THE EFFECTS OF ELECTRODE LOCATION AND BASELINE ANXIETY ON KINDLING-INDUCED ANXIETY IN THE RAT

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The Effects of Electrode Location and Baseline Anxiety on Kindling-Induced Anxiety in the Rat

by

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Abstract

A series of three experiments were completed to further study the relation of kindled site and baseline anxiety levels to plus maze behavior in Wistar rats. Results indicated that the behavioral outcomes found one week post kindling for animals kindled in central amygdala, the medial amygdala, the basolateral amygdala, and the nucleus basalis differed depending on the location of the kindled site. In addition, the results showed that the nature of behavioral outcomes for animals kindled in the right medial amygdala and basolateral amygdala were dependent on pre-kindling baseline anxiety levels, measured using a novel retest procedure. Taken together, the above results indicate that both location of kindled site and the pre-kindling baseline anxiety levels of rats play a role in determining post-kindling behavioral outcomes in the elevated plus maze. These results may explain inconsistencies in previous studies related to the behavioral effects of kindling.

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The work presented in this thesis has been previously published in three prior publications for which I am co-author. As a graduate student I was responsible for all aspects of the experimental processes (e.g. ordering materials, scheduling, surgeries and behavioral testing) and data analysis. The publications are as follows:

> Adamec R, Shallow T. Effects of baseline anxiety on response to kindling of the right medial amygdala. Physiology and Behavior 2000;70(1/2):67-80.
>
> Adamec R, Shallow T. Rodent anxiety and kindling of the central amygdala and nucleus basalis. Physiology and Behavior 2000;70(1/2):177-87.
>
> Adamec R, Shallow T, Burton P. Anxiolytic and anxiogenic effects of kindling – role of baseline anxiety and anatomical location of the kindling electrode in response to kindling of the right and left basolateral amygdala. Behavioural Brain Research 159 (2005) 73-88.

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1.0 Introduction and Overview

Human studies of limbic system epilepsy commonly identify anxiety and depression as the prominent affective disorders that accompany seizures [5, 54]. These characteristic behavioral effects of human epilepsy have become the focus of animal models developed to enable researchers to further study the behavioral changes associated with the seizures, and to potentially identify the biological factors that lead to the psychopathology. Of particular interest is the question related to whether the affective disorders are a product of the pathophysiology of epilepsy, or whether they are due to other associated psychosocial causes.

Kindling as a Model for Partial Seizure Disorder

For more than three decades the kindling procedure, involving the repeated delivery of brief, high-frequency trains of electrical impulses to the brain, has been used to study convulsive seizure activity. The kindling phenomenon is a process whereby repetition of previously non-convulsive stimulations results in these stimulations becoming convulsive [50]. This convulsive sensitization is long lasting, and has been found to persist up to one year after the stimulations have been stopped [75].

There are a number of components of the kindling model which mirror those implicated in the seizure activity of humans including the length of sensitization, the graded development of seizures and the presence of associated effects on cognition and affect. Goddard et al. [49] found that kindling of cortical and limbic areas of the brain resulted in motor convulsions which were longer in duration than the stimulus train.

Further research by Racine [74] has shown that kindling has a graded development with respect to both the duration and amplitude of the seizure, and the motor involvement. There is also a gradual spreading involvement of brain structures distal to the stimulated focus [75]. Kindling of limbic structures in animals is found to proceed rapidly, and limbic kindling has been found to be modulated most effectively by agents that are effective in treating complex partial seizure disorders in humans [5].

In addition, researchers have confirmed the applicability of the kindling model as a model for the psychopathology associated with limbic epilepsy given the observed associated changes in cognition and affect. Kindling-induced changes in cognition include: a short-term impairment of long-term memory [41]; a disruption in radial 8-arm maze performance in well-trained rats [63]; impairment in the passive-avoidance task and an effect on flavor-aversion conditioning [71]; and impaired place navigation by rats in the Morris water task [96]. Demonstrated changes in affect include: heightened defensiveness in cats [1, 25]; increased anxiety in rodents in the elevated plus-maze [12, 19]; persistent anxiety in human epileptics [5]; a decrease of non-social behavior and an increase in immobility in the social interaction test [53]; increased resistance to capture in an open field test [59]; and exaggerated fear-potentiated startle [86]. Of particular interest in the current study are the changes in anxiety-like behavior in rodents that follow kindling [12, 19, 59].

Role of Amygdala in Anxiety-Like Behavior

The effects of kindling on the underlying neuroanatomical structures involved in the generation of anxiety-like behaviors have been an important focus in the study of epilepsy. In recent years, much of the research related to anxiety has focused on one particular anxiety system, the fear system, and one corresponding anatomical focus, the amygdala. Davis et al. [36] summarized the high level involvement of the amygdala in the fear response. They noted that for the most part, electrical stimulation of the amygdala produces a series of behavioral changes that mimic those produced by fearful stimuli, and that lesions of the amygdala block innate or conditioned reactions to stress. This statement is supported by research in humans whereby Hermann et. al. [55] found that following the removal of the amygdala in an anterior temporal lobectomy, anxiety, as measured by the Mental Health Inventory, was reduced.

Animal studies have also confirmed the role of the amygdala in the generation of anxiety-like behavior. For example, in the basolateral nucleus of the rodent amygdala, Davis et. al. [36] reported that decreased opiate and GABA transmission and increased noradrenergic transmission lead to excitation of the amygdala and an improvement in aversive conditioning. They also found that an increase in opiate and GABA transmission and decreasing NMDA and noradrenergic transmission mitigates aversive conditioning and produces anxiolytic effects in appropriate animal tests. (For review see [89]).

Factors Which Influence the Effect of Kindling on Anxiety-Like Behavior

There are a number of factors that appear to contribute to the effects of amygdala

kindling on anxiety-like behavior. In particular, findings have pointed to hemisphere of focus, location of focus within a nucleus, and the baseline anxiety at time of initiation of kindling as variables that are important in determining the effects of kindling [12].

Hemisphere of Focus

Opposite effects on the anxiety-like behavior of rats have been observed following kindling in the left and right basolateral amygdala [19]. Kindled rats with electrodes located in the right basolateral amygdala tended to show increased anxiety-like behavior in the elevated plus-maze test [19, 53]. In contrast, kindling of the left basolateral amygdala (BLA) was anxiolytic in the elevated plus-maze [19, 59]. Witkin et al. [102] first reported an anxiolytic like effect of left BLA kindling on punished responding in rats trained to bar-press under a multiple schedule to produce food (every 30^{th} response) or food plus shock (every 10^{th} response). Similar differences were found following kindling in the right and left medial amygdalas which showed respective increases [6, 19] and decreases [19] in anxiety-like behavior for at least one week following termination of the seizures.

Anterior-Posterior Plane

While kindling of certain nuclei in the amygdala appear to produce uniform effects on anxiety (e.g. right basolateral nucleus) [12, 53], in others differing behavioral responses to kindling appear to depend on the anterior-posterior location of the kindled site. In 1993, Adamec and McKay [18] reported a decrease in the anxiety levels of rats kindled in the right posterior medial amygdala relative to implanted controls, while animals kindled in the right anterior medial amygdala showed increased anxiety. In a

similar kindling experiment, Adamec and Morgan [19] replicated the anterior-posterior plane effect in the right basomedial nucleus with kindling in the more anterior foci resulting in increased anxiety, while kindling of the posterior foci ranged from decreased anxiety to no significant effect. Anterior/posterior variances in kindling affect have also been demonstrated in the right cortical nucleus [18] and the right central nucleus of the amygdala [12].

Baseline Anxiety at Initiation of Kindling

Numerous studies have shown that the level of anxiety in cats at the beginning of the kindling process is also a strong predictor of behavioral consequence [1, 4, 5, 7]. In general, cats can be divided into four major categories [1]: rat killers - least defensive; non-rat killers which attack the rat - next least defensive; non-rat killers that do not attack rats but approach and sniff the rat - more defensive; and non-rat killers that withdraw abruptly and go into a covered shelter to observe the prey from a distance - most defensive. Behaviorally, the least defensive cats are minimally affected by threat whereas more defensive cats avoid potential threats such as vocal threats, novel environments and threatening prey.

When considering neural activity, Adamec reported two findings related to the characteristic groupings of these animals: 1) animals were found to vary in amygdaloid response when orienting to the live rat, with non-killers showing a higher integrated neural amygdaloid response; and 2) the increased amygdaloid response is accompanied by a decreased level of activity in the ventral hippocampus - an area known to facilitate predatory attack. In addition it has been found that hippocampal kindling of un-defensive

cats serves to increase predatory behavior while similar kindling in defensive cats further increases the defensive response. Overall, the research suggests that the response of the amygdala reflects the behavioral consequence and that the process is likely facilitated by the strength of amygdala efferent transmission to other areas of the brain - including the hypothalamus and the periacqueductal gray (PAG) [2, 3, 4, 8, 10, 11].

How then does the level of excitability of the amygdala and the strength of amygdala efferents lead to changes in the physiological and behavioral effects of seizures? It has been suggested that the behavioral changes are likely a product of both excitation and/or attenuation of transmission in several different pathways [24]. In 1991, Adamec confirmed that at least three different substrates control the approach-attack and defensive response to threat. The amygdalo-ventromedial hypothalamus (VMH) pathway was found to increase the defensive response of cats to rats. The amygdalo- bed nucleus of the stria terminalis (BNST) pathway was found to play a role in the suppression of some types of predatory aggression. Finally, changes in the inhibition in the ventral hippocampus, more specifically in areas CA1 and CA3, were implicated in changes in defensiveness and predatory aggression in cats. Further experiments have also pointed to a critical role of NMDA dependent long-term potentiation of the right amygdalo-peri-acqueductal gray (PAG) pathway in the prolonged increase of feline anxiety-like behaviors [13]. Taken together, the above results provide direct evidence for baseline behavioral effects which must be given careful consideration in the interpretation of kindling studies, particularly those measuring fear responses. While some consideration has been given to behavioral baseline effects in studies of feline defense, no such

consideration has been given to rodent investigations concerning kindling and anxiety-like behaviors.

Measuring Baseline Behavioral Effects in Rodents

A number of tests are used to measure anxiety in rodents. As new tests emerge they are typically validated pharmacologically, behaviorally and/or physiologically. File [42] noted that the anxiety tests, for the most part, fall into three high level categories: those based on conflict or conditioned fear; those in which anxiety is generated by a novel situation; and those that involve the use of chemicals to induce anxiety.

The elevated plus-maze is a validated measure of anxiety in the rat [72, 73] which permits the quantitative analysis of anxiety generated in a novel situation. The maze consists of two open arms located opposite one another, and two open-roofed, closed arms. The arms are elevated 50 cm above the ground and are joined in the middle by a 10 cm square platform. Pharmacologically, rats exposed to the plus-maze only show an increase in open-arm exploration following administration of clinically effective anxiolytics such as chlordiazepoxide and diazepam [72]. Physiologically, the plasma corticosterone concentrations are higher in rats confined to the open arms versus those confined to closed arms. The specific anxiety measured in the test is that of an approach-avoidance conflict generated by exposure to an open and elevated maze alley [33]. In general, anxiety-like behavior in the maze is related to the exploration of the open arms with increased anxiety exhibited as open-arm avoidance. In a study designed to systematically determine the anxiogenic role of the various stimuli in the plus-maze, Treit

et. al. [98] confirmed that it is the open space, rather than the height or novelty characteristics of the maze, which is the anxiogenic stimulus. Because behavioral analysis in the elevated plus-maze does not require long training procedures nor the use of aversive stimuli, it is believed to have clear advantages over other anxiety tests [72]. This lack of motivational artifacts, combined with the relative simplicity of the test, make it useful for the study of anxiety-like behavior.

To examine the behavioral baseline of rodents in kindling studies, researchers would be required to perform some form of pre-test prior to the initiation of kindling. The introduction of a plus-maze pre-test is complicated by two characteristics of rodent behavior in the elevated plus-maze. The first concerns the fact that rodents that are repeatedly exposed to the plus-maze continue to spend large amounts of time in the closed arms with no apparent habituation of the anxiogenic response [72, 98]. The second characteristic is that clinically-effective, anxiolytic benzodiazepines that normally increase open-arm activity on a first plus-maze test do not result in an anxiolytic behavioral response in rats that had been previously exposed to the plus-maze [43, 44, 46, 98]. Taken together, these findings would suggest that a repeated test in the plus-maze would no longer be a reliable measure of rodent anxiety.

To overcome this complication, the Adamec laboratory has developed a novel retest procedure that allows the re-testing of rodents in the plus-maze apparatus while maintaining test-retest reliability [current study]. In this procedure, rats are tested prior to the kindling/experimental process using normal protocol. At a minimum of three-weeks later, rats can then be re-tested in the plus-maze, providing the maze is located in a novel

room. The 3 week inter-test interval and the novel room are both required to stop the decrease in open-arm exploration. Re-testing using this process also restores the sensitivity to benzodiazepines thus maintaining pharmacological validity (unpublished data).

Purpose of Current Study

The purpose of the current study was three-fold and includes three separate experiments.

