days 1 and 2 of the kindling procedure, rats in the kindling groups received 1 stimulation of 400 uA peak to peak constant current square wave pulses of 1 msec pulse width, delivered in a train of 62.5 pulses per second for one second. Stimulation was administered to the right electrode, and rats were stimulated one at a time. After the train was delivered to each rat, the rat was observed and any seizure activity recorded. Definition of seizure activity was based on Racine (1978) and replicated Weiss et al. (1995) with the following progressive behaviors: Stage 1 - freezing behavior, Stage 2 - twitching and/or jaw movement, Stage 3 - unilateral forepaw clonus, Stage 4 - bilateral forepaw clonus, and Stage 5 - rearing and falling over or losing balance. During the stimulation and observation of seizure activity, operated control rats were hooked up to leads but were not stimulated, and the unoperated controls were without leads. Depending on the length of the seizures experienced by the rats, the processing of the batch took approximately 15-20 minutes. When the last rat in the batch to be stimulated had been completed, all rats were returned to their home cages for the remainder of the day.

The kindling procedure continued in the same way on days 3 and 4, except that the train duration was increased to two seconds for any rat in the kindling group that did not yet experience a stage 5 seizure. On day 5 and subsequent, rats in the kindling groups who had still not experienced a stage 5 seizure had the train duration increased to 3 seconds. Kindling stimulation at the established parameters continued daily until three Stage 5 seizures were experienced. Once a rat in a particular batch had its third Stage 5 seizure, it was not given any

further stimulation during the daily session until the remaining rats from kindling groups in the same batch also experienced three stage 5 seizures (or a maximum of 20 stimulations if no stage 5 seizures were experienced). Once all kindled rats in a particular batch reached this point, they were given one final stimulation, and seizure activity and duration recorded. All rats in that batch had then completed the kindling procedure and were ready for the LFS procedure.

Low frequency stimulation. On the day following the completion of the kindling procedure, the LFS procedure was begun. The rats were again processed in batches, with each batch containing one rat from each of the four test groups, the operated control group, and the unoperated control group. For every second batch (i.e., half the group), however, the controls were left in their home cages and did not experience the LFS procedure. This separation was made to determine whether the additional handling of rats in the sham LFS procedure may contribute to any reduction in anxiety. On later statistical analysis, no difference was found between the control groups left in their home cages, and those experiencing the sham LFS procedure. The controls were therefore collapsed back into two groups: one operated control, and one unoperated control.

As in the kindling procedure, unoperated controls were placed in the cart with no leads and operated controls were hooked up to leads but not stimulated. On day 1, stimulation was administered simultaneously to the right hemisphere of the rats in both the right and bilateral LFS groups. Stimulation was set at 400 μA peak to peak constant current square wave pulses of 100 μsec pulsewidth delivered in a train of 900 pulses at a rate of one pulse per second (total time = 15 minutes). Immediately following, stimulation with the same parameters was

administered to the left electrode of the rats in the bilateral LFS group. Rats were then returned to their home cages. This procedure continued daily for 7 consecutive days, with the order of electrode stimulation for the bilateral LFS rats being alternated daily.

Behavioral testing. On the day following the final LFS (i.e., one week following kindling), rats were randomly assigned to one of two testing rooms containing the holeboard and plus maze. Both rooms were contained in a similar area of the building, and had identical floors, ceilings, and walls, with standard fluorescent lighting and no windows. One room was large with no furnishings other than the testing apparatus and video equipment. The other room was small, with wall cabinetry and a room divider in close proximity to the testing apparatus and the video equipment. For each test, a rat was taken from its home cage and placed by a gloved hand into the center of the holeboard. It was then left alone and its activities videotaped for 5 minutes by a stationary camera mounted on a tripod. Immediately after completing the holeboard test, the rat was placed by gloved hand into the centre square of the plus maze, with the head facing an open arm. The rat was then left alone and its activities videotaped for 5 minutes, then returned to its home cage. The holeboard and plus maze were both thoroughly cleaned with an alcohol/water mix after each rat test to ensure no odors remained. All testing took place between 0830 and 1300 hrs.

Three weeks following the completion of the LFS period (i.e., one month following kindling), all rats were again tested in the holeboard and plus maze. Rats were tested in the room alternate to the one they had experienced on the first test. The procedure for testing was otherwise identical to the first test. Within 24 hrs of completion of the three week behavioral testing, all stimulated rats were given a final stimulation using parameters identical to their last

kindling stimulation. Seizure activity and duration were recorded.

Behavior analysis. After testing was completed, the videotapes were viewed and an inter-rater reliability of .90 was established before official scoring was undertaken. Commercial stopwatches and counters were checked for accuracy and used to facilitate scoring.

The holeboard was used to measure activity and exploratory tendency in accordance with File and Wardill (1975). Two measures of activity were taken. The first measure consisted of counting the number of rears performed during the 5 min test. A rat was considered to have made a rear if it rose up on its hind legs. A second measure of activity was taken by recording the time spent freezing (completely motionless), then deducting that time from the total time spent in the holeboard. Exploratory tendency was measured by counting the number of head dips (rat places or 'dips' nose into one of the holes in the floor) performed during the 5 min test.

The plus maze was used to measure exploratory tendency (Rogers & Johnson, 1995) and anxiety (Lister, 1987; Pellow et al., 1985). Exploratory tendency was measured by counting the total number of entries into the closed arms. A rat was considered to have entered an arm when all four paws were on the arm (i.e., no paw left in the central square).

Two standard anxiety measures were taken: ratio time and ratio entry. Ratio time was calculated by dividing time spent in the open arm (all four paws on the arm) by total time spent in all four arms (time in the central square is not included). Ratio entry was calculated by dividing the number of entries into the open arms by the total number of entries into all the arms.

A third anxiety measure, ratio risk, was taken based on risk assessment as described by Blanchard and Blanchard (1989). A rat was considered to be performing risk assessment if its nose and/ or head and at least one paw stretched out into the open while its body and at least two paws remained in the closed arm. Ratio risk was calculated by dividing the total number of risk assessments performed by the amount of time spent in the closed arms (the rat is only able to perform risk assessment coming from the closed arms).

Histology. After all testing had been completed, rats were deeply anaesthetized with sodium pentobarbital. Each rat was then injected with sodium nitrite (1%) and perfused transcardially with phosphate buffered saline and 4% paraformaldehyde. The brain was immediately removed and frozen in liquid nitrogen. It was then placed in a low-temperature freezer for storage until sectioning at a later date.

Brains were later removed from the low-temperature freezer and placed in a cryostat. Frozen 37 μM sections were taken, beginning at the decussation of the anterior commissure and continuing through to the electrode tracks. The sections were mounted on slides and stained with metachromatic cresyl violet. Using an image analyzer (Jandel, Mocha), the coordinates of the electrode tips in the lateral and vertical plane were measured. Lateral and vertical coordinates were normalized to the nearest corresponding atlas section (Paxinos & Watson, 1986) and then plotted. The AP plane position of the section through the tip of the electrode track was calculated by multiplying section number by thickness and subtracting that distance from the AP plane of the decussation of the anterior commissure.

Statistical analysis: Test results from subjects considered off-target after histological evaluation (outside the BLA) were omitted, leaving the following group membership for

purposes of statistical analysis:

1. Right kindling, no LFS - 9 rats.
2. Right kindling, sham LFS - 9 rats.
3. Right kindling, right LFS - 11 rats.
4. Right kindling, bilateral LFS - 10 rats.
5. Operated control (no kindling or LFS) - 10 rats.
6. Unoperated control (no kindling or LFS) - 15 rats.

The data of these subjects were analyzed using the NCSS 6.0 GLM ANOVA program. Behavior in the holeboard and plus maze, as well as seizure duration were analyzed for effects of kindling, right LFS, and bilateral LFS two-way repeated measures analysis of variance. T-tests were used for planned comparisons, and Bonferroni for unplanned.

Results

Main Findings

Right BLA kindling reliably induced anxious behavior in all measures for up to one month following cessation of kindling. Bilateral (but not right) LFS reversed kindling-induced anxious behavior to control levels for up to three weeks following stimulation, depending on the measure: Risk assessment was increased to control levels on the day following the cessation of LFS, and this effect was still evident three weeks later. While both measures of open arm exploration (ratio time and ratio entry) were also increased to control levels on the day following LFS, this effect was no longer evident three weeks later.

Control Analysis

The two unoperated control groups (unoperated, sham kindled, no LFS; and unoperated, sham kindled, sham LFS) and the two operated control groups (operated, sham kindled, no LFS; and operated, sham kindled, sham LFS;) were analyzed by a two-way repeated measures ANOVA comparing groups over test 1 and test 2 on weight, measures of activity and exploration tendency in the holeboard (rearing, head dips, time active) and plus maze (closed entries), and measures of anxious behavior in the plus maze (ratio entry, ratio time, and ratio risk).

There were test effects for rearing in the holeboard and weight. The number of rears during the five-minute test declined in all groups from an overall mean ± SEM of 37.41 rears ± 1.19 when tested at one week post-kindling, to 32.82 rears ± 1.21 when tested four weeks

post-kindling [F(1,20) = 6.60, p<.05]. However, there was no significant group effect or group x test interaction [F(3,21) = .32, p>.05 and F(3,20) = .33 p>.05 respectively]. Weight increased from a mean ± SEM of 428.78 gm ± 3.60 one week post-kindling to 483.25 gm ± 3.75 four weeks post-kindling [F (1,19) = 99.62, p<.01]. There were no significant group or group x test interactions, with F (3,21) = 1.28, p>.05, and F (3,19) = .24, p>.05 respectively.

There were no other test effects, and no between group differences or group x test interactions for any measures.

Experimental Groups

Only histologically confirmed on-target subjects were used for statistical analysis. As the two operated control groups (sham kindled, no LFS; and sham kindled, sham LFS) did not differ on analysis, they were combined into one operated control and analyzed with the four remaining experimental groups. There were thus five groups for analysis: 1)operated controls; 2) right kindled, no LFS; 3) right kindled, sham LFS; 4) right kindled, right LFS; and 5) right kindled, bilateral LFS. Analysis was performed using a two-way repeated measures ANOVA comparing groups over test 1 and test 2.

Behavioral Measures in the Elevated Plus Maze

Ratio entry. On analysis of ratio entry (number of entries into open arms divided by total entries in all arms) there was a significant group effect and group x test interaction with F(4,44) = 2.65, p < .05, and F(4,43) = 2.66, p < .05 respectively, but no test effect [F(1,43) = .17, p>.05]. Planned comparisons between groups performed by t-tests revealed that on

both Test 1 and 2, kindled groups that received either no LFS, sham LFS, or right LFS had reduced ratio entry measures compared to controls [$t(43) \geq 2.98$, $p<.01$], indicating that kindling had decreased open arm exploration, and right LFS had not counteracted that effect. Bilateral LFS increased ratio entry to that of controls on Test 1 [$t(43)=.73$, $p>.05$]. By Test 2, however, ratio entry had dropped from control level [$t(43)=4.05$, $p<.01$] to that of the other kindled groups (Figure 1).