Experiment 1: Differences in location of kindled site and resulting behavioral outcomes

Experiment 1 was designed to further analyze the effects of kindling on various nuclei including an analysis of any potential anterior-posterior effects. Two particular areas of focus were chosen given their documented involvement in behavioral affect.

The first was the central nucleus of the amygdala. It has been suggested that connections between the right basolateral amygdala and the central nucleus are necessary for the anxiogenic response seen as a result of right BLA kindling [12]. In addition a number of researchers have found that the central nucleus plays a role in the development of anxiety in rodents [34, 35, 37, 61]. To date studies have found conflicting results as to the behavioral effects of kindling the central nucleus, with both anxiogenic [12, 52] and anxiolytic [59] results reported. It has been suggested [12] that differing effects of central nucleus kindling on anxiety may depend on location of the kindled site in the anterior-posterior plane. This study was designed to test this idea.

The second location of study was the nucleus basalis due to its proposed role in both affective and cognitive functions. Electrolytic and cell lesions in this nucleus have been found to produce effects like those seen with damage to the anterior central nucleus. Small bilateral lesions of the nucleus basalis were found to produce deficits in passive avoidance of drinking [68]. While these deficits suggest a decrease in fearfulness, the change in passive avoidance caused by the damage is also associated with signs of increased anxiety in agonist social encounters [69, 80].

Previous findings related to these locations suggest that kindling of the nucleus basalis and the anterior central amygdala should produce an anxiolytic effect while kindling of other nuclei of the central amygdala shown to facilitate anxiety should produce an anxiogenic effect.

Experiment 2: Relation of baseline anxiety to response to kindling of the right medial amygdala

Experiment 2 was designed to study the importance of baseline anxiety on response to right medial amygdala kindling. There were three purposes of this study. The first was to confirm that behavior in the plus-maze using the retest procedure is stable on the retest. The second was to use a retest paradigm with the elevated plus-maze to examine the relation of baseline anxiety-like behavior to response to medial amygdala kindling. Finally, the experiment also examined the effects of electrode implantation on behavior.

Experiment 3: Relation of baseline anxiety and electrode location to the response to kindling in the right and left basolateral amygdala

Experiment 3 was designed to extend the study of how baseline anxiety affects response to kindling in the right and left basolateral amygdala. This study was designed to provide a better understanding of the generality of the importance of baseline anxiety effects in limbic kindling.

2.0 Materials & Methodology

2.1 Experiment 1 - Differences in location of kindled site and resulting behavioral outcomes.

2.1.1 Subjects & Handling Procedure

Experimental subjects were one hundred and twenty male Wistar rats weighing 150g to 170g upon arrival in the laboratory. Rats were individually housed in clear plastic cages measuring 47 cm x 24.5 cm x 21 cm with wood chip bedding. Commercial rat chow pellets and water were available ad libitum in home cages. All rats were maintained on a 12 hour light/dark cycle with lights on at 0700 h. Animals weighed between 200 and 250 grams at the beginning of the experiment.

Rats were adapted to the laboratory for four days prior to the initiation of the experiment. During the first day of adaptation, animals were left undisturbed. On each of the three remaining days, animals were handled for a one minute period. The handling procedure involved picking the animal up with a gloved hand, and positioning the animal on the opposite arm while maintaining gentle restraint, with the gloved hand, around the shoulder area. The restraint was loosened or tightened based on the level of mobility. Following the one-minute handling procedure, rats were returned to their home cages. Handling continued every second day of the experiment up to the day the animals began adaptation to the kindling apparatus.

2.1.2 Experimental Groups

Four brain areas were targeted in the study, with the experimental groups determined based on brain area, and the presence or absence of kindling. All animals received right

Table 1: Handling Timeframes

Timeframe	Handling Period
Day 1 in Laboratory	No handling
Day 2 to Day 4	Handled once per day for 1 minute
Day 5 to surgery date for implanted animals. (Controls also handled at same periods).	Every second day for 1 minute
Surgery date to the adaptation to kindling apparatus	No handling
Adaptation to kindling apparatus	Once per day for 2 days. Handling period required to attach electrode lead (controls handled as well)
Kindling process	As required until animal reached 4th stage 5 seizure (controls were matched)
Post stage 5 seizure to behavioral testing	No handling

Note: Animals were also were transferred by hand to behavioral testing apparatus for all behavioral testing procedures.

hemisphere electrodes implanted in either the: anterior central nucleus (ACN) of the amygdala; mid central nucleus (MCN) of the amygdala; posterior central nucleus (PCN) of the amygdala, or nucleus basalis (NB). The groups were divided as follows: 1 - ACN kindled; 2 - ACN non-kindled; 3 - MCN kindled; 4 - MCN non-kindled; 5 - PCN kindled; 6 - PCN non-kindled; 7 - NB kindled; and 8 - NB non-kindled. The non-kindled animals served as implanted controls.

Given the large number of groups, the study was completed in stages with all groups represented during each stage.

2.1.3 Surgical Procedure

Rats were anesthetized with Somnotol (60mg/kg) and Atropine (0.5mg/kg) administered via an intraperitoneal injection, and then placed in a stereotaxic instrument with the incisor bar positioned at "skull flat". Once in position, the surgical area was locally anesthetized using Marcaine, and the incision was made. Then the zero horizontal

elevation of the head was confirmed by checking both bregma and lambda.

Coordinates for the target brain areas were adapted from the atlas of Paxinos and Watson [70]. Targets were selected to fall within particular anterior-posterior positions based on the findings in a recent review by Adamec [12]. The anterior-posterior ranges acceptable for each brain area, and the lateral and vertical coordinates are provided in Table 1. A hole was drilled in the skull immediately above the target area and a 0.125 mm twisted bipolar stainless steel stimulating electrode (Plastics One) was implanted. Following electrode placement, an antibiotic spray was applied around the wound area. The electrode was secured in place with dental acrylic cement secured to the skull with three stainless steel skull screws. Once the cement had dried, a dust cap was positioned onto the top of the electrode and the animal was given a subcutaneous injection of chloramphenicol (10 mg). Rats were then returned to their holding rooms and were given a one week recovery period prior to the start of the experiment.

Table 2: Target coordinates for central nucleus and nucleus basalis placements.

Structure	Anterior-posterior range	Target Coordinates		
		AP	Lateral	Vertical
Posterior central amygdala	-2.8 to -3.3	-3.0	4.2	7.9
Mid central amygdala	-2.3 to -2.79	-2.5	4.2	7.4
Anterior central amygdala	-1.8 to -2.3	-2.1	4.1	7.6
Nucleus basalis	-1.8 to -2.3	-1.8	3.2	7.2

Anterior-posterior ranges and coordinates are in mm relative to Bregma. Vertical coordinates are in mm below the dura.

2.1.4 Kindling Procedure

Following the recovery period, rats were adapted to the kindling box for two days. The kindling box consisted of a large grey rectangular wooden structure that was divided into eight individual sections. Within each wooden section, was a wire mesh cage with an open top. On each of the two adaptation days, each rat was placed in a compartment, and remained there for approximately 10 minutes. Rats were then picked up and placed on the forearm where they were gently restrained so that the stimulating lead could be connected. Once placed back into the compartment, the rats remained in the kindling box for an additional 10 minutes. Following the adaptation, stimulating leads were disconnected and the animals were returned to their home cages. During the kindling procedure, rats were placed into the kindling box with stimulating leads attached. Kindling stimulation was applied twice daily using a batch process whereby eight rats at a time, one for each experimental group, were placed in the kindling box. Implanted controls were handled exactly like kindled animals, except for the lack of stimulation. Kindling occurred during two specific time periods of the day. The first procedure occurred between 0900 and 1100 h, and the second occurred between 1400 and 1600 h. Stimulation consisted of trains of balanced biphasic 1 millisecond pulses of 400μA peak-to-peak amplitude with a train rate of 62.5 pulses per second. The duration of the train was set to 1 second for the first two stimulations, and then increased to 2 seconds for the remainder of the kindling process.

Rats were kindled twice a day, until they reached 3 stage 5 seizures as defined by Racine [75]. When a rat reached this point it was left undisturbed until all other kindled

animals in the batch had also shown 3 stage 5 seizures (approximately 1-4 days). Approximately 24 hours after the last animal of the batch had the third stage five seizure, all animals were returned to the kindling box and a fourth stage 5 seizure was evoked for all kindled animals. Following the fourth seizure, animals were returned to the holding rooms for one week.

2.1.5 Kindling Parameters

The number of stimulations to the first stage 5 seizure and the duration of the last (4^{th}) seizure were the two measures recorded during the kindling process.

2.1.6 Behavioral Testing

Following the seven-day, post-kindling rest period, anxiety-like behavior was tested in the hole board and the elevated plus-maze. The hole board is used to provide an independent measure of activity and exploratory tendencies that could have an effect on behavior in the plus-maze [45]. The hole board test involved placing a rat in the center of a plywood box. The box measured 60 cm per side, with walls rising 35 cm above a floor that was elevated 12 cm above the ground. Four evenly spaced holes measuring 2.5 cm each were drilled in the floor, with each hole placed 14 cm from a wall. The hole board was painted flat grey. Once placed in the box, the rat was videotaped remotely for 5 minutes from above.

Following the five minute hole board test, the animal was transferred immediately by gloved hand to the elevated plus-maze located behind a room divider. For the test,

animals were placed on the center platform of the maze always facing the same open arm and were videotaped remotely for a five-minute period from above. Rats were then returned to their home cages.

Behavioral testing was scheduled so that all groups were tested at the same average time of the day (1448 h ± 12 min). Between each test the hole board and the plus-maze were cleaned using a 10% alcohol solution.

2.1.7 Behavioral Measures

A number of behavioral measures were taken from videotaped records of the hole board and plus-maze tests. Activity measures in the hole board included the number of rears [45] and time active, i.e., time spent in motion [17, 18, 19]. The main exploratory measure of the hole board is the number of head dips, the placing of the nose down into one of the four holes in the floor [45]. In addition, the number of boli left behind in the hole board was counted as the rat was removed from the hole board.

Behavioral measures taken in the elevated plus-maze included measures of anxiety-like behavior (ALB). The measures of activity in the plus-maze are the number of entries into the closed arms of the maze known as closed-arm entries and the total number of entries into any arm of the maze. Anxiety-like behavior was analyzed using two additional measures. The first measure was ratio time, and was calculated by dividing the time spent in the open arms of the maze by the total time spent in any arm of the maze (rats are considered to be in an arm of the maze only when all four paws are in that arm). Animals spending less time in the open arms will have lower ratio times and are

considered to be exhibiting more anxiety-like behavior [19]. Risk assessment is the second measure of anxiety-like behavior in the plus-maze. Risk assessment was measured as the frequency and time the animal spent with hind paws located in the closed arm while stretching the head and front of the body into the open arm [29]. In the Adamec laboratory, risk assessment is presented as ratio frequency risk by dividing the time spent risk assessing by the time spent in closed arms of the maze. This measure was included to ensure that changes in risk assessment were truly changes in behaviour and not merely the result of an increased opportunity to engage in risk due to increased time spent in the closed arms. Fecal boli were also collected for the plus-maze.

2.1.8 Body Weight

All rats were weighed upon arrival at the laboratory, at the time of surgery and immediately following behavioral testing, thus allowing an assessment of treatment effects on growth. Monitoring body weight allowed observation as to whether kindled and control rats differed in weight gain patterns, i.e. whether amygdala functions, other than anxiety, were affected by amygdala kindling.

2.1.9 Histology

Once the behavioral testing was complete, rats were anesthetized with Somnotol and reconnected to the stimulator. An anodal DC lesioning current of 2 mA was delivered for a 2 second period. This process was used to deposit metallic ions to the lesioning site to aid in the electrode location process. Following the stimulation rats were perfused

transcardially using two solutions, phosphate buffered saline and a second solution composed of formalin (10%), sucrose (20%) and potassium ferrocyanide (1%) in phosphate buffered saline. The first solution was used to replace the blood. The formalin in the second solution acted as a fixative for the brain tissue facilitating removal of the brain. The potassium ferrocyanide reacted with the metallic ions deposited during the lesion to produce a blue dot which aided in the location of the electrode tip. Immediately following removal, the brains were stored in the perfusion solution in individual marked containers where they remained overnight in the refrigerator. The next day, the solution in each container was changed to a 20% sucrose solution and they were returned to the refrigerator for approximately 1-2 days, or until the brains sank to the bottom. Brains were then stored in the 4% formaldehyde phosphate buffered saline solution until sectioned.

Given the importance of the anterior-posterior plane, extreme care was taken in the sectioning process. Thirty-seven μM thick sections were cut beginning at the decussation of the anterior commissure and were counted as the sectioning proceeded through the electrode tracks. Sections were mounted and slides were labeled clearly. On the following day each slide was stained using metachromatic cresyl violet. Using the number of sections to the electrode tip, the known thickness of each section and the coordinates of the decussation (according to Paxinos and Watson [70]) it was possible to determine the AP position of the electrode tip to within 37 μm. An image analyzer (Jandel, Mocha) was used to measure the coordinates of the electrode tips in the lateral and vertical plane. The coordinates were then normalized to the nearest Paxinos and

Watson atlas section and plotted accordingly.

2.1.10 Statistical Analysis

Electrode tips were coded as either on target (within the central nucleus or nucleus basalis) or off target. The behavioral and histological data of on-target animals were analyzed using a two-way ANOVA (BMDP Solo program). The factors analyzed were kindling and brain area. A one-way ANOVA was used to analyze kindling parameters for the various brain areas. A combination of Post Hoc Duncan's and a priori t-tests were used to evaluate main effects and interactions.

2.2 Experiment 2 - Relation of baseline anxiety to response to kindling of the right medial amygdala

2.2.1 Subjects

The subject group consisted of fifty-seven male Wistar rats from Charles River Canada. Animals weighed approximately 150-170 g upon arrival at the laboratory. The housing conditions and adaptation/handling procedures were identical to those provided in Experiment 1. Animals weighed between 200 and 250 grams at the beginning of the experiment.

2.2.2 Experimental Groups

Following the three day adaptation, the animals were randomly assigned to one of three groups - kindled, implanted control and handled control. The kindled group

consisted of seventeen animals that received electrodes implanted in the anterior medial amygdala. This group was the only group in the experiment that was kindled. The implanted control group included fifteen animals with electrodes in the anterior medial amygdala. These animals were handled as kindled animals but did not receive electrical stimulation. The handled control group consisted of 25 animals that were handled as the other groups but did not have an electrode implanted. These animals were included to examine the effects of the surgical procedure on behavior. Following histological analysis, it was determined that two of the implanted controls had electrodes that were outside of the target area. The group was subsequently reduced to 15 animals.

2.2.3 Surgical Procedure

Coordinates for the right anterior medial amygdala were adapted from a previous study in the Adamec laboratory [6]. The target area was located 0.6 mm posterior to bregma, 4.0 mm lateral to the midline, and 8.6 mm ventral to dura. The surgical procedure carried out was as described in Experiment 1.

2.2.4 Behavioral Testing

Following the seven day recovery period, anxiety-like behavior was tested in the hole board and the elevated plus-maze as described for Experiment 1.

The second test of anxiety-like behavior using the hole board and elevated plus-maze occurred three weeks later, post kindling. This test was done using the same procedure, in the same hole board and plus-maze, but in a novel room. Given the number

of animals in each group, tests were once again completed in stages with each group represented during each stage. All tests were scheduled to occur during 1200 and 1300 h each day.

2.2.5 Behavioral Measures

Behavioral measures were assessed in both the hole board and plus-maze using the videotaped tests. The measures analyzed were identical to those described in Experiment 1, with the addition of one new measure. Relative time risk assessment was determined by dividing the time of risk assessment by the time spent in the closed arms of the maze. This ratio, similar to relative frequency risk assessment described in experiment one, was taken to control for the possibility that changes in time spent in the closed arm would affect the opportunity to engage in risk assessment rather than reflect a real change in risk assessment behavior.

2.2.6 Body Weight

All rats were weighed upon arrival at the laboratory, at the time of surgery and immediately following behavioral testing, thus allowing an assessment of treatment effects on growth.

2.2.7 Kindling Procedure

Following the first hole board and plus-maze test, rats were adapted to the kindling box for two days as described for Experiment 1.

The kindling procedure was as described in Experiment 1 with slight changes to accommodate the test-retest paradigm. After the third stage 5 seizure, kindled rats were left alone in their home cages for 1-4 days to allow other rats in the group to achieve three stage five seizures. Then a fourth stage 5 seizure was triggered in all kindled-group rats, and the rats were left unhandled for 1 week. The duration of the last stage 5 seizure was recorded. At the end of that week all rats were re-tested in the hole-board and plus maze. The total time interval between plus maze tests was three weeks. Handled and implanted controls were treated the same as kindled rats, except they were not stimulated. Implanted controls were plugged into the stimulating apparatus but were not stimulated.

2.2.8 Kindling Parameters

The number of stimulations to the first stage 5 seizure and the duration of the last (4^{th}) seizure were the two measures recorded during the kindling process.

2.2.9 Histology

The histological procedure for this experiment was the same as that described for Experiment 1.

Animals with electrodes found to be within the medial amygdala and anterior to 1.0 mm posterior to bregma were considered to be on target. Two implanted control animals were removed from the study because their electrodes were found to be outside of the target area.

2.2.10 Statistical Analysis

Behavioral analysis was completed for the first hole board and plus-maze tests. From the plus maze data, a median ratio time and median frequency risk assessment score were calculated. These scores were then used to divide the animals into categories of either below or equal to or above the median on the first test (Median Split). These categories were then used in all further analyses.

Three main analyses were conducted in the experiment. The first test was designed to determine whether the implantation of the electrode had an effect on behavior and as well to determine any differences in responding from test 1 to test 2. A three-way analysis of variance with repeated measures (test number) was used to examine the interactions. The independent variables tested were group (handled and implanted control), median split (below and equal to, or above median) and test number (Test 1 and Test 2). The second analysis compared implanted controls and animals that were scheduled to be kindled to make sure there were no behavioral differences. This test involved a two-way analysis of variance comparing group (implanted and to-be-kindled) with median split for Test 1. The third and final analysis was done to determine the effects of kindling on behavior. This also involved a two-way analysis of variance to compare group (implanted controls and kindled) with median split from Test 1 on Test 2 data. A combination of Post Hoc Duncan's and a priori t-tests were used to evaluate individual mean contrasts. The statistical program BMDP for PC-SOLO was used for the analyses.

2.3 Experiment 3: Relation of baseline anxiety and electrode location to the response to kindling in the right and left basolateral amygdala

Within this experiment there were two separate studies. The studies will be referred to as 3.1 and 3.2.

2.3.1 Experiment 3.1: Kindling of the Right Basolateral Amygdala

2.3.1.1 Subjects and Handling Procedure

The subject group consisted of fifty-four male Wistar rats received from Charles River Canada. Animals weighed approximately 150-170 g upon arrival at the laboratory. The housing conditions and adaptation/handling procedures were identical to those provided in Experiment 1. Animals weighed between 200 and 250 grams at the beginning of the experiment.

2.3.1.2 Experimental Groups

Following the three day adaptation, the animals were randomly assigned into one of three groups - kindled, implanted control and handled control. The kindled group consisted of 18 animals that had electrodes implanted in the right baslolateral amygdala. This group was the only group in the experiment that was kindled. The implanted control group included 18 animals with electrodes also in the right basolateral amygdala. These animals were handled as kindled animals but did not receive electrical stimulation. The handled control group consisted of 18 animals that were handled as the other groups but did not have an electrode implanted. These animals were included to examine the effects

of the surgical procedure on behavior. Following histological analysis, it was determined that one of the kindled animals had an electrode that was outside of the target area. The group was subsequently reduced to 17 animals.

2.3.1.3 Surgical Procedure

Coordinates for the right basolateral amygdala were 2.3 mm posterior to bregma, 4.8 mm lateral to the midline, and 8.5 mm ventral to dura. The surgical procedure was as described in Experiment 1.

2.3.1.4 Behavioral Testing

Following the seven day recovery period, anxiety-like behavior was tested in the hole board and the elevated plus-maze as described in Experiment 1.

The second test of anxiety-like behavior using the hole board and elevated plus-maze occurred three weeks later, post kindling. This test was done using the same procedure, in the same hole board and plus-maze, but in a novel room. Given the number of animals in each group, tests were once again completed in stages with each group represented during each stage. All tests were scheduled to occur during 1100 and 1300 h each day.

2.3.1.5 Behavioral Measures

Behavioral measures were assessed in both the hole board and plus-maze using the videotaped tests. The measures analyzed were identical to those described in

Experiment 1 for both the hole board and plus-maze. In this experiment an extra measure of resistance to capture was recorded. This measure identifies the animal's response as it is being picked up from the hole board to be placed in the plus-maze. The score recorded was a: 0 - no reaction; 1 - vocalize or shy away from the experimenter's hand; 2 - vocalize and shy away from hand; 3 - run away from hand, 4 - vocalize and run away from hand; 5 - bite or attempt to bite hand; or 6 - launch a jump attack at the hand. Resistance to capture has been reported to increase following extensive amygdala kindling [57, 74]. Behavioral measures analyzed in the elevated plus-maze were as described in Experiment 1. In addition to these measures, one additional measure was analyzed. This measure, known as ratio entry, is similar to ratio time and is defined as the number of entries into the open arms of the maze divided by the total number of entries into any arm of the maze. Rats exhibiting increased anxiety-like behavior tend to have fewer entries into the open arms of the maze and therefore typically have lower ratio entries.

2.3.1.6 Body Weight

All rats were weighed upon arrival at the laboratory, at the time of surgery and immediately following behavioral testing, thus allowing an assessment of treatment effects on growth.

2.3.1.7 Kindling Procedure

Following the first hole board and plus-maze test, the adaptation and kindling

procedure was carried out as described in Experiment 1. After the third stage five seizure rats were left alone in their cages for a period of 1-4 days to allow other rats to achieve 3 stage five seizures. Then a fourth stage five seizure was triggered in all kindled group rats, and the rats were then left unhandled for a week. At the end of this week all rats were tested in the hole board and plus maze for a second time. These tests occurred at least three weeks after the first.

2.3.1.8 Kindling Parameters

The number of stimulations to the first stage 5 seizure and the duration of the last (4^{th}) seizure were the two measures recorded during the kindling process.

2.3.1.9 Histology

The histological procedure used for this experiment was identical to that used for Experiment 1.

Animals with electrodes found to be within the right basolateral nucleus were considered to be on target. One kindled animal was removed from the study because the electrode was found to be outside of the target area.

2.3.1.10 Statistical Analysis

Behavioral analysis was completed for the first hole board and plus-maze tests. From the data, a mean ratio time score was calculated. Mean ratio time was the split factor since ratio time data were normally distributed (Mean Split). The mean ratio time

score was then used to divide the animals into two categories of either below or equal to or above the mean on the first test. These categories were then used in all further analyses.

Three main analyses were done. The first analysis was designed to determine whether the implantation of the electrode had an effect on behavior, and as well to determine any differences in responding from Test 1 to Test 2. A three-way analysis of variance with repeated measures (test number) was used to examine the interactions. The variables tested were group (handled and implanted control), mean split (below or equal to or above mean) and test number (Test 1 and Test 2). The second analysis compared handled and implanted controls and animals that were scheduled to be kindled to make sure there were no behavioral differences. This test involved a two-way analysis of variance comparing group (controls and to-be-kindled) by mean split for Test 1. The third analysis was done to determine the effects of kindling on behavior - this involved two parts. First a two-way analysis of variance was conducted for each group comparing Mean Split and Test with repeated measures on test. If groups showed a change over tests on the same measure, then a second two-way ANOVA was carried out comparing Group (handled control, implanted control, and kindled) with Mean Split. Post Hoc Tukey-Kramer or a-priori t-tests (planned comparison Fisher's LSD and t-tests) were used to evaluate individual mean contrasts. The statistical program BMDP for PC-SOLO was used for the analyses.

2.3.2 Experiment 3.2: Kindling of the Left Basolateral Amygdala

2.3.2.1 *Subjects and Handling Procedure*

The subject group consisted of fifty-four male Wistar rats received from Charles River Canada. Animals weighed approximately 150-170 g upon arrival at the laboratory. The housing conditions and adaptation/handling procedures were identical to those provided in Experiment 1. Animals weighed between 200 and 250 grams at the beginning of the experiment.

2.3.2.2 *Experimental Groups*

Following the three day adaptation, the animals were randomly assigned into one of three groups - kindled, implanted control and handled control. The kindled group consisted of 18 animals that received electrodes implanted in the left basolateral amygdala. This group was the only group in the experiment that was kindled. The implanted control group included 18 animals with electrodes also in the left basolateral amygdala. These animals were handled as kindled animals but did not receive electrical stimulation. The handled control group consisted of 18 animals that were handled as the other groups but did not have an electrode implanted. These animals were included to examine the effects of the surgical procedure on behavior. Following histological analysis, it was determined that four of the kindled animals had electrodes outside of the target area. The group was subsequently reduced to 14 animals. In addition, the analysis revealed that six of the implanted control animals had electrodes outside of the target area, thus reducing the number of subjects in this group to 12.

2.3.2.3 *Surgical Procedure*

The surgical procedure for this experiment was the same as that described for Experiment 1.

Coordinates for the left basolateral amygdala were 2.3 mm posterior to bregma, 4.8 mm lateral to the midline, and 8.5 mm ventral to dura.

2.3.2.4 *Behavioral Testing*

The behavioral testing procedures for this experiment were identical to that described in Experiment 3.1.

2.3.2.5 *Behavioral Measures*

The behavioral measures assessed in this experiment were identical to those described in experiment 3.1.

2.3.2.6 *Body Weight*

All rats were weighed upon arrival at the laboratory, at the time of surgery and immediately following behavioral testing, thus allowing an assessment of treatment effects on growth.

2.3.2.7 *Kindling Procedure*

The kindling procedure for this experiment was identical to that described in experiment 3.1.

2.3.2.8 Kindling Parameters

Similar to Experiment 3.1, the number of stimulations to the first stage 5 seizure and the duration of the last (4^{th}) seizure were the two measures recorded during the kindling process.