Ratio time. There was a significant group x test interaction for ratio time (time in open arms divided by total time) [$F(4,43) = 2.75$, $p<.05$], but no significant group [$F(4,44) = 1.54$, $p>.05$] or test [$F(1,43) = 1.42$, $p>.05$] effects. On planned comparisons and Bonferroni, kindling decreased ratio time in all but the bilateral LFS group on Test 1, with the bilateral LFS group dropping back by test 2 [all $t(43) \geq 2.75$, $p < .01$], a pattern very similar to that of ratio entry (See Figure 2).

Ratio risk. Ratio risk (number of risk assessments divided by time in the closed arms) increased from test 1 to test 2, with means ± SEM of 0.108 ± 0.007 and 0.147 ± 0.007 respectively. Test effects were significant with $F(1,42) = 14.46$, $p < .01$, as were group effects [$F(4,44) = 2.88$, $p < .05$], but there were no group x test interactions [$F(4,42 = 1.02$, $p > .05$]. Planned comparisons of kindled groups (sham or no LFS) with the control group revealed a decrease in risk assessment from control level for both Tests 1 and 2 [$t(43) \geq 3.13$, $p<.01$], indicating that kindling had increased anxious behavior. As in the open arm measures, bilateral LFS (but not right LFS) increased performance to control level [$t(43) = 1.06$, $p>.05$; $t(43) = 2.16$, $p<.05$;] respectively (see Figure 3). Unlike open arm time and entries, however, this effect still remained on test 2.

Closed arm entries. Closed arm entry (number of entries into the closed arms) remained the same with means ± SEM of 10.76 and 10.80 entries ± 0.40 and 0.40 on Tests 1 and 2 respectively. There was also no group effect or group x test interaction [$F(4,44) = 1.92$, $p>.05$ and $F(4,43) = 1.32$, $p>.05$ respectively], indicating that the reduction of activity in the holeboard (see below) did not carryover to the plus maze (see Figure 4).

Behavioral Measures in the Holeboard

Rearing. As in controls, rearing declined in all experimental groups from test 1 to test 2, with means ± SEM of 35.97 rears ± 1.00 one week post-kindling declining to 29.85 rears ± 1.01 four weeks post-kindling [$F(1,43) = 18.56$, $p<.01$]. However, there was no significant group effect or group x test interaction [$F(4,44) = 1.86$, $p>.05$] and [$F(4,43) = .71$, $p>.05$, respectively] (see Figure 5).

Time active. There was a small but statistically significant decline in time active, with a mean ± SEM of 295.797 secs ± 1.259 one week post kindling compared to 291.297 secs ± 1.259 four weeks post kindling [$F(1,42) = 6.35$, $p < .05$]. There was no group effect [$F(4,44) = .64$, $p > .05$] or group x test interaction [$(F(4,42) = 1.68$, $p > .05$]. However, it should be noted that there was a decline in time active in the control analysis as well that approached significance [$F(1,19) = 3.89$, $p>.05$ (but $<.06$)].

Head dips. As in controls, head dips declined slightly but non-significantly in all experimental groups from test 1 to 2, with means ± SEM of 14.34 dips ± 1.08 and 12.28 dips ± 1.08 respectively [$F(1,42) = 1.79$, $p > .05$]. There was no significant group effect or group x test interaction [$F(4,44) = .39$ $p > .05$; $F(4,42) = .46$, $p > .05$, respectively] (see Figure 6).

Seizure Duration

Neither right nor bilateral LFS had any effect on seizure duration. Contrary to expectations, the four experimental groups did not differ, indicating that LFS failed to block seizures. There was, however, an overall decrease in seizure duration within groups from Test 1 to Test 2. Mean seizure duration $\pm$ SEM decreased from 108.875 secs $\pm$ 5.207 immediately following kindling to 86.697 secs $\pm$ 5.764 four weeks later (see Figure 7). This test effect was significant at $F(1,27) = 8.08$, $p<.01$, but there was no significant group effect or group x test interaction [$F(3, 34) = .05$, $p>.05$; $F(3,27) = .09$, $p>.05$, respectively].

Histology

Rats were considered on-target if electrode tracks were located either in the BLA or BLA-LA border (see Figures 8A and 8B). In accordance with Adamec (1998), on-target co-ordinates were subjected to a two-way ANOVA comparing groups on plane and hemisphere of focus.

AP plane. On analysis of AP plane by group and day, there was a significant side effect [$F(1,44) = 7.79$, $p<.01$] and group x side interaction [$F(4,44) = 3.31$, $p <.05$], but no group effect [$F(4,44) = .67$, $p >.05$] (see Figure 9). However, there were no between group differences on unplanned comparison using Bonferroni corrected t-tests. The interaction was a result of a within-group difference in the sham LFS group. In this group, electrodes on the left side were positioned more posterior than those on the right side, causing the interaction.

Pearson correlations relating open arm time, open arm entries, and number of risk

assessments to left and right AP plane in each group were all non-significant, with all $p > .05$.

Lateral plane. There were no group or side effects and no group x side interaction on analysis of lateral plane [$F(4,44) = .91$, $p > .05$; $F(1,44) = 1.60$, $p > .05$; and $F(4,44) = .60$, $p > .05$ respectively].

Vertical plane. There were also no group or side effects, and no group x side interaction on analysis of vertical plane [$F(4,44) = .99$, $p > .05$; $F(1,44) = 1.01$, $p > .05$; and $F(4,44) = .31$, $p > .05$ respectively].

Weight

As expected, weight increased in all groups from a mean ± SEM of 419.3 gm ± 1.6 one week post-kindling to 476.6 gm ± 1.6 gm four weeks post-kindling [$F(1,42) = 615.65$, $p < .01$]. There was no significant group effect [$F(4,44) = .45$, $p > .05$] or group x test interaction [$F(4, 42) = .84$, $p > .05$].

Discussion

The current study found that bilateral LFS administered to the BLA reversed kindling-induced anxious behavior in rats, while right LFS had no effect. The duration of the reversal varied, having dissipated in the open arm measures three weeks later, but remaining evident in the risk assessment. Neither right nor bilateral LFS blocked seizures. As in other studies, right BLA kindling was reliably anxiogenic, and this effect was shown to last for at least one month after kindling ceased.

Right LFS

It had been hypothesized that right LFS would depotentiate kindling-induced LTP in the right hemisphere, leading to a reversal of LTP-induced anxious behavior. Contrary to expectations, right LFS had no effect on kindling-induced anxious behavior. Either the LFS protocol had little or no effect in reversing kindled-induced potentiation, or potentiation was reversed but did not result in behavioral change.

Based on the findings of Weiss et al. (1995), it was expected that the LFS protocol would have blocked seizures. Weiss et al. (1995) had found that LFS completely blocked the development and progression of seizures and afterdischarges, and suppressed seizures that had already developed (i.e., afterdischarge and seizure thresholds were increased by the LFS, lasting anywhere from 2 to 6 weeks)[1]. However, LFS applied in the current study had no

[1] On further testing, Weiss, Eidsath, Li, Heynen & Post (1998) attributed these effects to DC leakage rather than LFS. Nonetheless, Figure 1 of their paper shows that LFS without DC stimulation did substantially suppress afterdischarge duration compared to controls. This effect lasted for up to a week, gradually dissipating thereafter, but no statistical analysis or discussion of this result are contained in the paper.

effect on seizures as measured. It is possible that seizure thresholds were raised in the current study, but were not detectable due to the stimulation protocol. Stimulation was set at kindling parameters for all rats, while Weiss et al. (1995) were kindling at the afterdischarge threshold for each rat. If rats had an afterdischarge threshold that had increased but was still less than the kindling parameters, seizures would have been elicited despite the increase in the threshholds. Unfortunately, seizure threshold was not measured in the current study. However, as seizure duration did not decrease, it appears unlikely that kindling-induced potentiation was reversed to any significant degree. In support of this conclusion, Wang and Gean (1999) found that LFS-induced LTD in the BLA (at the lateral-BLA synapse) was negligible in slices from kindled rats and significantly less than LTD induced in age-matched controls.

In their discussion, Wang and Gean (1999) noted that they were unable to induce the 'quenching' phenomenon Weiss et al. (1995) described, although they had used the same LFS protocol as the Weiss et al.(1995) study. The authors suggested that the conflicting results could relate to the different nature of the preparations (*in vivo* vs *in vitro*), and that other LFS protocols not investigated by them may be able to induce amygdala LTD in slices from kindled rats. However, a closer look at the Weiss et al. (1995) protocol raises another possibility that may contribute to both the Wang and Gean results and the results of the current study. An examination of the Weiss et al. (1995) method section reveals that LFS was administered to kindled rats *immediately* after the cessation of afterdischarge or seizure activity following the administration of the kindling stimulation. In other words, Weiss et al. (1995) *primed* the LFS on the first day with the high-frequency stimulation of the kindling protocol, then followed

with six more days of LFS.

Priming is achieved by administering a brief, high-frequency stimulation just prior to the LFS. In the current study, LFS began on the day following the cessation of kindling stimulation; in the Wang and Gean study, the kindled rats were rested from 3 - 7 days before being sacrificed for the slice preparations. Obviously, there was no priming stimulation in either Wang and Gean or the current study. However, the kindling stimulation administered just prior to the first LFS procedure in Weiss et al. (1995) would have undoubtedly primed the LFS. This may be an important difference in the protocols. Some studies of LTD have found priming necessary to induce *in vitro* LTD in both the hippocampus (Wagner & Alger, 1995) and the amygdala (Li et al., 1998) of adult rats. Other studies have found priming to enhance both LTD (Holland & Wagner, 1998, Wetzlar & Stanton, 1993) and depotentiation (Holland & Wagner) in slices taken from adult rats. In fact, Wetzlar and Stanton found that priming LFS could double the amount of LTD induced. Although Wang and Gean were able to induce LTD with unprimed LFS in amygdala slices from unkindled adult rats, the magnitude was significantly less than that induced in slices from unkindled young rats. Such studies suggest that the LFS protocol used in the Wang and Gean study, as well as in the current study, may have induced a negligible or minimal reversal of potentiation. In the Weiss et al. (1995) study, the inadvertent administration of a priming stimulation could have catalyzed LTD or depotentiation, or significantly enhanced any minimal LTD or depotentiation produced.

Although Adamec (1999) was able to reverse potentiation with unprimed LFS in felines, the animals in his study were only partially kindled; there were no generalized seizures as is the case in full kindling and the current study. While partial kindling does produce

behavioral change, it is not as pronounced as after full kindling (Witkin et al., 1988), and biomolecular changes are not as extensive (Chiasson, Dennison & Robertson, 1995). It seems likely, then, that the unprimed LFS protocol used by Wang and Gean and the current study may not have been able to counteract the robust potentiation induced by the full kindling paradigm, and therefore was unable to catalyze the biomolecular events necessary for behavioral change. The effect of primed LFS in the Weiss et al. (1995) protocol is difficult to determine, as Weiss et al. (1998) demonstrated that unintended leakage of DC current throughout the LFS administration was the major contributor to the robust 'quenching' effect reported. Although there also appeared to be a less-robust LFS-induced suppression of afterdischarge duration, no statistical analysis or discussion of that result was provided.