2.3.2.9 Histology

The histological procedure for this experiment was identical to that described in Experiment 3.1.

2.3.2.10 Statistical Analysis

Behavioral analysis was completed for the first hole board and plus-maze tests for handled controls and all on-target rats. There were too few off target rats to analyze above and below the split. Thus a mean ration time split value of .57 arose from considerations of the results of Experiment 3.1. Mean ratio time was the split factor since ratio time data were normally distributed (Mean Split). This score was used to divide the animals into categories of either below or equal to or above the mean on the first test. These categories were then used in all further analyses.

The three main analyses procedures conducted were identical to those described in Experiment 3.1.

3.0 Results

3.1 Experiment 1

Results in this experiment showed that kindling lastingly changed two measures of anxiety in the elevated plus-maze. The nature of the change depended on the location of the kindled site. Kindling of the posterior central nucleus decreased both open-arm exploration and frequency of risk assessment in the elevated plus-maze 1 week after the fourth stage 5 seizure. Kindling of the middle parts of the central nucleus was without behavioral effects. Kindling of the anterior central nucleus and the anterior nucleus basalis increased risk assessment. Changes in risk assessment produced by kindling of the central nucleus were dependent on open-arm avoidance, whereas the effects of nucleus basalis kindling were independent of open-arm avoidance. Changes in plus-maze behavior were independent of changes in exploration or activity in either the plus-maze or hole board. Detailed analyses are provided in the following sections.

3.1.1 Electrode Locations

The statistical analysis of electrode placements identified significant group differences in the anterior-posterior, vertical and lateral planes [all $F(3, 80) > 6.30$, $p<0.001$] as would be expected given different target coordinates. There were however, no kindling effects or interactions. There were no significant differences in the location of electrodes found between kindled animals and implanted controls. Information regarding the placement of electrodes including the mean location and the ns of on target animals by group is provided in Table 2.

Table 3: Table of electrode locations[a]

Area	Anterior-posterior[b] plane	Lateral[c] Plane	Vertical Plane	n's[d]	
				K	C
Posterior central amygdala	-2.85 ± 0.06	4.21 ± 0.09	7.44 ± 0.16	9	10
Mid central amygdala	-2.44 ± 0.05	4.21 ± 0.08	7.58 ± 0.13	14	12
Anterior central amygdala	-1.98 ± 0.05	4.13 ± 0.08	7.80 ± 0.14	9	15
Nucleus basalis	-2.10 ± 0.06	3.58 ± 0.10	6.90 ± 0.17	9	10

[a] All coordinates are averaged over kindled and implanted controls, which did not differ for a given target. Values are means ± SEM.
[b] Data are in mm posterior to bregma.
[c] Lateral position is in mm lateral to bregma, vertical is mm below the dura.
[d] Number of rats in the kindled (K) and implanted control (C) groups with on target electrodes.

3.1.2 Analysis of kindling parameters

There were significant differences by brain area in the number of stimulations required to elicit the first stage 5 seizure, [$F(3, 36) = 3.99, p < 0.02$ (Fig. 1.1)]. Animals with electrodes implanted in the nucleus basalis required more stimulations than those with electrodes located in the mid and anterior central nucleus. Animals with electrodes located in the posterior central nucleus required a number of stimulations that fell between the number required for those with electrodes located in the nucleus basalis and those with electrodes located in the mid and anterior central nucleus (Duncan test, $p < 0.05$). There were no significant differences in the durations of the final stage five seizures.

3.1.3 Effects of kindling the right central amygdala and right nucleus basalis on behavior in the hole board

The analysis of the hole board data revealed no main effects or interactions for

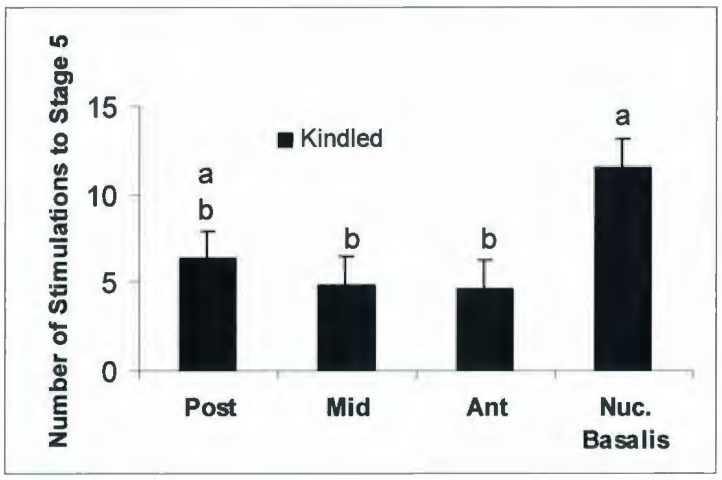


Fig. 1.1. The figure provides the means ± SEM of the number of stimulations required to produce the first stage 5 seizure. The means are provided for kindled animals in each brain area group. The means marked with the same letter (a or b) do not differ, but they do differ from means marked with a different letter. Means marked with two letters fall between means marked with either letter.

any behavioral measure. Therefore it is concluded that kindling did not affect activity or exploration in the hole board.

3.1.4 Effects of kindling the right central amygdala and right nucleus basalis on behavior in the elevated plus-maze

The analysis of the plus maze data revealed that kindling did not have an effect on exploration/activity in the plus maze as measured by arm entry measures. Kindling also had no effect on the number of boli left in the maze. There was however, a significant interaction between brain area and kindling for risk assessment, [$F(3,80) = 4.08$, $p< 0.01$]. Planned comparisons comparing implanted controls with kindled animals for each brain area revealed the following findings. Kindling in the posterior central amygdala reduced risk assessment, kindling in the mid-central amygdala had no effect on risk assessment and kindling in the nucleus basalis and the anterior central amygdala increased risk

assessment over controls [Fig 1.2, all $t(80) > 2.01$, $p < 0.05$ two-tailed tests]. The analysis also revealed a probable effect of electrode implantation on risk assessment for control animals with electrodes placed in the posterior central amygdala. This group exhibited an increased level of risk assessment over other implanted controls, which did not differ from each other (Duncan test, $p < 0.05$).

There was a marginal effect of kindling on ratio time, [$F(3, 80) = 1.35$, $p < 0.28$; brain area by kindling interaction]. Further analysis of ratio time showed a tendency toward a difference between control and kindled rats with electrodes in the posterior central amygdala, $t(80) = 1.43$, $p < 0.08$, one-tailed test (Fig. 1.2). For ratio time there was no effect of implantation found and control groups did not differ.

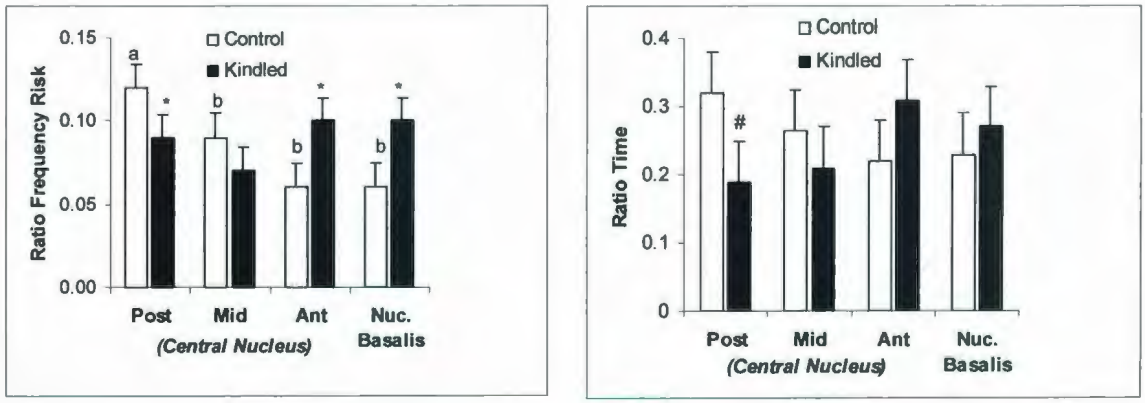


Fig. 1.2. The figure illustrates the means + SEM of ratio frequency risk assessment (left histogram) and ratio time (right histogram). The means are provided for implanted controls and kindled animals by brain area. The kindled group means marked with a "*" in the top histogram are significantly different from the control mean for the corresponding brain area. The control means in the top histogram marked with a "b" do not differ from each other but do differ from control group marked with an "a". The kindled group means in the bottom histogram marked with a "#" tend to differ from the control mean (one-tailed test).

As seen in figure 1.2, there are similar tendencies in the mean scores for ratio time and ratio frequency risk. Based on these similarities, it was felt that the findings related to risk

assessment could be better understood if further analysis was used to assess the extent to which they were dependent on ratio time. This was carried out through an analysis of covariance which removed the effects of ratio time. Findings showed that the group x kindling interaction remained, [$F(1,79) = 2.74, p < 0.05$]. There was however, a change in the pattern of mean differences. The effects on risk assessment following kindling of the posterior and anterior central nuclei were removed (Fig. 1.3). The findings related to mid-central nucleus and the nucleus basalis groups did not change as a result of this analysis.

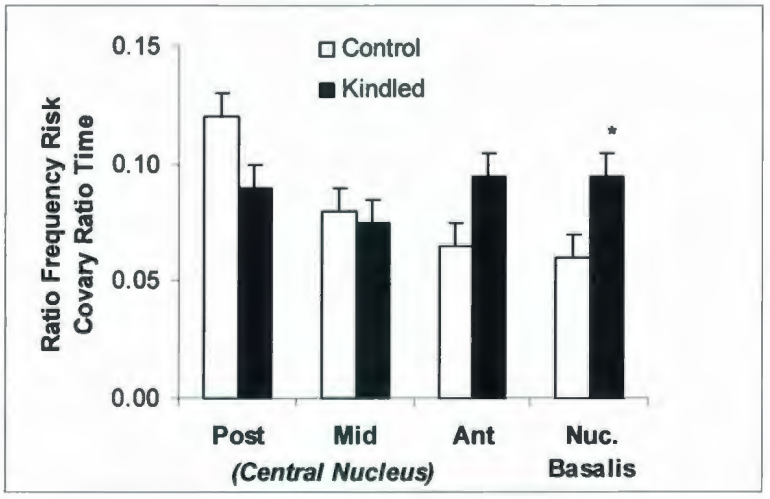


Fig.1.3. The means + SEM of ratio frequency risk are plotted in the figure with the effect of ratio time removed by analysis of covariance. The kindled group mean marked with an "*" differs from the corresponding control mean.

Covariance analysis suggests that common circuitry in posterior and anterior central nucleus changed by kindling may mediate changes in risk assessment and ratio time. In contrast, nucleus basalis kindling appears to alter only risk assessment acting on separate circuitry.

The above proposals were also supported by factor analysis. Two-factor analyses were carried out using data from the central nucleus groups in the first and data from

nucleus basalis rats in the second. All measures of activity/exploration in the hole board and plus maze were entered, in addition to ratio time and ratio frequency risk assessment used in the previous analyses. The results of the analysis are provided in Table 3. The test selected was a principle components analysis with varimax rotation. The number of factors was selected using a scree analysis. The cutoff for factor loading was set at 0.5 and overall both analyses accounted for 99% of the variance.

The analysis revealed a two-factor structure for central nucleus rats. The first factor, the "anxiety factor" loaded ratio frequency risk assessment and ratio time. The second factor, the "activity" factor was an orthogonal factor which loaded total arm entries in the plus maze and rearing in the hole board.

Results for nucleus basalis rats showed a three-factor structure. For these rats, only ratio time loaded on the "anxiety" factor. A risk assessment /exploration factor loaded ratio frequency risk assessment and total arm entries in the plus maze. A third factor, "risk assessment/activity" was also loaded by ratio frequency risk assessment as well as rearing in the hole board. Ratio frequency risk assessment and ratio time fell on independent factors, consistent with the notion that kindling effects on these behaviors are mediated through different circuitry in these animals.

Table 4: Table of factor loadings from factor analysis[a]

Central Nucleus Groups[b]						
Factors			**Anxiety**		**Activity/Exploration**	
			Ratio time	0.70	Rear	0.65
			Frequency risk assess	0.72	Total arm entries	0.70
Nucleus Basalis Groups						
Factors	**Risk assessment /exploration**		**Anxiety**		**Risk Assessment /activity**	
	Frequency risk assess	0.61	Ratio time	0.58	Frequency risk assess	0.62
	Total arm entries	0.76			Rear	0.52

[a] Numbers reflect factor loadings

[b] Variables entered: head dips, rears, arm entries (total), ratio time, and relative frequency of risk assessment.

3.1.5 Plus-maze behavior and electrode location

3.1.5.1 Risk assessment and electrode location in kindled controls

Results showed a significant correlation between ratio frequency risk assessment of implanted controls and location of electrode in the AP plane (Pearson $r = -0.44$, $p < 0.01$). This correlation included all planes across controls in all brain areas, with more posterior locations showing higher ratio frequency risk.

3.1.5.2 Risk assessment and electrode location in kindled animals

Given that controls in the different brain area groups differed with respect to ratio frequency risk assessment, it was necessary to use a ratio of control risk assessment in order to conduct a correlation of risk assessment in kindled animals. The ratio was determined by dividing ratio frequency risk assessment of kindled rats by the average

ratio frequency risk assessment of their appropriate controls, as has been done in previous studies [19]. The ratio was then correlated with electrode location.

Analysis of variance was redone to ensure validity of the new ratio measure. A significant brain area by kindling interaction, [$F(3, 80) = 3.86, p < 0.02$], was found once again, and the kindled and control groups differed as in the previous analysis of ratio frequency risk assessment [Fig 1.4, compare with Fig. 1.2, marked kindled groups differ from control values of 1, all $t(80) \geq 2.70, p < 0.03$, planned comparison two-tailed tests].

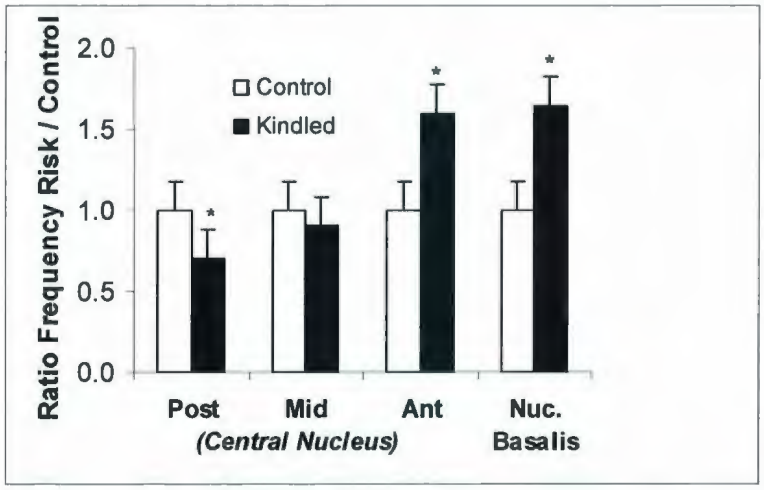


Fig. 1.4. The figure identifies means +SEM of ratio frequency risk assessment divided by the average ratio frequency risk assessment of controls for each brain area. Kindled group means marked with an "*" are significantly different from their relevant control means.

The results showed a correlation between the ratio of control risk assessment and the AP plane ($r = 0.53, p < 0.01$) and lateral plane ($r = -0.36, p < 0.02$). These two variables accounted in total for 38% of the variance of ratio control risk assessment [multiple $r = 0.62$, $F(2, 40) = 12.15, p < 0.001$]. There was no correlation found between the ratio of control risk assessment and the vertical plane location of kindling electrodes.

The correlations and subsequent prediction equation indicate that as the location moves anterior, kindling increases risk assessment relative to the control. Additionally, the correlations suggest that as you move away from the midline, kindling serves to decrease risk assessment [prediction equation: risk = 0.96 AP (mm) – 0.53 lateral (mm) + 5.59, weights and intercept differ from zero, all $t(40) \geq 2.47, p < 0.02$].

3.1.6 Body weight

The groups did not differ with respect to body weight at the time of behavior testing (mean ± SEM, 438.8 ± 5.2 grams). This finding along with the fact that the groups did not differ at the start of the experiment suggests that kindling did not have an effect on body weight.

3.2 Experiment 2

3.2.1 Relation of baseline behavior to response to medial amygdala kindling

In general, the results of this experiment showed that kindling lastingly increased anxiety (decreased open-arm exploration) in the elevated plus-maze 1 week after the last kindled seizure. Changes in anxiety were independent of changes in exploration or activity in either the plus-maze or hole board. In addition, kindling induced behavioral changes were found to be dependent on baseline behavior. Detailed analyses are provided in the following sections.

3.2.1.1 Effects of median split along ratio time, retesting and kindling on behavior in the plus-maze and hole board.

Test 1. The only group difference found between implanted controls and the rats in the kindled group on Test 1 was in boli deposited in the hole board. Rats to be kindled left more boli behind in the hole board [$F(1, 26) = 5.41$, $p < 0.03$, 0.00 ± 0.3 vs. 0.96 ± 0.26, implanted versus kindled groups, respectively]. These groups, however, did not differ on the number of boli produced in the plus-maze.

There were no interactions with any behavioral measure. Group means on Test 1 in the hole board (head dips and rears) along with closed-arm entries in the plus-maze are plotted in Figure 2.1. Figure 2.2 (top panel) shows open-arm exploration and risk assessment means in the plus maze from Test 1.

There were several median split main effects on Test 1. The first being ratio time [$F(1,26) = 42.14$, $p < 0.001$]. The median was based on all animals including controls and the effect, without a group interaction, proved that the value was appropriate to capture the median of all animals in the experiment. The n's in the median split were also equal at 15 animals each. The n's across groups were as follows: implanted controls, n = 13 with eight below and five above the median; Kindled, n = 17 with seven below and ten above the median. Although small, these n's are comparable to group sizes used in previous studies [6, 19]. The second measure to show a median split effect was total arm entries in the plus maze [$F(1,26) = 4.82$, $p < 0.04$]. The third median split effect was found for open arm entries in the plus maze [$F(1, 26) = 52.04$, $p < 0.001$]. Based on the

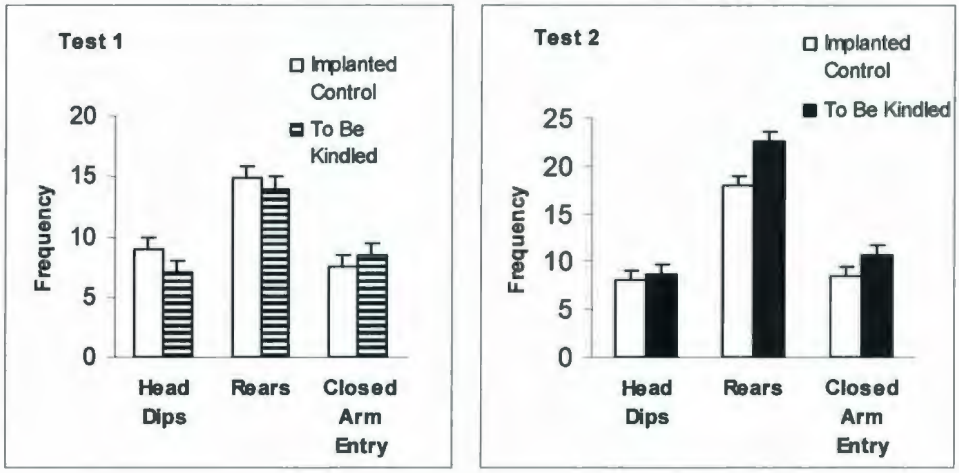


Fig. 2.1. This histogram provides the means + SEM of hole-board exploration and activity (head dips and rearing) and plus-maze activity/exploration (closed arm entries). The Test 1 histogram includes the "implanted controls" and the "to be kindled" groups. The "to be kindled" groups are "kindled" in the histogram for Test 2. There were no differences found between groups.

data, it is apparent that the total arm entry effect was caused by open-arm entries as closed arm entries did not differ. This effect is expected because rats above the median split on ratio time would enter the open arms more than rats below the median split.

Test 2. Kindled rats showed an anxiogenic response on Test 2. Rats with ratio time scores above the median on Test 1 showed a decrease in ratio time on Test 2 [Fig. 2.2, Ratio Time Test 2]. Kindled animals with ratio time scores below the median on Test 1 showed no change in ratio time on Test 2 in relation to implanted controls (Group × Median Split interaction, $[F(1,26) = 5.58, p < 0.03]$; mean contrasts (Duncan test, $p < 0.05$ and $t(26) \geq 1.99, p < 0.04$ planned comparisons). The significant effects noted above were specific to open-arm avoidance in the plus maze. There were no further kindling or median split effects, or interactions.

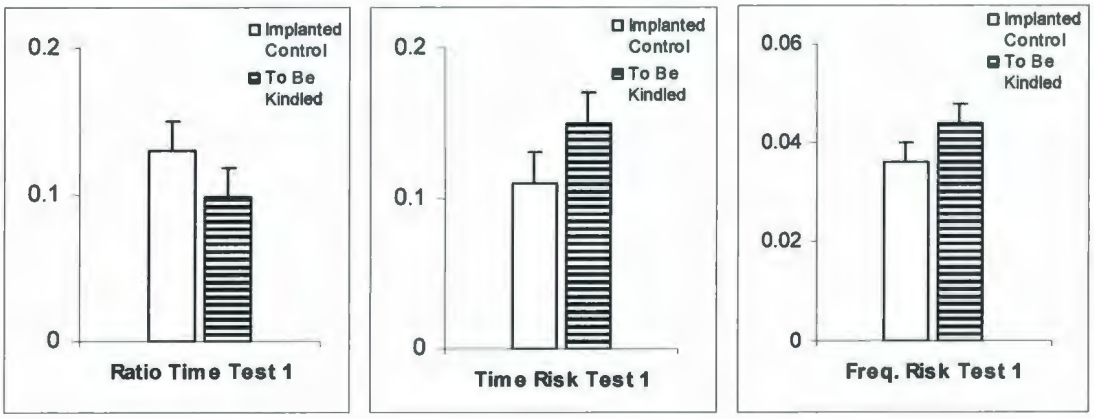
The lack of an effect of kindling on risk assessment (Fig. 2.2) was of interest given previous findings [19]. A median split on ratio time was also without a median split

effect on risk assessment. Based on this result, the animals were reassigned to new groups using a relative frequency risk assessment median split, and the above analysis was repeated.

3.2.1.2 Effects of median split along frequency of risk assessment, retesting and kindling on behavior in the plus maze and hole board

Test 1 No differences were found between implanted controls and kindled rats for hole board and plus maze behavior or for boli counts in each apparatus. There were however, median-split effects for risk assessment, total arm entries and closed arm entries [all $F(1,26) \geq 6.57$, all $p < 0.02$]. The subject numbers across groups were 13 for implanted controls – split seven below and six above the median, and 17 for kindled – split nine below and eight above the median. These effects did not include group interactions. There were no effects of median split or group interactions for any other measures.

Test 2 Kindling did have an effect on total and closed arm entries (Fig 2.3). Test 2 showed Group X median split interactions for total arm entries [$F(1,26) = 12.28$, $p<0.002$] and closed-arm entries [$F(1,26) = 12.84$, $p<0.002$]. In both cases, rats that were above the median on Test 1 were not affected by kindling; however those that were below the median on Test 1 showed an increase in arm entries. It should be noted that the increase in the number of arm entries caused by kindling exceeded the number of arm entries of above-median rats (Fig 2.3, bottom panel, Duncan test, $p<0.05$).



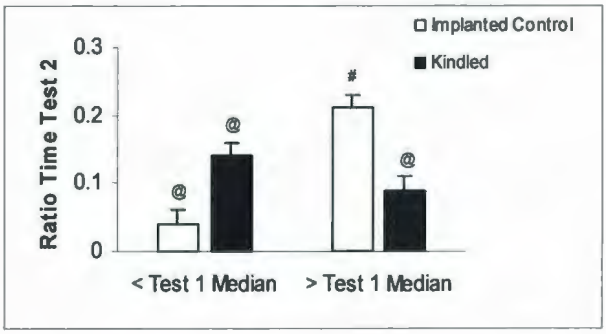
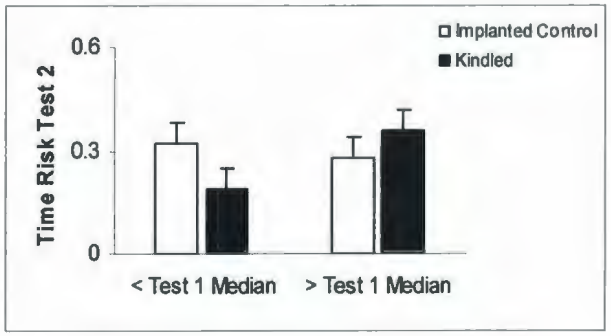
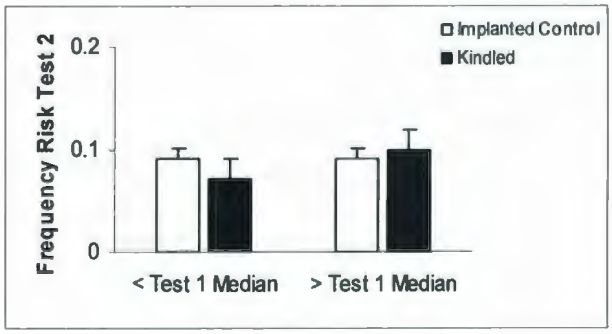


Fig. 2.2. The means and SEM of ratio time, relative time risk and relative frequency risk are plotted for both Test 1 and Test 2. The three lower histograms for Test 2 have both implanted controls and kindled (to be kindled from Test1) groups from above and below the median split on Test 1 using median ratio time. Ratio time means marked with @ do not differ but together differ from the mean marked with # (Duncan test, $p <$ 0.05).