Bilateral LFS

Open arm behavior. In the current study, bilateral LFS reversed anxious behavior but did not block seizures or alter seizure duration in the kindled rats. If the unprimed LFS protocol was unable to elicit either depotentiation or LTD as hypothesized above, then the results of the bilateral LFS appear contradictory. However, Li et al. (1998) found that LFS actually produced a small potentiation in the amygdala unless primed. If unprimed amygdala LFS in the current study produced a potentiation, then right LFS would have added a small potentiation to an already intensely potentiated area known to be anxiogenic. As the rats were already showing substantial anxious behavior, there would likely have been little behavioral change. A small left potentiation repeatedly produced during bilateral LFS, though, may significantly add to a small potentiation already present due to seizure spread from the right

hemisphere. The additive effect could result in a significant change to an area not already intensely activated, resulting in a potentiation of the left BLA. As noted by Adamec (1998), left BLA kindling is known to be anxiolytic. A potentiation of the left BLA would thus be expected to have an anxiolytic effect, as it did in the current study. The anxiolytic effect of bilateral LFS on open arm behavior in the current study was no longer evident three weeks later. In fact, a review of Figures 1 and 2 will illustrate that both measures of open arm behavior had substantially (though non-significantly) dropped below all other groups, including the kindled-only group. Although the drop had not reached statistical significance, it is of particular interest to note the similarity of these results to left BLA kindling in Kalynchuck et al. (1997): Left BLA long-term kindling was found to be anxiolytic on open arm behavior when tested initially, but anxiogenic when tested one month later. In the current study, the anxiolytic effect of bilateral LFS had reversed by three weeks and behavior had dropped below the kindled groups. Had testing been extended to one month or later, open arm behavior may have continued to fall until statistically less than kindled groups. The results of Kalynchuck et. al. suggest that, in the current study, administering repeated unprimed LFS (15 min a day for seven days) to the left BLA following right BLA kindling (which would have resulted in some spread to the left hemisphere) may have acted like long-term kindling of the left BLA. This would account for the initial increase in open arm behavior (an anxiolytic effect), the reversal by three weeks, and the drop in behavior below other kindled groups (an anxiogenic effect).

Risk assessment. Similar to open arm behavior, bilateral LFS increased risk assessment to control level, but the effect did not dissipate and was still evident when tested three weeks later. The longevity of this effect appears contradictory to the short-lived results on open arm

behavior. However, Kalynchuck et al. (1997) did not measure risk assessment, and the results of the current study are in line with several studies suggesting that risk assessment may be measuring a different aspect of anxiety from that of open arm behavior. Cruz et al. (1994) found that while risk assessment loaded on the same factor as percentage of open arm entries and time (anxiety), it also loaded heavily on two other factors, one being central square time (decision making) and the other being grooming (displacement), a behavior associated with approach avoidance conflict (Blanchard & Blanchard, 1989). In addition, risk assessment was decreased by the anxiolytics nitrazepam and midazolam, but was not significantly affected by the anxiogenics FG 71142 and pentylenetetrazol, in contrast to open arm behavior. Furthermore, pharmacological and behavioral factorial separability have also been shown in mice. Rodgers et al.(1992) found the anxiogenics mCPP and TFMPP enhanced risk assessment in mice on the elevated plus maze, but did not significantly affect open-arm entries and time. Rodgers and Johnson (1995) found risk assessment behavior of mice to load on a separate factor from open arm entries and time (anxiety) and closed arm entries (activity).

In addition, hemisphere differences may play a role in behavioral separability. In rats, the NMDA receptor blocker MK-801 reduced risk assessment but did not affect open arm time (Adamec et al., 1999). This result was obtained when MK-801 was administered to either the left BLA or bilateral BLA prior to cat exposure, but not when administered to the right BLA. While that particular study used predator stress instead of kindling to induce anxious behavior, it is interesting to note that the current study found a similar separability of open arm behavior and risk assessment on a hemisphere basis: Bilateral LFS reduced open arm behavior for a short time and risk assessment for an extended period, whereas right LFS had

no effect on either measure.

AP Plane

Supporting previous studies, kindling of the right BLA was anxiogenic. As suggested by Adamec (1998), AP plane of focus in the BLA was irrelevant: behavioral measures were not correlated with AP plane of kindling focus in the right BLA or LFS focus in the left BLA.

Repeated Testing

With the elevated plus maze located in a different room on the second test than the first test, activity in the plus maze (closed arm entries) did not decline. This result supports the findings of Adamec et al. (1999) and Dawson et al. (1994) that the increased open arm avoidance found by many researchers on the second test is due to habituation of exploratory behavior. Closed arm entries have been shown to load exclusively on an activity factor (Cruz et al., 1994), making it a valid measure of plus maze activity.

While the measure of activity in the plus maze did not decline, a measure of activity in the holeboard did. Rearing significantly declined from test 1 to test 2 in all control and experimental groups. One possible explanation for this decline could be the nature of the apparatus. Like the plus maze, the holeboard was placed in a different room for the second test. However, the holeboard has four high walls over which the rats cannot see. The rats only view into the room is the opening in the top of the box, through which the ceiling could be viewed (except for the head-dip holes which view only a small patch of flooring). The ceilings and floors are similar in both rooms. If the only change in surroundings was the ceiling

view and the flooring visible through the holes, the difference would have been very small and may not have been enough to overcome habituation. In support of this idea, Rodgers et al., (1997) found no effect of moving the plus maze to a different room when testing mice under dim red lighting. In their discussion, they mention that the lighting may have made distal cues less discernable, thereby counteracting the effect of the different room. With regard to the holeboard in the current study, the similar ceiling and floor views may have also counteracted the effect of a different room. This would not have been the case for the elevated plus maze, as the layout and content of the two rooms were very different, and fully visible from the arms of the maze.

Regardless of the reason for the reduction in rearing behavior, Fernandes and File (1996) found that activity measured in the plus maze and activity measured in the holeboard loaded on different factors. As noted, the closed arm entries in the plus maze have been confirmed as a good measure of activity by factor analysis. It is therefore reasonable to assume that retesting in a different room overcomes the problem of exploratory habituation on the second test when using the elevated plus maze in rat testing.

Conclusion

Whether depotentiation or LTD was produced in the current study by LFS is not known, although it appears unlikely. Clearly, bilateral LFS reversed kindling-induced anxious behavior in this study, while right LFS had no effect. Neither right nor bilateral LFS had any effect on the occurrence of seizures. Differences between primed and unprimed LFS, and hemisphere differences no doubt played a significant role in the results obtained. A major

problem in interpretation is that there have been relatively few studies of amygdala LTD, either *in vivo* or *in vitro*. Furthermore, *in vitro* studies have not distinguished slices on the basis of hemisphere. There is currently little doubt that functional differences exist in the amygdala based on hemisphere, as well as nuclei and AP plane (Adamec, 1998). It is therefore highly likely that the characteristics and induction paradigms of amygdala LTD also vary in this regard. As a result, theories based on a few *in vitro* studies that do not differentiate between hemisphere are not likely to be accurate.

Based on the foregoing discussion, it is hypothesized that, in the current study, unprimed left BLA-LFS administered during the bilateral LFS protocol potentiated the left BLA (Li et al., 1998). As predicted by Adamec (1998), this potentiation initially resulted in an anxiolytic effect on the kindling-induced anxious behavior as measured by open arm behavior and risk assessment in the elevated plus maze. Similar to Kalynchuck et al. (1997), the anxiolytic effect later dissipated on open arm behavior and appeared to be reversing to an anxiogenic effect. The effect on risk assessment remained, illustrating a separability in function similar to the finding of Adamec et al. (1999). It is also hypothesized that the LFS protocol failed to suppress developed seizures because the unprimed LFS was, as found by Wang & Gean (1999) in kindled neurons, unable to produce LTD.

Further studies are needed to compare left, right, and bilateral primed and unprimed LFS with regard to changes in seizure parameters, LTP expression, and behavioral manifestation.

References

Abraham, W. C., & Bear, M. F. (1996). Metaplasticity: Plasticity of synaptic plasticity. Trends in Neuroscience, 19, 126 - 130.

Adamec, R. (1990a). Amygdala kindling and anxiety in the rat. Neuroreport, 1, 255-258.

Adamec, R. (1990b). Does kindling model anything clinically relevant? Biological Psychiatry, 27, 249-279.

Adamec, R. (1991). Partial kindling of the ventral hippocampus: Identification of changes in limbic physiology which accompany changes in feline aggression and defense. Physiology and Behavior, 49, 443-453.

Adamec, R. (1993a). Partial kindling of the ventral hippocampus: The role of the benzodiazepine receptor in changes in limbic physiology which accompany changes in feline defense. Physiology and Behavior, 49, 443-453.

Adamec, R. (1993). Partial limbic kindling - Brain, behavior, and the benzodiazepine receptor. Physiology & Behavior, 54, 531-545.

Adamec, R. (1998). Amygdala kindling and rodent anxiety. In Kindling 5, M. E. Corcoran & S. L. Moshe (Eds.), Kindling 5 (pp. 327-348). New York: Plenum Press.

Adamec, R. (1999). Evidence that limbic neural plasticity in the right hemisphere mediates partial kindling induced lasting increases in anxiety-like behavior: Effects of low frequency stimulation (Quenching?) on long term potentiation of amygdala efferents and behavior following kindling. Brain Research, 839, 133-152.

Adamec, R., Burton, P., Shallow, T., & Budgell, J. (1999). Unilateral block of NMDA receptors in the amygdala prevents predator stress induced lasting increases in anxiety-like behaviour and unconditioned startle - effective hemisphere depends on the behaviour. Physiology & Behavior, 65, 739-751.

Adamec, R. E., & McKay, D. (1993). Amygdala kindling, anxiety, and corticotrophin releasing factor (CRF). Physiology and Behavior, 54, 423-431.

Adamec, R. E., & Morgan, H. D. (1994). The effect of kindling of different nuclei in the left and right amygdala on anxiety in the rat. Physiology and Behavior, 55, 1-12.

Adamec, R., & Stark-Adamec, C. (1983). Partial kindling and emotional bias in the cat: Lasting after-effects of partial kindling of ventral hippocampus. II: Physiological changes. Behavioral Neural Biology, 38, 223-239.

Adamec, R., & Young, B. A. (2000). Neuroplasticity in specific limbic system circuits may mediate specific kindling induced changes in animal affect - Implications for understanding anxiety associated with epilepsy. Neuroscience and Biobehavioral Reviews, 24, 705-723.

Bechara, Al, Tranel, D., Damasio, H., Adolphs, R., Rockland, C., & Damasio, A. R. (1995). Double dissociation of conditioning and declarative knowledge relative to the amygdala and hippocampus in humans. Science, 269, 1115-1118.

Blanchard, D. C., & Blanchard, R. J. (1972). Innate and conditioned reactions to threat in rats with amygdaloid lesions. Journal of Comparative and Physiological Psychology, 81, 281-290.

Blanchard, R. J., & Blanchard, D. C. (1989). Antipredator defensive behaviors in a visible burrow system. Journal of Comparative Psychology, 103, 70-82.

Blanchard, D. C., Blanchard, R. J., Tom, P., & Rodgers, R. J. (1990). Diazepam changes risk assessment in an anxiety/defense test battery. Psychopharmacology, 101, 511-518.

Chapman, P. F., & Bellavance, L. L. (1992). Induction of long term potentiation in the basolateral amygdala does not depend on NMDA receptor activation. Synapse, 11, 310-318.

Chiasson, B. J., Dennison, Z., & Robertson, H. A. (1995). Amygdala kindling and immediate-early genes. Molecular Brain Research, 29, 191-199.

Christie, B. R., & Abraham, W. C. (1992). NMDA-dependent heterosynaptic long-term depression in the dentate gyrus of anaesthetized rats. Synapse, 10, 1-6.

Christie, B. R., Kerr, D. S., & Abraham, W. C. (1994). Flip side of synaptic plasticity: Long-term depression mechanisms in the hippocampus. Hippocampus, 4, 127-135.

Clugnet, M. C., & LeDoux, J. E. (1990). Synaptic plasticity in fear conditioning circuits: Induction of LTP in the lateral nucleus of the amygdala by stimulation of the medial geniculate body. Journal of Neuroscience, 10, 2818-2824.

Cruz, A. P. M., Frei, F., & Graeff, F. G. (1994). Ethopharmacological analysis of rat behavior on the elevated plus-maze. Pharmacology Biochemistry and Behavior, 49, 171-176.

Davis, M. (1992). The role of the amygdala in fear and anxiety. Annual-Review-of-Neuroscience, 15, 353-375.

Davis, M. (1997). Neurobiology of fear responses - the role of the amygdala. Journal of Neuropsychiatry and Clinical Neurosciences, 9, 382-402.

Davis, M., Rainnie, D., & Cassell, M. (1994). Neurotransmission in the rat amygdala related to fear and anxiety. Trends in Neuroscience, 17, 208-214.

Dawson, G. R., Crawford, S. P., Stanhope, K. J., Iversen, S. D., & Tricklebank, M. D. (1994). One-trial tolerance to the effects of chlordiazepoxide on the elevated plus maze may be due to locomotor habituation, not repeated drug exposure. Psychopharmacology, 113, 570-572.

Dudek, S. M., & Bear, M. F. (1993). Bidirectional long-term modification of synaptic effectiveness in the adult and immature hippocampus. Journal of Neuroscience, 13, 2910-2918.

File, S. E. (1990). One-trial tolerance to the anxiolytic effects of chlordiazepoxide in the plus-maze. Psychopharmacology, 100, 281-282.

File, S. E. (1991). The biological basis of anxiety. In: Meltzer HY, Nerozzi D (eds). Current practices and future development in the pharmacotherapy of mental disorders. Excerpta Medica, Amsterdam, pp 159-166.

File, S. E. (1993). The interplay of learning and anxiety in the elevated plus-maze. Behavioural Brain Research, 58, 199-202.

File, S. E., Mabbutt, P. S., & Hitchcott, P. K. (1990). Characterisation of the phenomenon of "one-trial tolerance" to the anxiolytic effect of chlordiazepoxide in the elevated plus-maze. Psychopharmacology, 102, 98-101.

File, S. E., & Wardill, A. G. (1975). Validity of head-dipping as a measure of exploration in a modified hole-board. Psychopharmacology, 44, 53-59.

File, S. E., Zangrossi, H. Jr., Viana, M., & Graeff, F. G. (1993). Trial 2 in the elevated plus-maze: A different form of fear. Psychopharmacology, 111, 491-494.

Fox, R. J., & Sorenson, C. A. (1994). Bilateral lesions of the amygdala induced by diverse environmental challenges. Brain Research, 648, 215-221.

Gean, P.-W., Chang, F-C., Huang, C-C., Lin, J-H., & Way, L-J. (1993). Long-term enhancement of EPSP and NMDA receptor-mediated synaptic transmission in the amygdala. Brain Research Bulletin, 31, 7-11.

Gloor, P. (1978). Inputs and outputs of the amygdala: What the amygdala is trying to tell the rest of the brain. New York: Plenum Press.

Goddard, G. V., McIntyre, D., & Leech, C. (1969). A permanent change in brain function resulting from daily electrical stimulation. Experimental Neurology, 25, 295-330.

Helfer, V., Deransart, C., Marescaux, C., & Depaulis, A. (1996). Amygdala kindling in the rat - anxiogenic-like consequences. Neuroscience, 73, 971-978.

Henke, P. G., & Sullivan, R. M. (1985). Kindling in the amygdala and susceptibility to stress ulcers. Brain Research Bulletin, 10, 833-837.

Hermann, B. P., & Whitman, S. (1984). Behavioral and personality correlates of epilepsy: A review, methodological critique, and conceptual model. Psychology Bulletin, 95, 451-497.

Holland, L. L., & Wagner, J. J. (1998). Primed facilitation of homosynaptic long-term depression and depotentiation in rat hioppocampus. The Journal of Neuroscience, 18, 887-894.

Kalynchuck, L. E., Pinel, J. P. J., & Treit, D. (1998). Long-term kindling and interictal emotionality in rats: effect of stimulation site. Brain Research, 779, 149-157.

Kalynchuck, L. E., Pinel, J. P. J., Treit, D., & Kippin, T. E. (1997). Changes in emotional behavior produced by long-term amygdala kindling in rats. Biological Psychiatry, 41, 438-451.

Kamal, A., Biessels, G. J., Gispen, W. H., & Urban, I. J. (1998). Increasing age reduces expression of long-term depression and dynamic range of transmission plasticity in CA1 field of the rat hippocampus. Neuroscience, 83, 707-715.

Kandel, E. R. (1991). Cellular mechanisms of learning and the biological basis of individuality. In: E. R. Kandel, J. H. Schwartz, & T. M. Jessell, (Eds.) Principles of Neural Science, 3rd ed. (pp. 1009-1031). East Norwalk: Appleton & Lange.

LaBar, K. S., LeDoux, J. E.,Spencer, D. D., & Phelps, E. A. (1995). Impaired fear conditioning following unilateral temporal lobectomy in humans. Journal of Neuroscience, 15, 6846-6855.

Lehmann, H., Ebert, U., & Losher, W. (1998). Amygdala-kindling induces a lasting reduction of GABA-immunoreactive neurons in a discrete area of the ipsilateral piriform cortex. Synapse, 29, 299-309.

Li, H., Weiss, S. R. B., Chuang, D-M., Post, R. M., & Rogawski, M. A. (1998). Bidirectional synaptic plasticity in the rat basolateral amygdala: Characterization of an activity-dependent switch sensitive to the presynaptic metabotropic glutamate receptor antagonist 2S-alpha-ethylglutamic acid. The Journal of Neuroscience, 18, 1662-1670.

Lister, R. G. (1987). The use of a plus-maze to measure anxiety in the mouse. Psychopharmacology, 180-185.

Loscher, W., Ebert, U., Wahnschaffe, U., & Rundfeldt, C. (1995). Susceptibility of different cell layers of the anterior and posterior part of the piriform cortex to electrical stimulation and kindling: Comparison with the basolateral amygdala and "area tempestas." Neuroscience, 66, 265-276.

Maren, S. (1996). Synaptic transmission and plasticity in the amygdala: An emerging physiology of fear conditioning circuits. Molecular Neurobiology, 13, 1-22.

Maren, S., & Fanselow, M. S. (1995). Synaptic plasticity in the basolateral amygdala induced by hippocampal formation stimulation *in vivo*. The Journal of Neuroscience, 15, 7548-7564.

Maren, S., & Fanselow, M.S. (1996). The amygdala and fear conditioning: Has the nut been cracked? Neuron, 16, 237-240.

Marks, I. M. (1987). Fears, phobias and rituals. Oxford University Press, New York.

Martin, J. H. (1991). The collective electrical behavior of cortical neurons: The electroencephalogram and the mechanisms of epilepsy. In: E. R. Kandel, J. H. Schwartz,& T. M. Jessell,(Eds.) Principles of Neural Science, 3rd ed. (Pp. 777-791). East Norwalk: Appleton & Lange.

McIntyre, D.C. (1978). Amygdala kindling and muricide in rats. Physiology and Behavior, 21, 49-56.

McKernan, M. G., & Shinnick-Gallagher, P. (1997). Fear conditioning induces a lasting potentiation of synaptic currents *in vitro*. Nature, 390, 607-611.

Mittan, R. J. & Locke, G. E. (1982). Fear of seizures: Epilepsy's forgotten problem. Urban Health, 40, 40-41.

Nieminen, S. A., Sirvioe, J., Teittinen, K., Pitkaenen, A., Airaksinen, M. M., & Riekkinen, P. (1992). Amygdala kindling increased fear-response but did not impair spatial memory in rats. Physiology and Behavior, 51, 845-849.

Paxinos, G., & Watson, C. (1982). The Rat Brain in Stereotaxic Coordinates. Sydney: Academic Press.

Paxinos, G., & Watson, C. (1986). The Rat Brain in Stereotaxic Coordinates. San Diego, CA: Academic Press.

Pellow, S., Chopin, P., File, S. E., & Briley, M. (1985). Validation of open:closed arm entries in an elevated plus-maze as a measure of anxiety in the rat. Journal of Neuroscience Methods, 14, 149-167.

Phillips, R. G., & LeDoux, J. E. (1992). Differential contribution of amygdala and hippocampus to cued and contextual fear conditioning. Behavioral Neuroscience, 106, 274-285.

Pinel, J. P.(1981). Spontaneous kindled motor seizures in rats. In J. A. Wada (Ed.). Kindling 2, Raven Press, New York.

Pinel, J. P. J. & Rovner, L. I. (1978). Experimental epileptogenesis: Kindling-induced epilepsy in rats. Experimental Neurology, 58, 335-346.

Pitkanen, A., Tuunanen, J., Kalviainen, R., Partanen, K., & Salmenpera, T. (1998). Amygdala damage in experimental and human temporal lobe epilepsy. Epilepsy Research, 32, 233-253.

Prince, D. A. (1978). Neurophysiology of epilepsy. Ann Rev. Neurosci., 1, 395-415

Racine, R. (1978). Kindling: The first decade. Neurosurgery, 3, 234-251.

Racine, R. J., Milgram, N. W., & Hafner, S. (1983). Long-term potentiation phenomena in rat limbic forebrain. Brain Reseaarch, 260, 217-231.

Rodgers, R. J., Cole, J. C., Cobain, M. R., Daly, P., Doran, P.J., Eells, J. R., & Wallis, P. (1992). Anxiogenic-like effects of fluprazine and eltoprazine in the elevated plus-maze: Profile comparisons with 8-OH-DPAT, CGS 12066B, TFMPP and mCPP. Behaviorial Pharmacology, 3, 621-634.

Rodgers, R. J. & Johnson, J. T. (1995). Factor Analysis of Spatiotemporal and Ethological Measures in the Murine Elevated Plus-Maze Test of anxiety. Pharmacology, Biochemistry and Behavior, 52, 297-303.