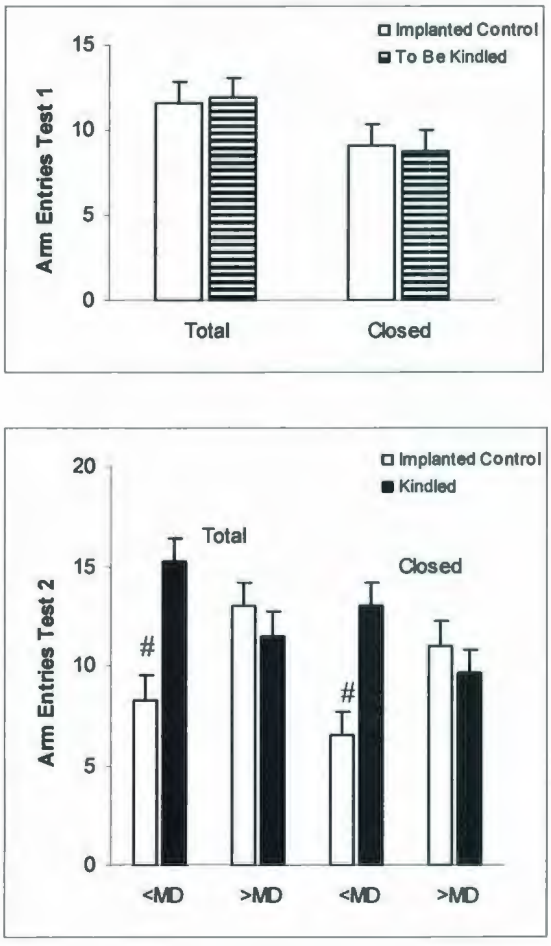


Fig. 2.3. Plotted are the means + SEM of total arm entries (TOTAL), and closed arm entries (CLOSED) in the elevated plus maze. The top panel shows arm entries of kindled (To Be Kindled) and implanted controls on Test 1. There was no difference between the groups on this test. The bottom panel shows arm entries for implanted controls and kindled animals with those below the median (<MD) and above the median (>MD) of relative frequency risk plotted separately. Means marked with a # differ from unmarked means, which do not differ (Duncan test, $p<0.05$).

There was no effect of kindling on ratio time or risk assessment measures in Test 2. There was also no main effect of median split on Test 2. This may be due to a rise in risk assessment on retest which will be addressed below in the control analyses. This study also showed no effect of medial amygdala kindling on risk assessment which is contrary to previous findings in this laboratory [19]. This however, may be due to the kindling location, a concept that will be expanded further in the discussion.

3.2.1.3 Electrode Locations

The AP, lateral and vertical plane coordinates of electrodes for implanted controls and kindled groups did not differ. The average coordinates for all animals included in the behavioral analysis are provided in Table 4. Coordinates from a previous study in this laboratory on the effects of medial amygdala kindling on anxiety [19] are also provided and the mean differences between the locations in both studies are compared using t-tests. As identified in the table, there were differences in the lateral and vertical plane coordinates. Kindled locations in this study were more lateral and less deep than in the previous study.

Table 5: Electrode locations in the right medial amygdala

Study	Anterior-Posterior[a] plane	Lateral[b] plane	Vertical plane
This Study	0.79 ± .03	4.21 ± .06	9.25 ±.06
Adamec and Morgan 1994	0.69 ± .07	3.89 ± .09	9.69 ± .10
Difference[c]	0.10	0.32	-0.44
t-values[d] [$t(36)$]	1.40, $p>0.05$	2.45, $p<0.05$	3.58, $p<0.05$

All coordinates are averaged over kindled and implanted controls, which did not differ in either study.
Values are means ± SEM
[a] Data are in mm posterior to bregma
[b] Lateral position is in mm lateral to midline, vertical is mm below the dura
[c] Coordinates in the present study minus those in Adamec and Morgan 1994.
[d] *T*-tests comparing the two studies

It was considered possible that the kindling locations used in this study were outside of the circuitry that affects risk assessment. If this was true then risk assessment and ratio time should load on orthogonal factors in a factor analysis. This was tested by carrying out separate analyses on implanted and kindled rats in Test 1 and Test 2. Hole board and plus maze variables were entered into a principle components analysis with varimax rotation. Scree analysis showed a three-factor solution for all analyses. The

factor loading cutoff was set at 0.5. All solutions accounted for greater than 99% of the variance. The factors and their loadings are provided in Table 5.

Table 6: Table of factor loadings from factor analysis of plus-maze and hole-board behavior

	Risk Assessment		Anxiety		Activity/Exploration	
Implanted controls, Test 1[a]	Total arm entries	.74	Ratio time	.78	Rear	.72
	Closed arm entries	.83	Total arm entries	.62	Time Active	.70
	Time risk assessing	.85				
	Frequency risk assessing	.92				
Implanted controls, Test 2	Time risk assessing	.96	Ratio time	.98	Total arm entries	.88
	Frequency risk assessing	.90	Total arm entries	.51	Closed-arm entries	.78
Kindled animals, Test 1	Time risk assessing	.91	Ratio time	.92	Total arm entries	.89
	Frequency risk assessing	.92			Closed-arm entries	.96
Kindled animals, Test 2	Time risk assessing	.94	Ratio time	.92	Total arm entries	.72
	Frequency risk assessing	.94	Total arm entries	.67	Closed-arm entries	.98

Numbers are factor loadings

[a] Variables entered were: head dips, rears, time active (all hole board), arm entries (total and closed arms), ratio time, and relative time and frequency of risk assessment (plus maze)

As identified in the table, there were some changes in factor loadings between Test 1 and Test 2 concerning entries in the plus maze and activity in the hole board. However, there was consistency in the loadings for risk assessment and ratio time, with these measures loading on separate factors in all analyses, thus supporting the idea that they are controlled by separate circuitry.

3.2.1.4 Effects of other median split criteria

Analyses above identified a median split effect for arm entries using risk assessment as the median criterion variable, but the split was not along the median of arm entries. It was therefore decided to redo the Test 2 analyses of kindling effects using either total arm entries or closed arm entries as criterion. This would allow us to determine whether the effect on arm-entries observed in Figure 2.3 was due to an absolute level of entries or could be identified by a median split of arm entries. The reanalysis showed no effect of kindling on arm entries or any other behavior. The means of implanted controls for total and closed arm entries were 10 and 8 – above the values of these measures using the frequency risk median split (8.2 and 6.4 respectively). Therefore, it appears accidental that the median split using risk assessment produced arm entry values low enough to produce the kindling effect. Other criteria tested, including head dips, time active and rearing in the hole board did not identify any median split effects of kindling.

3.2.1.5 Effect of kindling on body weight

There were no differences in weight found between implanted controls and kindled animals on Test 1. However, animals did gain weight by Test 2 and while there were no group x median split interactions on Test 2 using ratio time or risk assessment, there were group effects. Significant effects were found for ratio time split [$F(1,26) = 4.87$, $p<0.04$] and risk assessment split [$F(1,26) = 4.52$, $p<0.05$]. Overall, kindled rats weighed more than implanted controls on Test 2 (Fig 2.4).

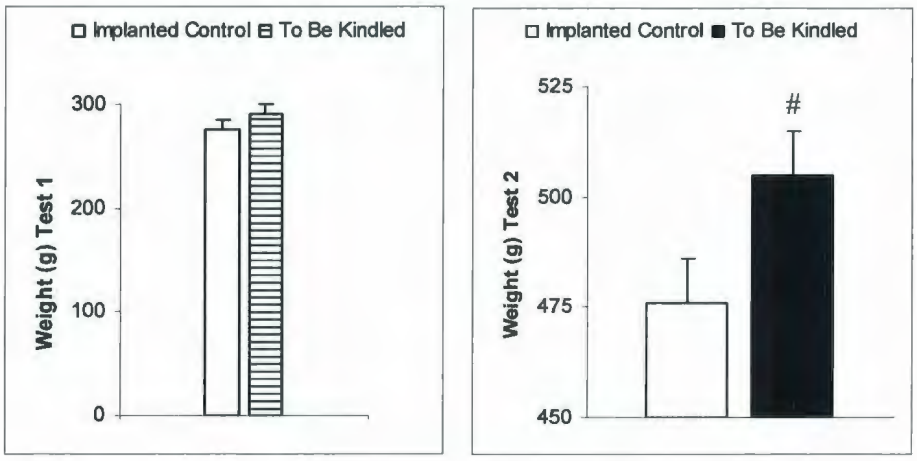


Fig. 2.4. The means + SEM for body weight are provided in the histogram. The left panel compares body weight of implanted controls and to be kindled animals for Test 1, for which there were no significant differences. The right panel shows the results for Test 2 where to be kindled rats were significantly heavier than implanted controls.

3.2.1.6 Effect of kindling parameters on behavioral changes

Ratio time and frequency risk assessment median splits were used as criteria in the analyses. The number of seizures to the first stage five seizure did not result in a median split effect using either criteria. Overall, it took 8.7 ± 0.8 (mean ± SEM) stimulations for kindled rats to reach the first stage five seizure.

There was however, a median split effect in duration of the fourth stage 5 seizure using frequency risk to set the median [$F(1,3) = 6.00$, $p<0.03$; 74.0 ± 3.5 vs. 62.5 ± 3.2 for below and above median groups respectively]. Further analysis revealed that this difference does not likely account for the effects of kindling on arm entries because below- and above-median kindled groups did not differ in their behaviors, and covarying seizure duration out of the analysis for arm entries did not change the pattern of results.

3.2.2. Relation of baseline behavior to response to electrode implantation

3.2.2.1 Effects of retesting, median split along ratio time, and electrode implantation on behavior and body weight of controls

Control group n's The handled control group consisted of 25 animals, split 14 below and 11 above the median, while the implanted control group included 13 animals, split 8 below and 5 above the median.

Hole board behavior Analysis of the control groups revealed that electrode implantation did not affect exploration (head dips), activity (time active) or boli in the hole board. However, implanted controls did rear more on Test 1 and 2 than handled controls [group effect, $F(1,34) = 6.83$, $p<0.02$, Figure 2.5]. Overall, only two measures changed from Test 1 to Test 2. Head dips decreased equally over both handled and implanted controls on Test 2 [Test effect, $F(1,34) = 7.00$, $p<0.01$], and rearing increased in both groups on Test 2 [Test effect, $F(1,34) = 19.61$, $p<0.01$].

Plus maze behavior Analyses revealed that risk assessment measures were affected by electrode implantation and retesting. There were significant test and group x test interactions for relative frequency and time risk assessment. Group x test interactions showed that handled and implanted controls did not differ on Test 1 on either frequency

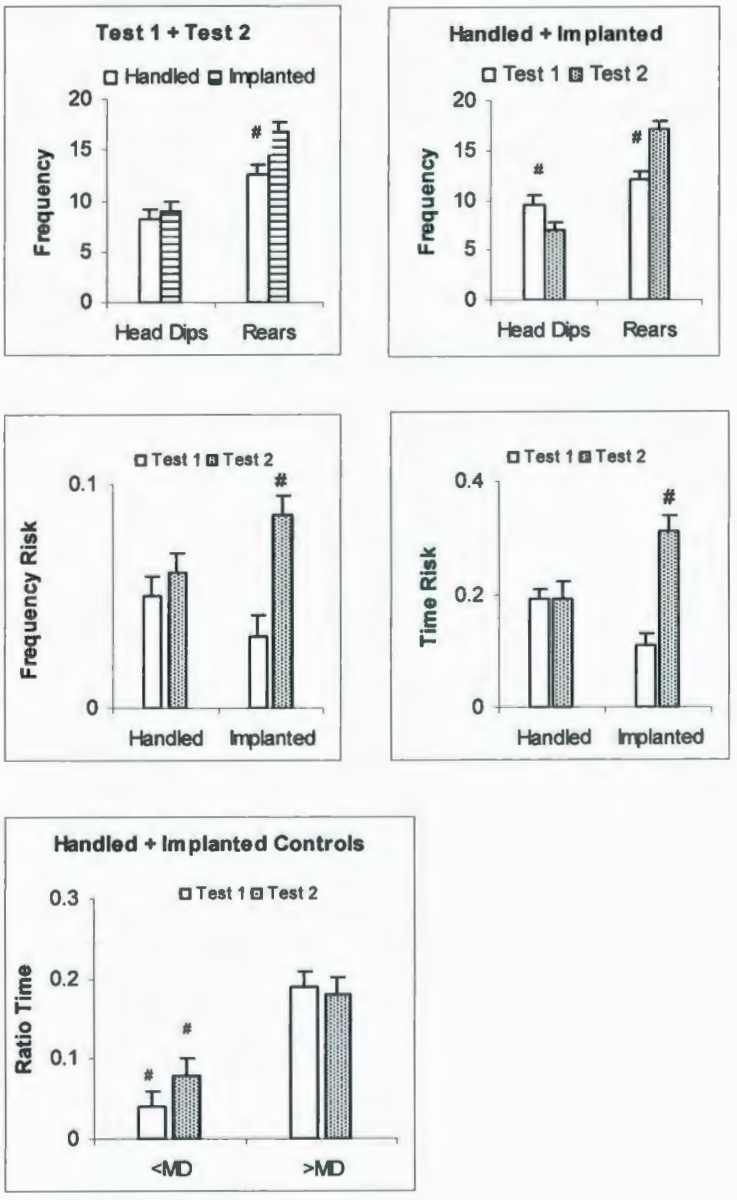


Fig. 2.5. Plotted are the means + SEM for behaviors in the hole board and plus maze. The top two panels compare head dips and rears for handled and implanted control rats on Test 1 and Test 2. The left panel shows results collapsed over tests and indicates that rearing is less for handled animals. The right panel shows results collapsed over groups and illustrates a difference between Test1 and the comparison Test 2 mean. The middle plots compare handled and implanted controls on frequency risk and time risk for Test 1 and Test 2. The means from implanted controls on Test 2 (marked with #) differ from unmarked means which do not differ from each other. The bottom plot shows ratio time means for Test 1 and Test 2 collapsed across handled and implanted controls. The groups are also divided into those that were below (<MD) and above (>MD) the median on Test 1. Means marked with # do not differ from each other. Unmarked means also do not differ amongst each other but do differ from marked means.

or time risk (Fig 2.5, middle panels). However, implanted controls increased their risk assessment on Test 2 beyond handled controls and their own Test 1 levels [Group x Test interactions $F(1,33) = 10.55, 10.38, p<0.001$ for frequency and time risk respectively]. Risk assessment of handled controls was stable over tests 1 and 2.