Rodgers, R. J., Johnson, N. J. T., Carr, J., & Hodgson, T. P. (1997). Resistance of experientially-induced changes in murine plus-maze behavior to altered retest conditions. Behavioural Brain Research, 86, 71-77.

Rogan, M.T., & LeDoux, J. E. (1996). Emotion: Systems, cells, synaptic plasticity. Cell, 85, 469-475.

Rogan, M. T., Staubli, U. V., & LeDoux, J. E. (1997). Fear conditioning induces associative long-term potentiation in the amygdala. Nature, 390, 604-607.

Rosen, J. B, & Schulkin, J. (1998). From normal fear to pathological anxiety. Psychological Review, 105, 325-350.

Shindou, T., Watanabe, S., Yamamoto, K., & Nakanishi, H. (1993). NMDA receptor-dependent formation of long-term potentiation in the rat medial amygdala neuron in an *in vitro* slice preparation. Brain Research Bulletin, 31, 667-672.

Stark-Adamec, C., & Adamec, R. E. In B. K. Doane and K. E. Livingston (Eds.) The Limbic System: Functional Organization and Clinical Disorders. New York, Raven Press, (1986), pp 217-227.

Strauss, E. (1989). Ictal and interictal manifestations of emotions in epilepsy. In: F. Boller, & J. Grafman, (eds.). Handbook of Neuropsychology, vol. 3. New York: Elsevier Publishers.

Treit, D., Menard, J., & Royan, C. (1993). Anxiogenic stimuli in the elevated plus-maze. Pharmacology, Biochemistry and Behavior, 44, 463-469.

Wagner, J. J. & Alger, B. E. (1995). GABAergic and developmental influences on homosynaptic LTD and depotentiation in rat hippocampus. The Journal of Neuroscience, 15, 577-1586.

Wang, S-P., & Gean, P-W (1999). Long-term depression of excitatory synaptic transmission in the rat amygdala. The Journal of Neuroscience, 19, 10656-10663.

Watanabe, Y., Ikegaya, Y., Saito, H., & Abe, K. (1996). Opposite regulation by the Beta-adrenoceptor-cyclic AMP system of synaptic plasticity in the medial and lateral amygdala *in vitro*. Neuroscience, 71, 1031-1035.

Watanabe, Y., Saito, H., & Abe, K. (1995). Roles of GABAA, NMDA and muscarinic receptors in the induction of long-term potentiation in the medial and lateral amygdala *in vitro*. Neuroscience Research, 21, 317-322.

Watanabe, Y., Saito, H., and Abe, K. (1995). Brain Research, 688, 233-236.

Weiss, S. R. B., Eidsath, A., Li, X.-L., Heynen, T., & Post, R. M. (1998). Quenching revisited: Low level direct current inhibits amygdala-kindled seizures. Experimental Neurology,154, 185-192.

Weiss, S. R. B., Li, X.-L., Rosen, J. B., Li, H., Heynen, T., & Post, R. M. (1995). Quenching: inhibition of development and expression of amygdala kindled seizures with low frequency stimulation. Neuroreport, 6, 2171-2176.

Wetzlar, E. M., & Stanton, P. K. (1993). Priming of homosynaptic long-term depression in hippocampus by previous synaptic activity. Neuroreport, 4, 591-594.

Witkin, J. M., Lee, M. A., and Walczak, D. D. (1988). Anxiolytic properties of amygdaloid kindling unrelated to benzodiazepine receptors. Psychopharmacology, 96, 296-301.

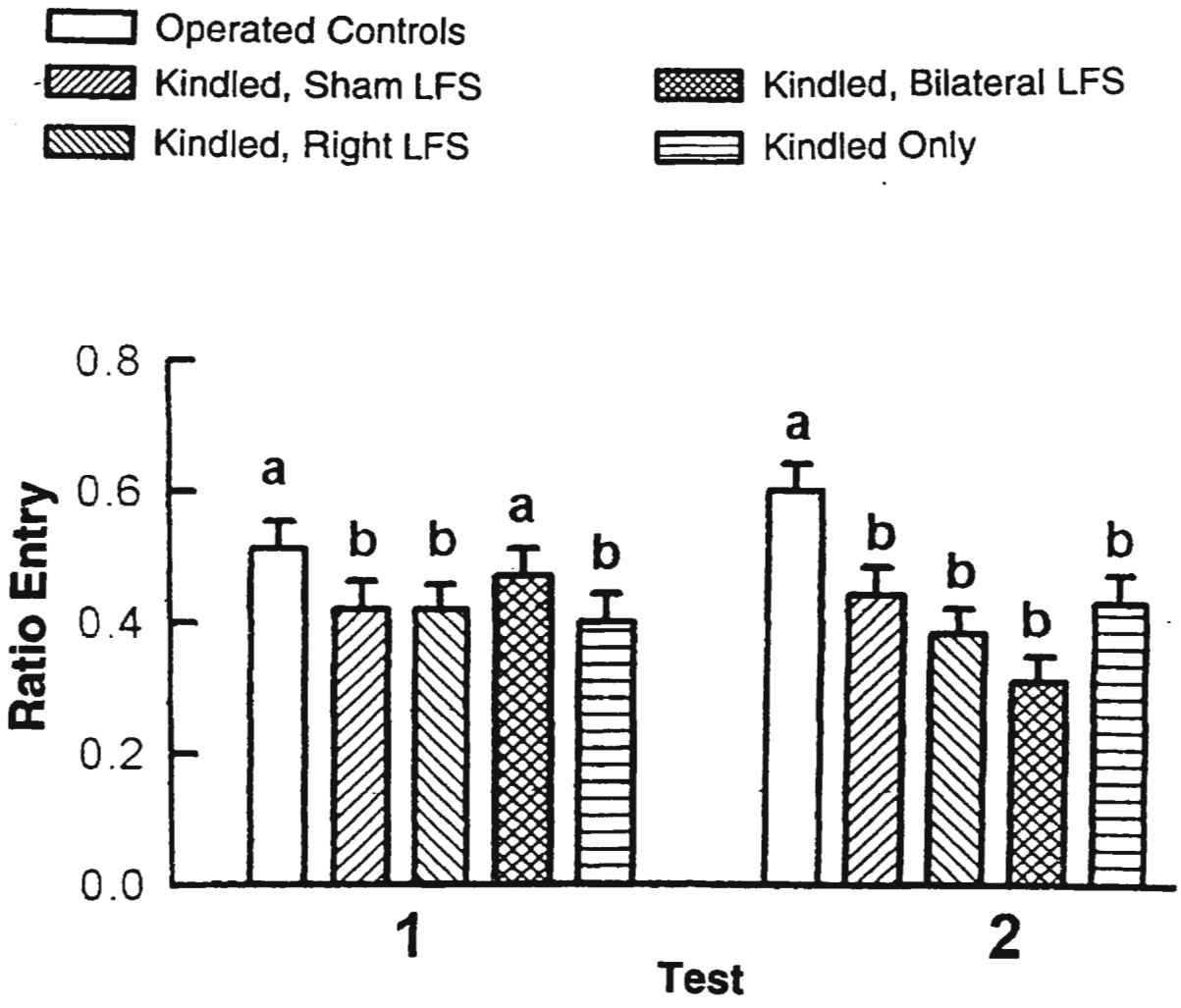

Figure 1. Mean Open Arm Entries in the Elevated Plus Maze. Anxious behavior measured as a ratio of entries in the open arm over total entries in the open and closed arms of the elevated plus maze (means ±SEM). Rats receiving kindling and bilateral LFS are similar to controls (a), showing significantly more open arm entries than kindled rats receiving sham, right, or no LFS (b). Three weeks later (test 2), open arm entries have dropped below all groups, and significantly less than controls.

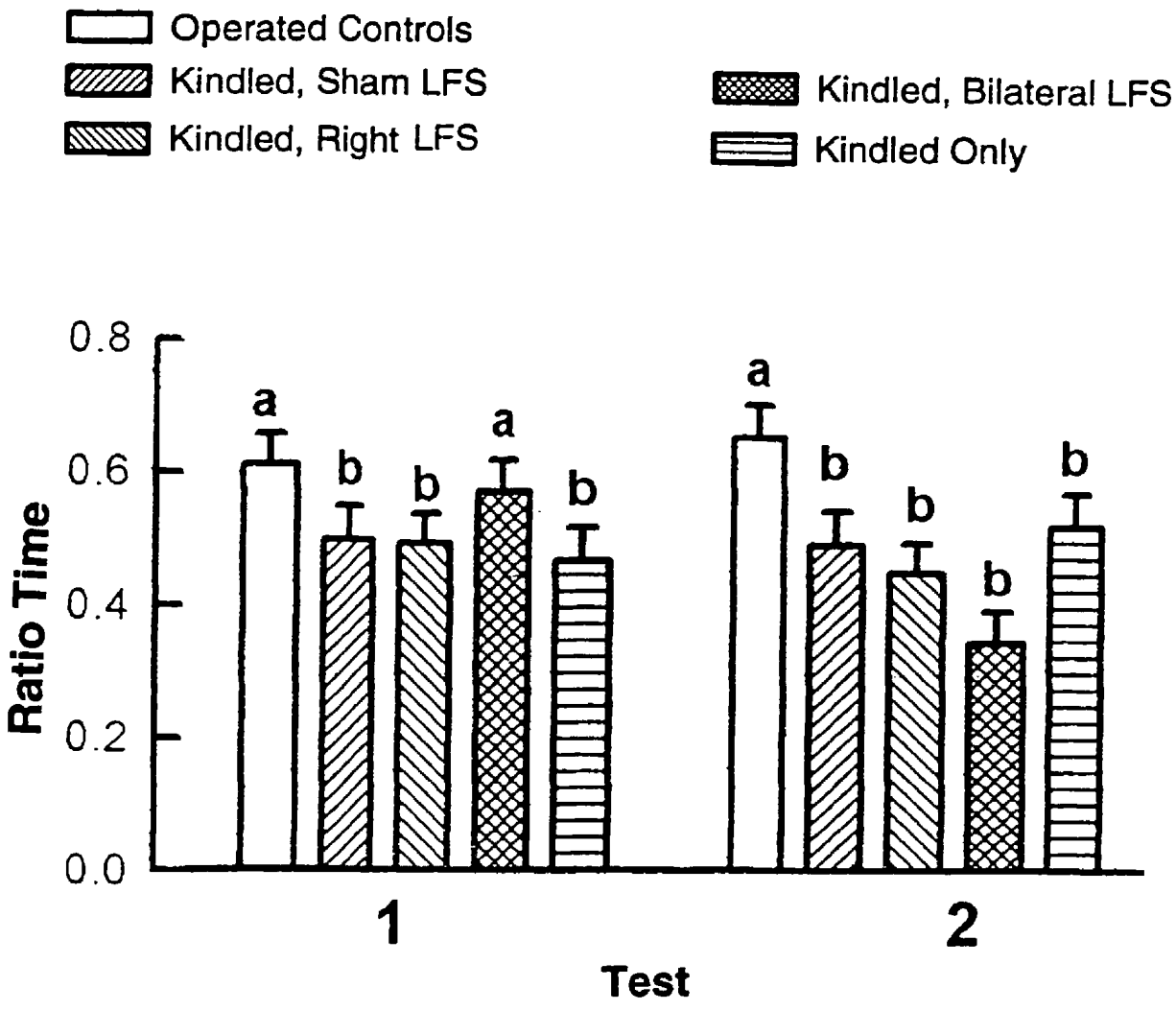

Figure 2. Mean Open Arm Time in the Elevated Plus Maze. Anxious behavior measured as a ratio of time in the open arm over total time in the open and closed arms in the elevated plus maze (means ± SEM). Rats receiving kindling and bilateral LFS are similar to controls (a), showing significantly more open arm time than kindled rats receiving sham, right, or no LFS (b). Three weeks later (test 2), open arm time has dropped below all groups, and significantly less than controls.

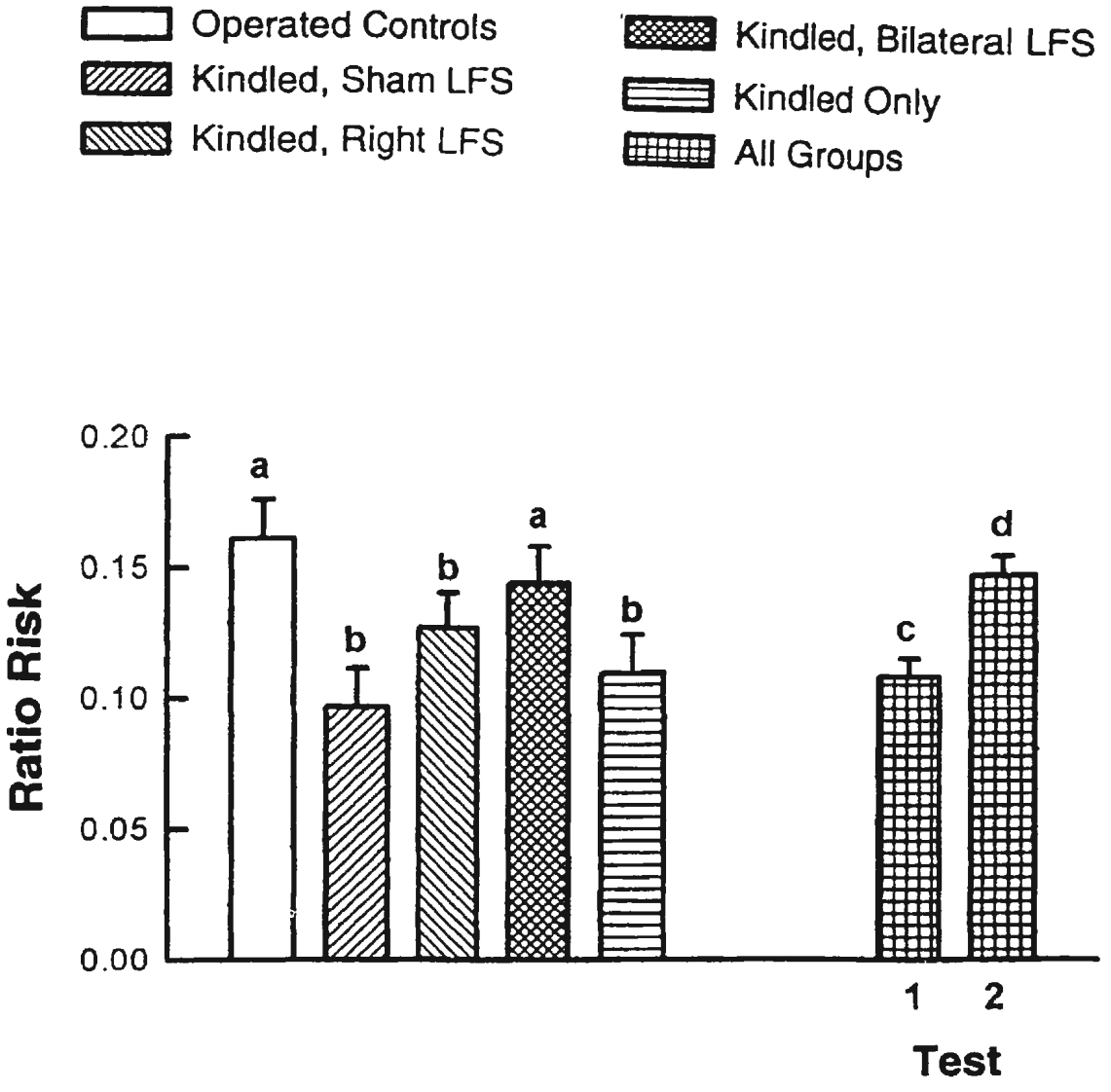

Figure 3. Mean Risk Assessments in the Elevated Plus Maze. Anxious behavior measured as a ratio of risk assessments performed over the total amount of time spent in the closed arms of the elevated plus maze (means ± SEM). Rats receiving kindling and bilateral LFS are similar to controls (a), showing significantly more risk assessments than kindled rats receiving sham, right, or no LFS (b). Risk assessment increased overall from test 1 (c) to test 2 (d), but there was no change in group distribution.

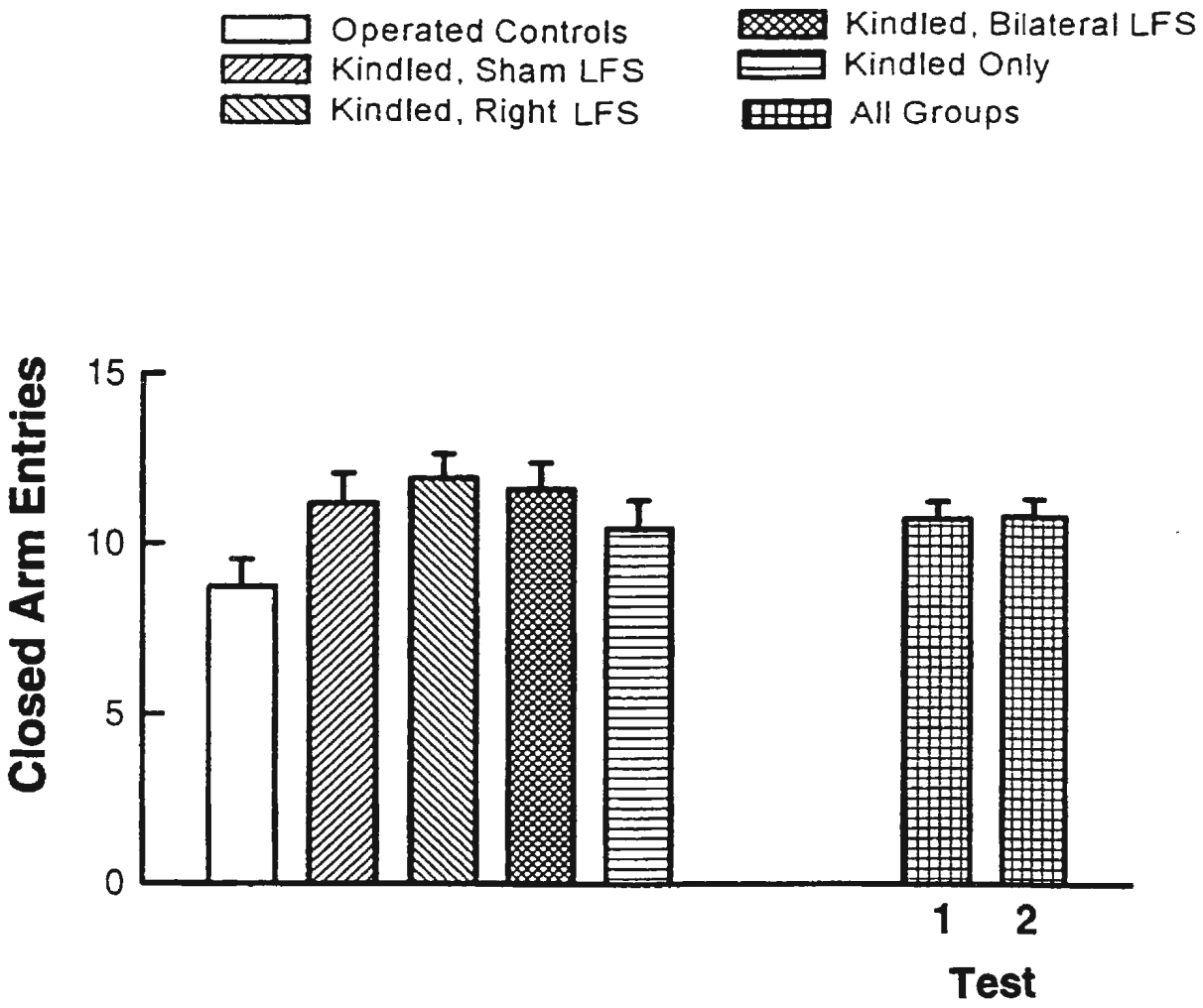

Figure 4. Mean Closed Arm Entries in the Elevated Plus Maze. Level of activity as measured by closed arm entries in the elevated plus maze (means ± SEM). There was no significant effect of group and no change between initial testing and retesting three weeks later.