There was no effect of implantation or retesting on total or closed-arm entries, or ratio time. There were also no effects or interactions for ratio time or arm entries. The only significant median effect was for ratio time [median split effect $F(1,34) = 23.29$, $p<0.001$, Fig 2.5].

Body Weight There was a significant three-way interaction for body weight [group x median split x Test $F(1,33) = 19.76, p<0.001$, Fig 2.6]. The interaction occurred as a result of a greater increase in body weight on Test 2 for below-median handled controls. The other mean differences represent the expected increase in weight in all groups during the timeframe between Test 1 and Test 2.

3.2.2.2 Effects of retesting, median split along risk assessment, and electrode implantation of behavior and body weight of controls

Control group n's. The handled control group consisted of 25 animals, split 9 below and 16 above the median, while the implanted control group included 13 animals, split 7 below and 6 above the median.

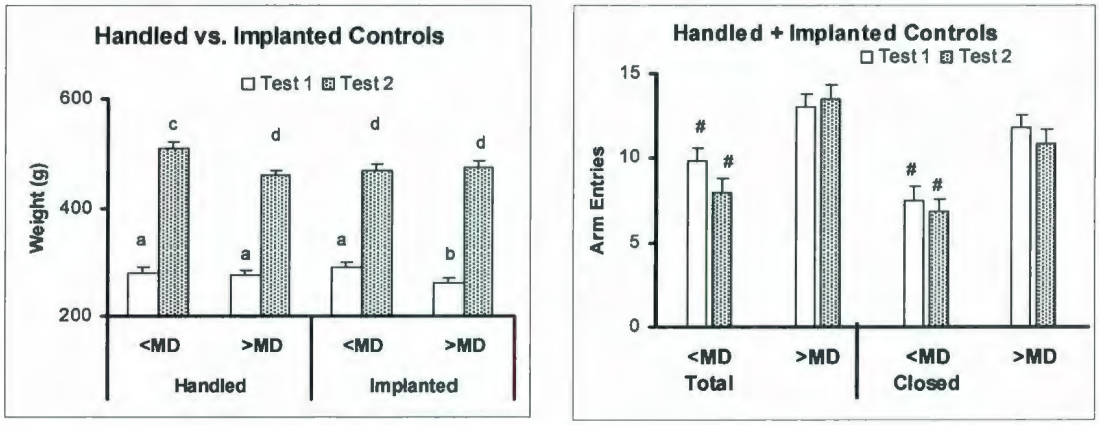


Fig. 2.6. Plotted in the histogram are means + SEM for body weight in handled and implanted controls and total and close-arm entries. The left panel shows the group x median split x test interaction using ratio time as the criterion to determine the median split. Means with the same letter are similar and differ from means marked with a different letter. The right panel shows the means for total and closed arm entries separated into below and above-median groups using relative frequency risk from Test 1 to determine the split. Means marked with # are similar but differ from unmarked means which do not differ from each other.

Effects on behavior and body weight. Overall the pattern of results found using risk assessment to set the median split were similar to those found when using ratio time as the criterion. However there were four differences found. The first difference was the lack of a three way interaction for body weight as described above. This was replaced by a test effect [$F(1,33) = 1211.25, p<0.001$] where as expected rats were heavier at the time of Test 2 than they were at Test 1. There were also, as expected, main median-split effects for risk assessment measures [$F(1,26) = 15.16, 6.67, p<0.001, 0.02$ for frequency and time risk respectively]. Thirdly, there were group x test interactions for risk assessment as described above (Fig. 2.5) and there were median-split effects for total and closed-arm entries in the plus maze [$F(1,26) = 18.36, 15.46, p<0.001, 0.02$, for closed-arm entries and total entries respectively]. Given that these effects were constant across tests without any group interactions, it was concluded that retesting and electrode implantation had no

effect on arm entries.

3.3 Experiment 3

3.3.1 Results of Experiment 3.1 – kindling of the right basolateral amygdala

In general, the results of this experiment showed that right BLA kindling of high baseline anxiety rats was anxiolytic one week after kindling. Right BLA kindling of low baseline anxiety rats was anxiogenic. In addition, left BLA kindling was either anxiogenic or without effect on plus maze anxiety, depending on baseline anxiety. Detailed analyses are provided in the following sections.

3.3.1.1. Effects of electrode implantation on behavior and the development of the ratio time split

There was no effect of electrode implantation found on any behavioral measures on Test 1 for any of the three groups. While there were some test effects (described later), there were no interactions found between handled and implanted controls and therefore they were combined as a single control group on subsequent tests.

Ratio time from Test 1 was used as the split criterion. The mean ratio time (.439) was used to group rats as below the mean (more anxious) or at or above the mean (less anxious). Data were normally distributed so this mean split is comparable to a median split.

3.3.1.2 *Effect of BLA kindling on anxiety in rats above and below ratio time split*

The response to kindling was assessed using ANOVA to identify Test effects and Mean Split effects. Significant behavioral effects were found for ratio time and ratio entry with rats below the mean split on test one showing an increase in ratio time

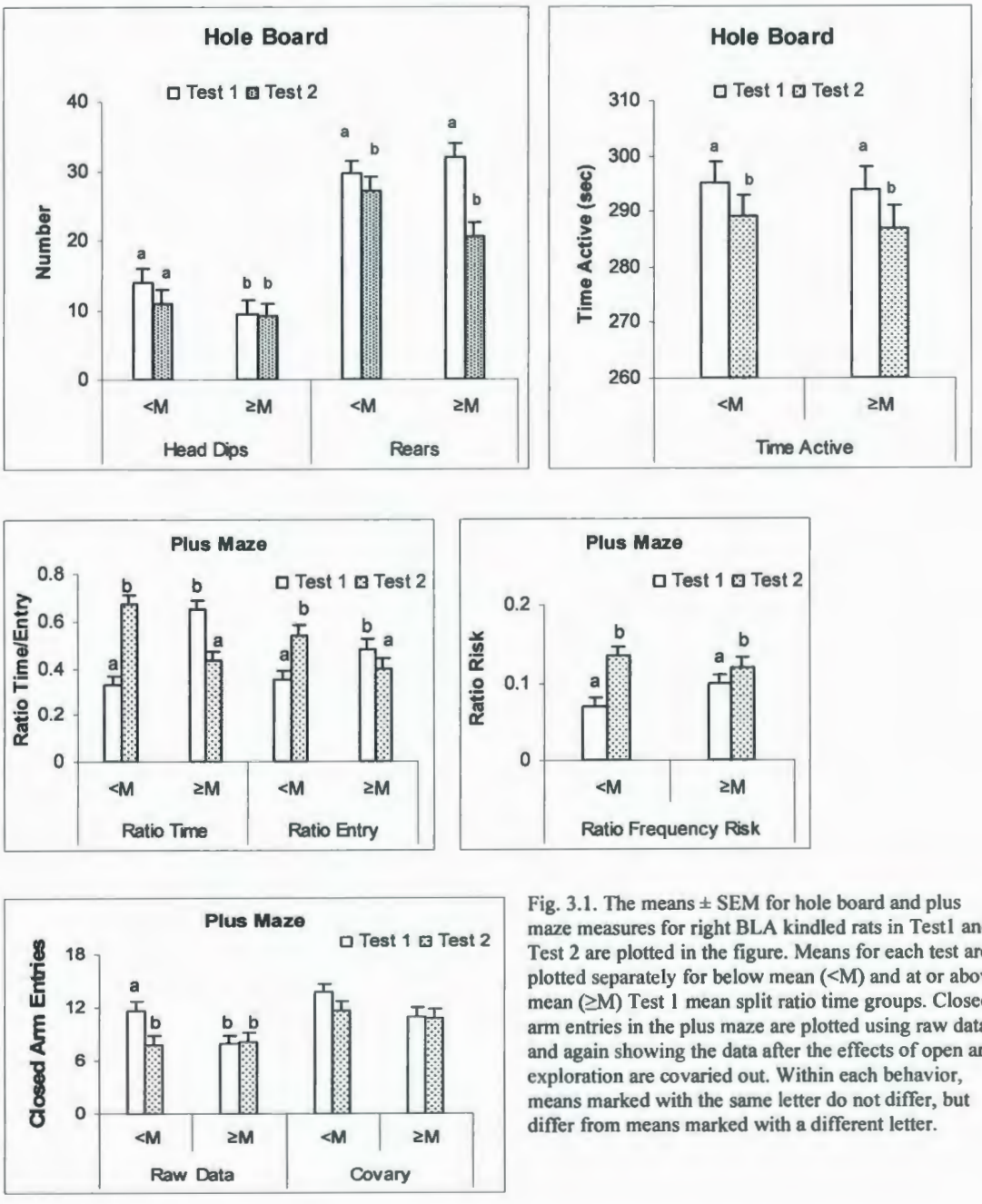


Fig. 3.1. The means ± SEM for hole board and plus maze measures for right BLA kindled rats in Test1 and Test 2 are plotted in the figure. Means for each test are plotted separately for below mean (<M) and at or above mean (≥M) Test 1 mean split ratio time groups. Closed arm entries in the plus maze are plotted using raw data and again showing the data after the effects of open arm exploration are covaried out. Within each behavior, means marked with the same letter do not differ, but differ from means marked with a different letter.

and ratio entry (an anxiolytic effect) on Test 2. Whereas rats above the Mean Split on Test 1 showed a decrease in ratio time and ratio entry (an anxiogenic like effect) on Test 2 [Fig. 3.1; Test x Mean Split interactions all $F(1,15) \geq 16.44$, $p<0.001$; planned comparisons, Fisher's LSD, $p< 0.05$]. In contrast, kindling increased risk assessment equally in above and below Mean Split rats [Test Effect only, $F(1,15) = 14.07$, $p<0.002$; Fig. 3.1]. This finding supports other findings from this lab whereby it appears that risk assessment and open arm exploration are controlled by separate neural circuitry.

Analysis of the hole board data showed no Test x Mean Split interactions. There were consistent decreases over tests in rears and time active [Test Effects only, all $F(1,15) \geq 6.12$, $p<0.03$), Fig. 3.1] characteristic of habituation to the hole board. There was no test effect on head dips but above Mean Split rats head dipped less than below Mean Split rats [Mean Split Effect, all $F(1,15) = 5.68$, $p<0.03$), Fig. 3.1]. In addition, kindling did have a significant effect on exploration in the plus maze [Test x Mean Split for closed arm entries, $F(1,15) = 8.20$, $p<0.02$]. Kindling decreased closed-arm entries in below Mean Split rats to the pre- and post-kindling level of above Mean Split rats (Fig. 3.1). Further covariance analysis revealed that the change in closed arm entries was due to the increase in open arm exploration in this group. When ratio time and ratio entry were covaried out from the closed arm analysis the interaction was eliminated [$F(1,13) = 2.48$, $p<0.14$), Fig. 3.1, covary plot]. Kindling did not have an effect on closed arm entries in above Mean Split rats.

There was an interaction effect between kindling and baseline anxiety on defensive resistance to capture [Test x Mean Split $F(1,15) = 10.44$, $p<0.006$, Fig 3.2,

capture score]. Kindling resulted in a decreased resistance to capture score only in above Mean Split rats.

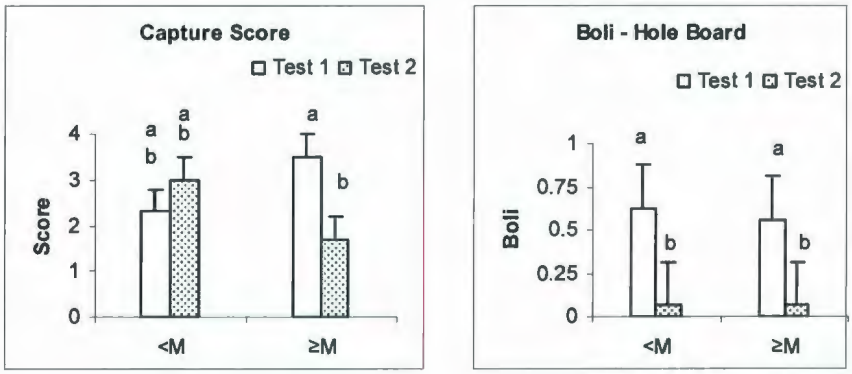


Fig. 3.2. The histograms show the means ± SEM of capture score and boli left in the hole board of right BLA kindled rats. Means for each test are plotted separately for below mean (<M) and at or above mean (≥M) Test 1 mean split ratio time groups. Within each behavior, means marked with the same letter do not differ, but differ from means marked with a different letter. Means marked with two letters fall between means marked with either letter.