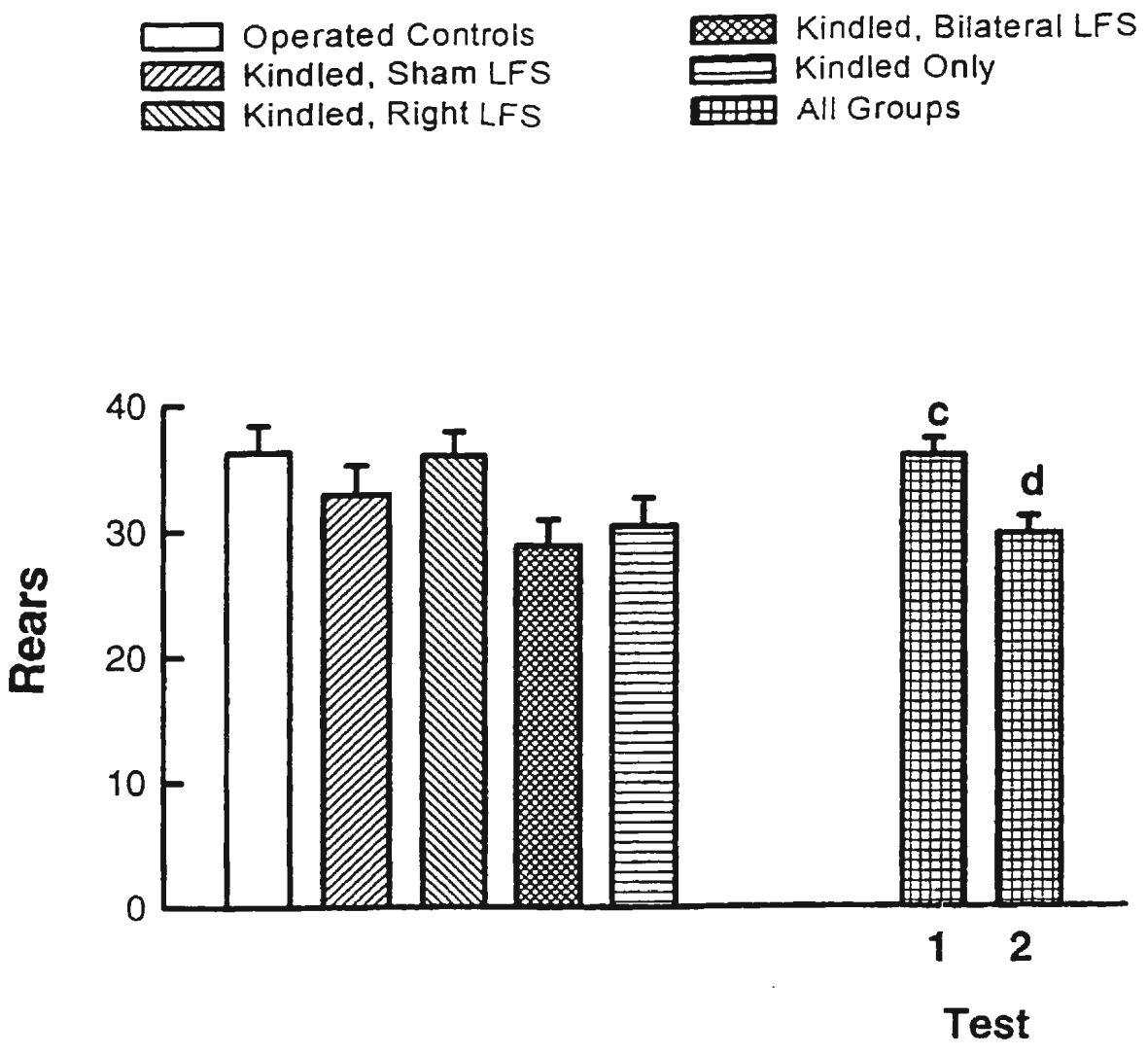

Figure 5. Mean Rears in the Holeboard. Activity in the hole board as measured by number of rears (means ± SEM). There were no group differences. Activity levels were significantly decreased on test 2 (d) three weeks after the initial test (c).

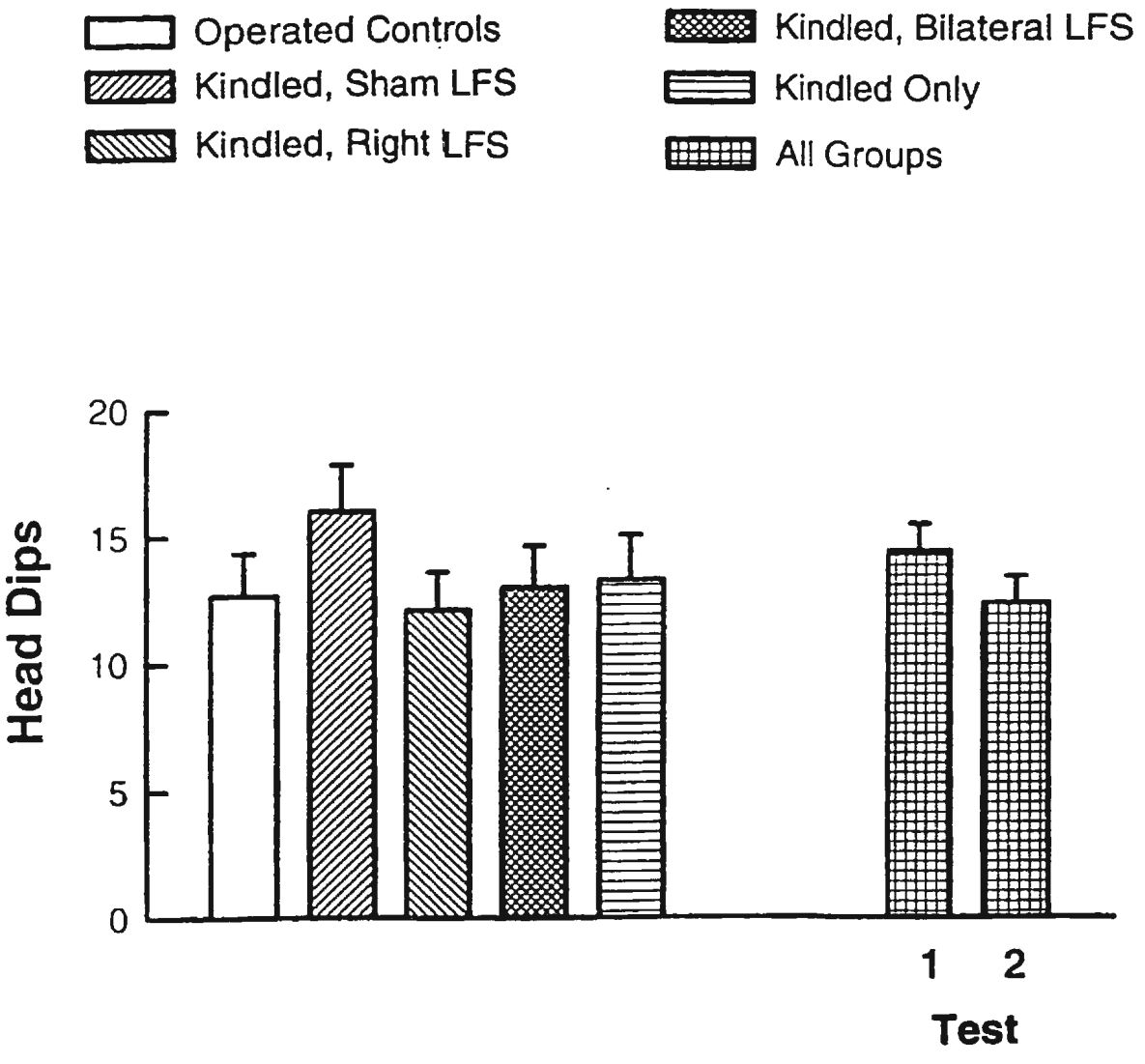

Figure 6. Mean Head Dips in the Holeboard. Exploration tendency as measured by head dips in the holeboard (means ± SEM). There were no significant differences between groups or on repeated testing.

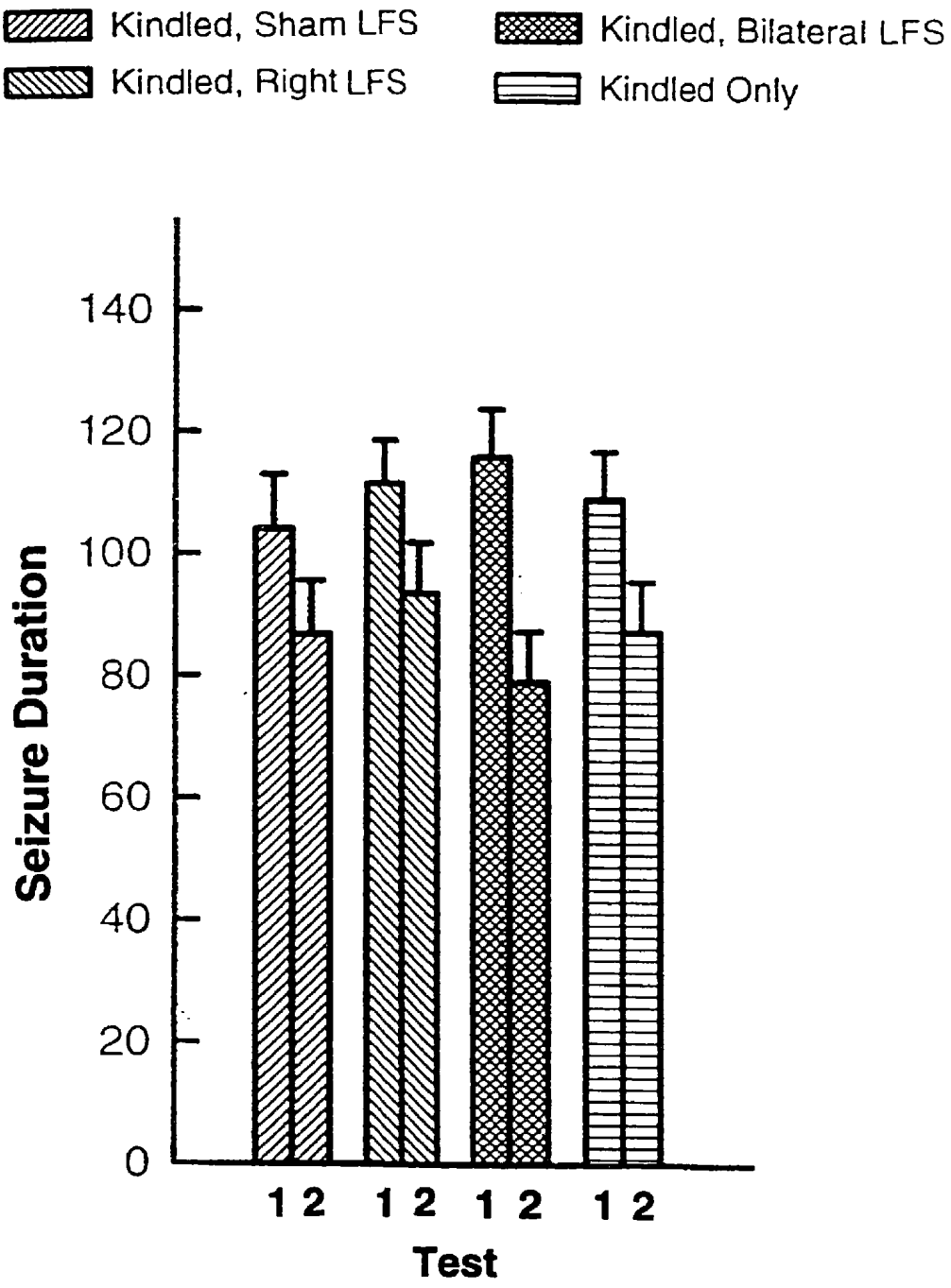

Figure 7. Mean Seizure Duration. Seizure duration for all kindled groups in seconds (means ± SEM). There are no significant group differences, although seizures declined overall when tested one month later.

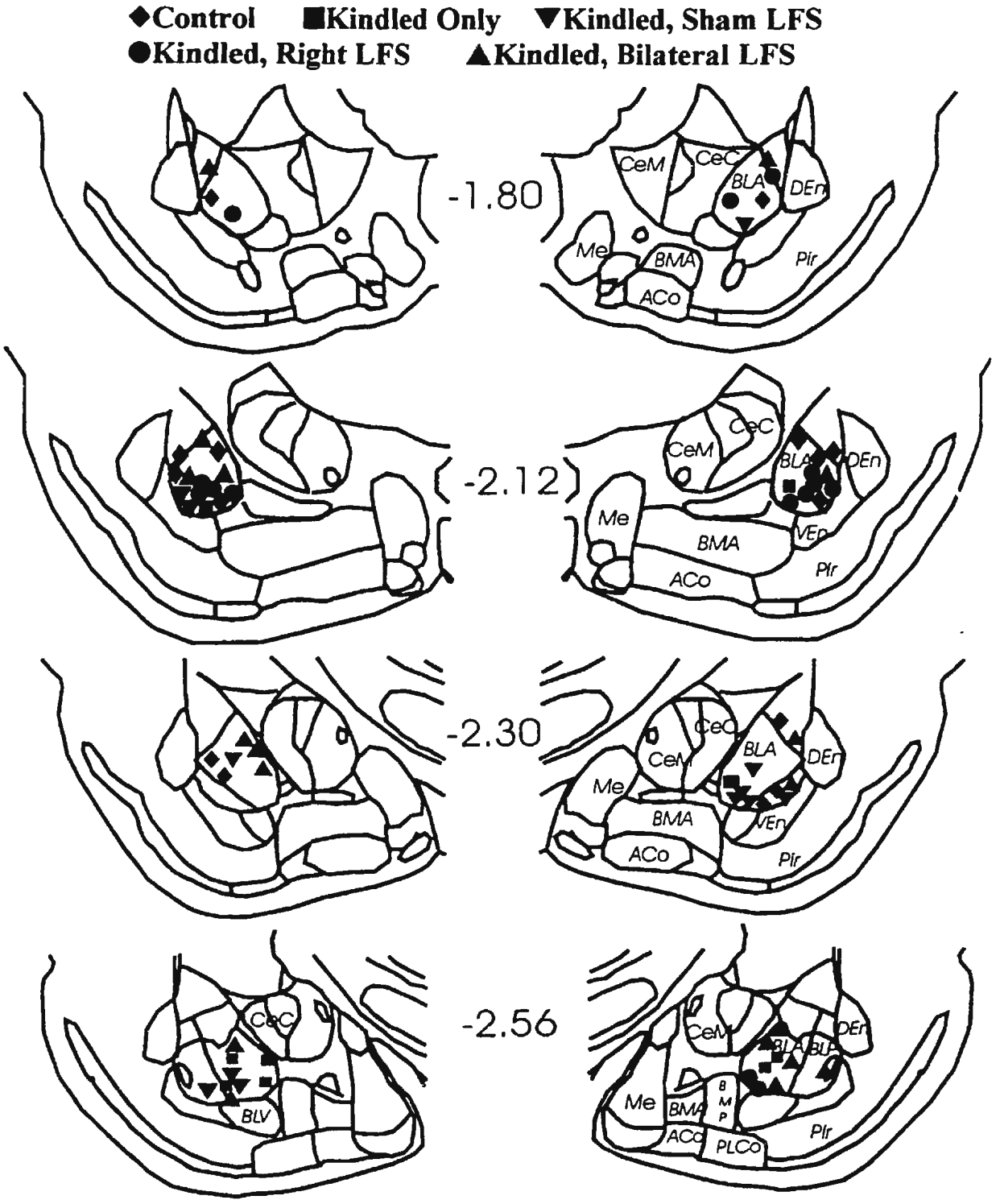

Figure 8(a). Locations of Electrode Tips. Locations are for all on-target rats as plotted onto plates of the Paxinos and Watson atlas for plate positions -1.80 to -2.56 mm posterior to bregma. Abbreviations are those used by Paxinos and Watson (1986).

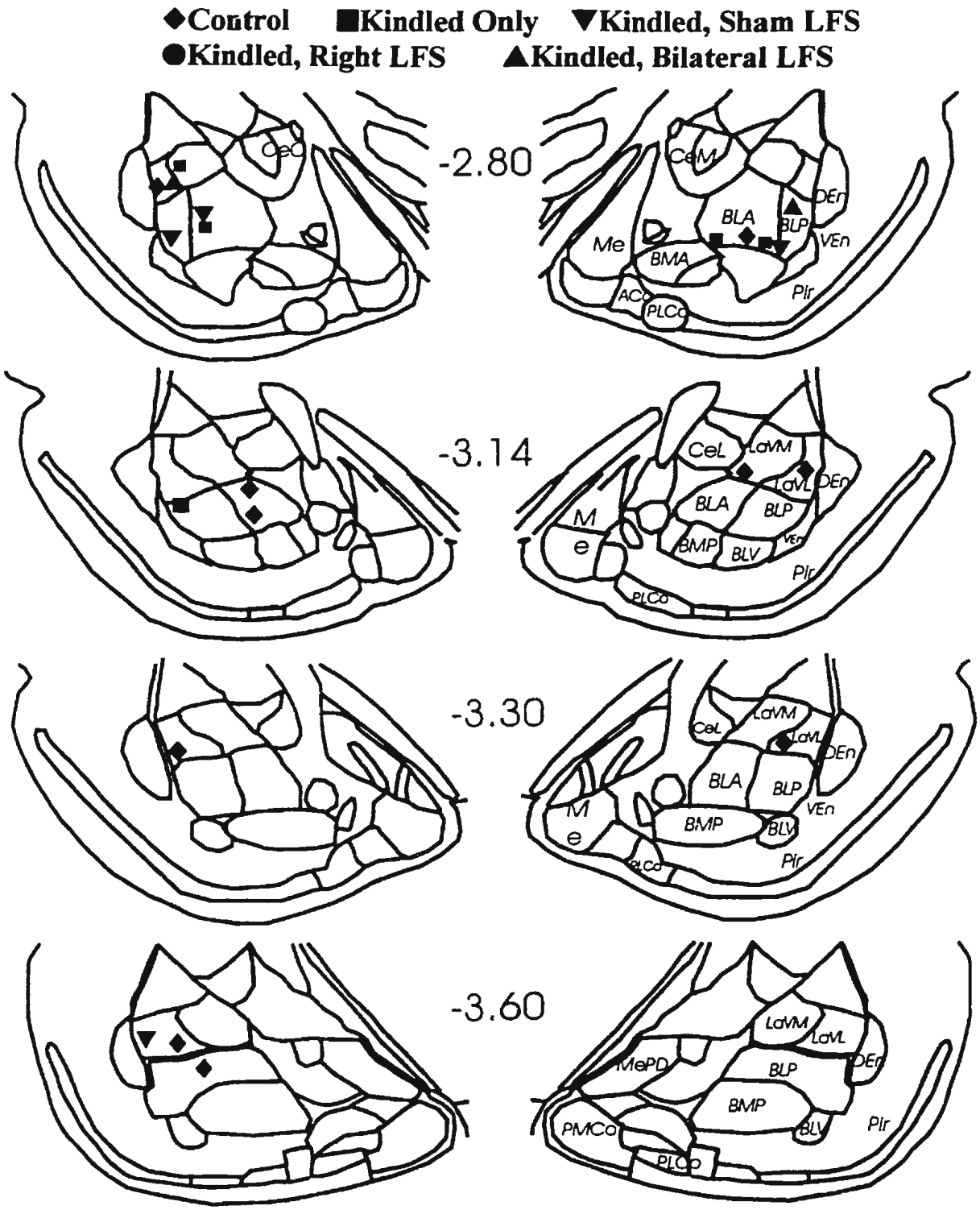

Figure 8(b). Locations of Electrode Tips (Continued). Locations are for all on-target rats as plotted onto plates of the Paxinos and Watson atlas for plate positions -2.80 to -3.60 mm posterior to bregma. Abbreviations are those used by Paxinos and Watson (1986).

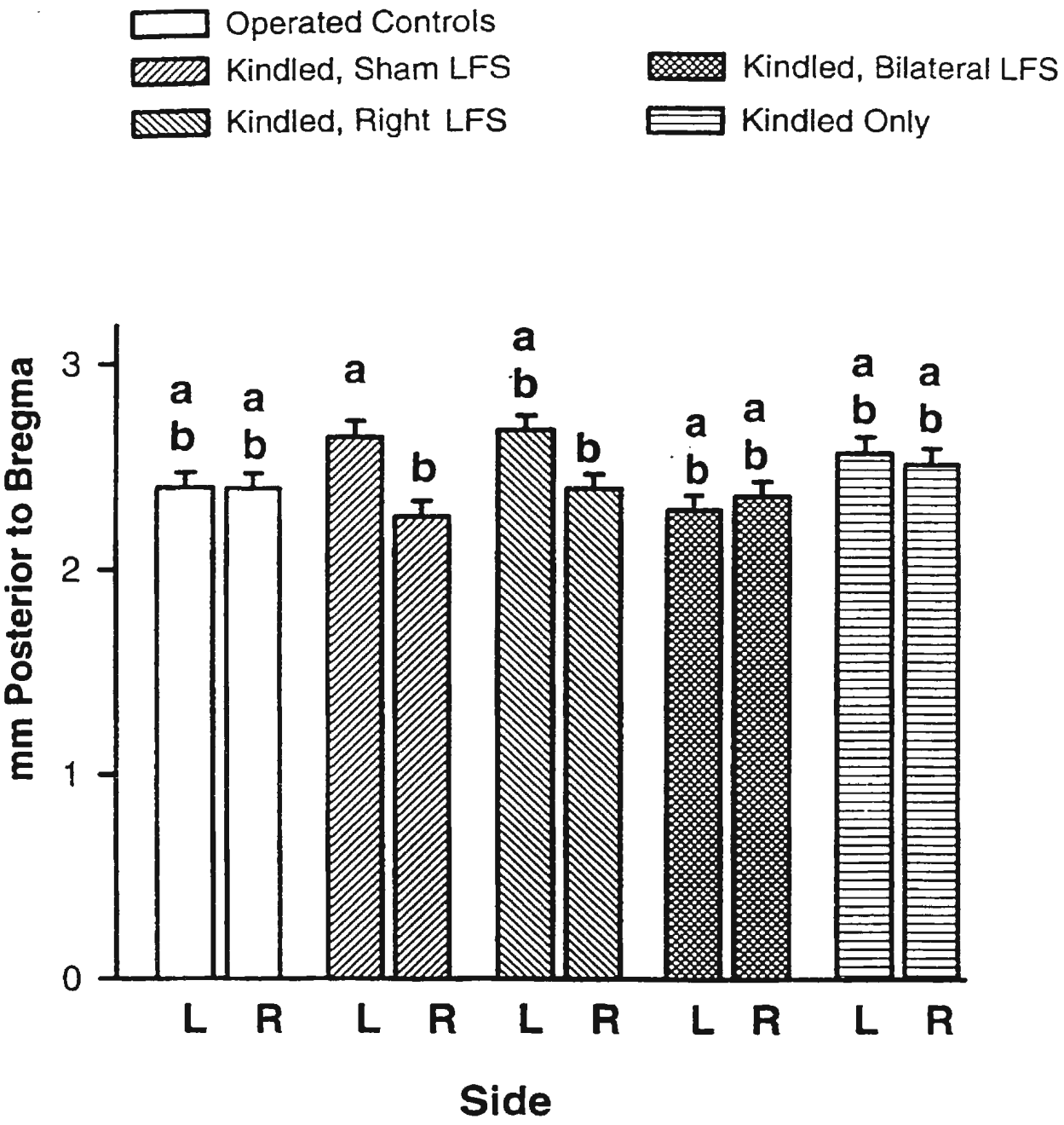

Figure 9. Mean AP Plane Co-ordinates of Electrode Tips. AP plane coordinates (means ± SEM) of electrode tip locations for all on-target rats in mm posterior to bregma . There was a significant side effect due to a difference in the sham LFS group.