Kindling also led to a decrease in the number of boli left in the hole board [Test Effect, $F(1,15) = 5.50$, $p<0.04$, Fig 3.2].

3.3.1.3 Effects of retesting on behavior in controls and control and kindled group comparisons on Test 2

A number of further analyses were carried out to determine whether the Test x Mean Split effects in kindled rats were due to kindling or whether they were reflecting a repeated testing effect in the hole board and plus maze.

Considering controls there was no Test x Mean Split interaction found for open arm exploration (ratio time and ratio entry), however there were Mean Split and Test Effects [all, $F(1,32) \geq 8.66$, $p<0.01$]. Post hoc Tukey-Kramer tests ($p<0.05$) revealed that rats below the Mean Split increased open arm exploration from Test 1 to Test 2 while rats above the Mean Split remained stable over tests (Fig 3.3, upper left panel).

Further comparisons were conducted to determine the extent to which the increase in open arm exploration for controls accounted for the increase over tests in below Mean Split kindled rats. Control and kindled rats were compared with respect to open arm exploration on Test 2. An ANOVA revealed significant Kindling x Mean Split Effects for ratio time and ratio entry [all $F(1,47) \geq 8.64$, $p<0.006$, Fig 3.3, upper right panel]. As well, the pattern of change in open arm exploration observed in control rats above, increase for below Mean Split kindling and decrease for above Mean Split kindling was preserved when kindled and control rats were compared on Test 2 (mean contrasts Fisher's LSD, $p < .05$). Thus, the increase in open arm exploration observed in Test 2 for Below Mean Split control rats was increased further in kindled rats, while the lack of a change in Above Mean Split controls was replaced by a decrease in open arm exploration in kindled rats.

Defensive resistance to capture, head dipping and rearing measures did show repeated testing effects in controls [Test Effects only, $F(1,32) \geq 5.39$, $p<0.03$, Fig 3.3, middle panel]. Capture scores were higher on Test 2 for control groups. Both kindled and control groups changed in their response to capture across tests so they were compared in Test 2 [Figure 3.3, lower left panel]. No significant main effects or interactions were found, however planned comparisons did show that Below Mean Split kindled and control rats did not differ but Above Mean Split kindled rats were less reactive to capture than controls (Fisher's LSD, $p < .05$).

Head dipping decreased in controls on Test 2 but there was no change in kindled rats (Fig. 3.1) so the expected habituation observed in controls did not occur for kindled

animals. Rearing decreased in controls on Test 2 (Fig 3.3) however, a further analysis comparing kindled and control rats on Test 2 showed no effects of kindling on rearing (Fig 3.3, bottom right panel).

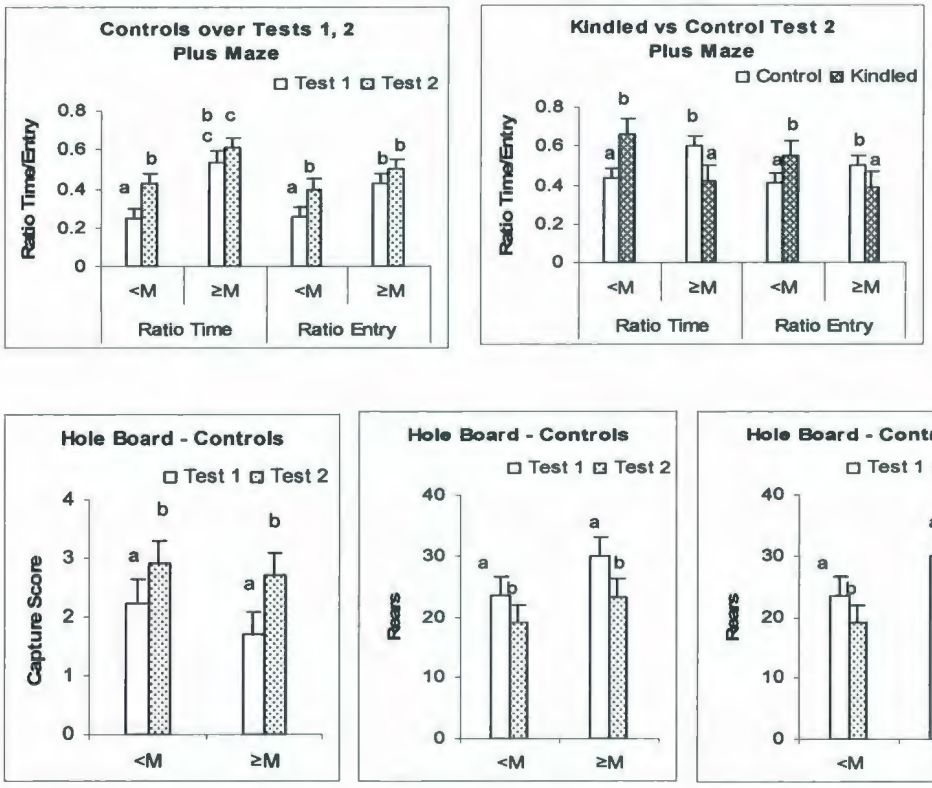


Fig. 3.3. Plotted in the top left and middle panels are means ± SEM for behaviors measured on Test 1 and Test 2 for right BLA control animals (handled and implanted controls combined). Plotted in the top right and bottom panels are means ± SEM of behaviors for controls (handled and implanted combined) and right BLA kindled rats on Test 2. Behaviors plotted are those which changed over tests in controls. Means are plotted separately for below mean (<M) and at or above mean (≥M) Test 1 mean split ratio time groups. Within each behavior, means marked with the same letter do not differ, but differ from means marked with a different letter. Means marked with two letters fall between means marked with either letter alone.

The remaining measures of behavior – risk assessment, closed arm entries in the plus maze and time active in the hole board did not change over tests for control groups. Therefore the effects found in kindled animals were due to kindling and not repeated testing. In addition, the number of boli left in the hole board was also consistent over tests for control animals, thus indicating that kindling caused the observed decrease in boli.

3.3.1.4 Effects of kindling on body weight

A comparison of body weights for handled and implanted controls and to be kindled rats showed no difference and thus confirmed that electrode implantation did not have an impact on body weight. An ANOVA contrasting these groups (to be kindled becomes kindled in Test 2) and mean ratio time split across tests found only a test effect [$F(1,12) \geq 38.97$, $p<0.001$]. This result was anticipated as animals would be expected to gain weight over tests (mean ± S.E.M. weight, tests 1 and 2 respectively: 334.1 ± 4.9 g versus 430.5 ± 11.0 g). Thus kindling did not affect body weight.

3.3.1.5 Anatomical considerations – experiment 3.1

In order to determine whether the location of the electrode tip contributed to the Mean Split ratio time effects on response to kindling, the electrode tip coordinates were analyzed. Three two-way ANOVAs were carried out comparing Kindled (Kindled and Implanted Controls) and Mean Split on AP plane, lateral and vertical coordinates. The electrode tip locations were projected onto the nearest plates of the Paxinos and Watson atlas [70]. The only significant effect found in the analyses was a Kindled effect in the

AP plane [$F(1,29) = 5.37, p<0.03$] with the electrodes of implanted controls appearing in a somewhat more anterior plane than kindled rat electrodes [mean ± SEM (mm) posterior to bregma: 1.92 ±.07 for controls versus 2.15 ± 0.6 for kindled]. There was no Mean Split or Mean Split by Kindled interactions for any coordinate. Given that there was no effect of implantation found on behavior on Test 1, the AP plane difference was not considered to be behaviorally significant. The lack of a Mean Split x Kindled interaction using electrode coordinates also supports the view that the differences found in the groups were not caused by electrode location.

3.3.1.6 Kindling Parameters

An ANOVA comparing Above and Below Mean Split kindled rats on: the number of stimulations to the first stage five seizure (mean ± SEM for Below and Above Mean Split groups: 8.9 ± 1.1`versus 9.4 ± 1.2 respectively) and duration of fourth stage five seizure (mean ± SEM for Below and Above Mean Split groups: 73.5 ± 4.3`versus 69.5 ± 4.5 s respectively) showed no significant group differences. Therefore, these parameters did not contribute to the differential kindling effects found.

3.3.2 Results of Experiment 3.2 – kindling of the left basolateral amygdala

3.3.2.1. Effects of electrode implantation on behavior and the development of the ratio time split

Electrode implantation was without effect on behavior in Test 1. Kindled rats did not differ from control groups, and because there were no differences or interactions

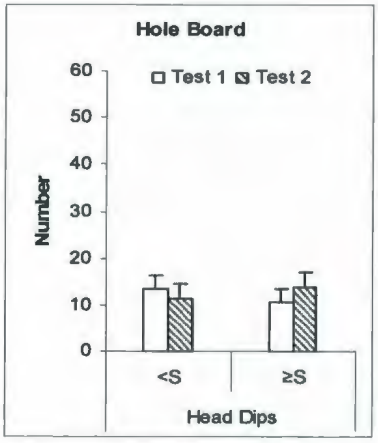
found between handled and implanted controls, they were combined for all further analyses.

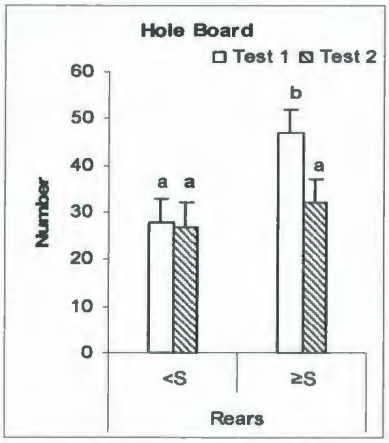
The data from experiment 3.1 and findings in the literature were used to develop the ratio time split. Previous findings from this laboratory suggested that right BLA kindling is anxiogenic and left BLA kindling is anxiolytic [19]. The present findings however, indicate that the effect of right BLA kindling on anxiety is dependent on the pre-kindling ratio time rather than hemisphere. If this applies to the left BLA then one could expect that left BLA kindled rats with pre-kindling ratio times at or above .57 (average of pre-kindling Test 1 ratio times from Above Mean Split, less anxious to be kindled rats and tests 1 and 2 ratio times of less anxious controls, all of which do not differ) would be anxiogenic. In addition, the Adamec and Morgan study [18] found that left BLA kindled rats showed an increase in ratio time to .34 versus controls that had a low ratio time of .21. This anxiolytic effect in left BLA kindled rats does not differ from the post-kindling anxiogenic effect found for right BLA kindled rats in experiment 3.1. Thus left BLA kindling effects may depend on low open arm exploration tendencies. This expectation led to the development of three left BLA baseline groups: High Anxiety with Test 1 ratio times of .21 or below; Low Anxiety with Test 1 ratio times at .57 or above; and Mid Anxiety with ratio times in between. From these groups it was predicted that left BLA kindling would reduce ratio time in Low Anxiety baseline rats (an anxiogenic effect); increase ratio time in High Anxiety baseline rats (anxiolytic effect) and have no effect on Mid Anxiety rats. The data from Test 1 showed that there were no High Anxiety rats in this study so animals were divided into the Low and Medium Anxiety groups.

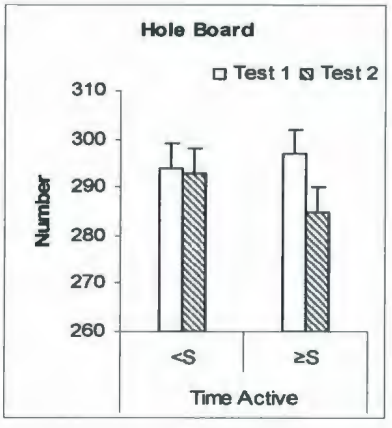
3.3.2.2. *Effects of left BLA kindling on anxiety based on ratio time split*

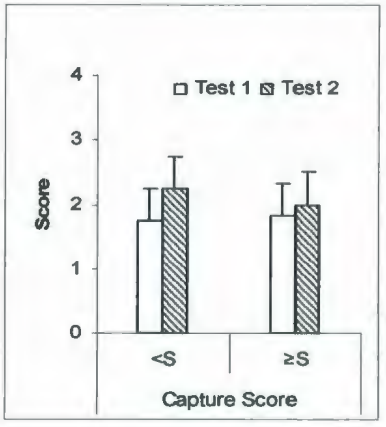
ANOVA was used to determine the effects of kindling comparing Test (T) and Mean Split (S) effects. The analyses revealed a significant T x S interaction for ratio time in the plus maze ($F\{1,12 = 4.80, p \leq 0.01$; Figure 3.4). In addition the interaction for ratio entry tended toward significance ($F\{1,12 = 2.30, p < 0.16$).

Hole Board









Plus Maze

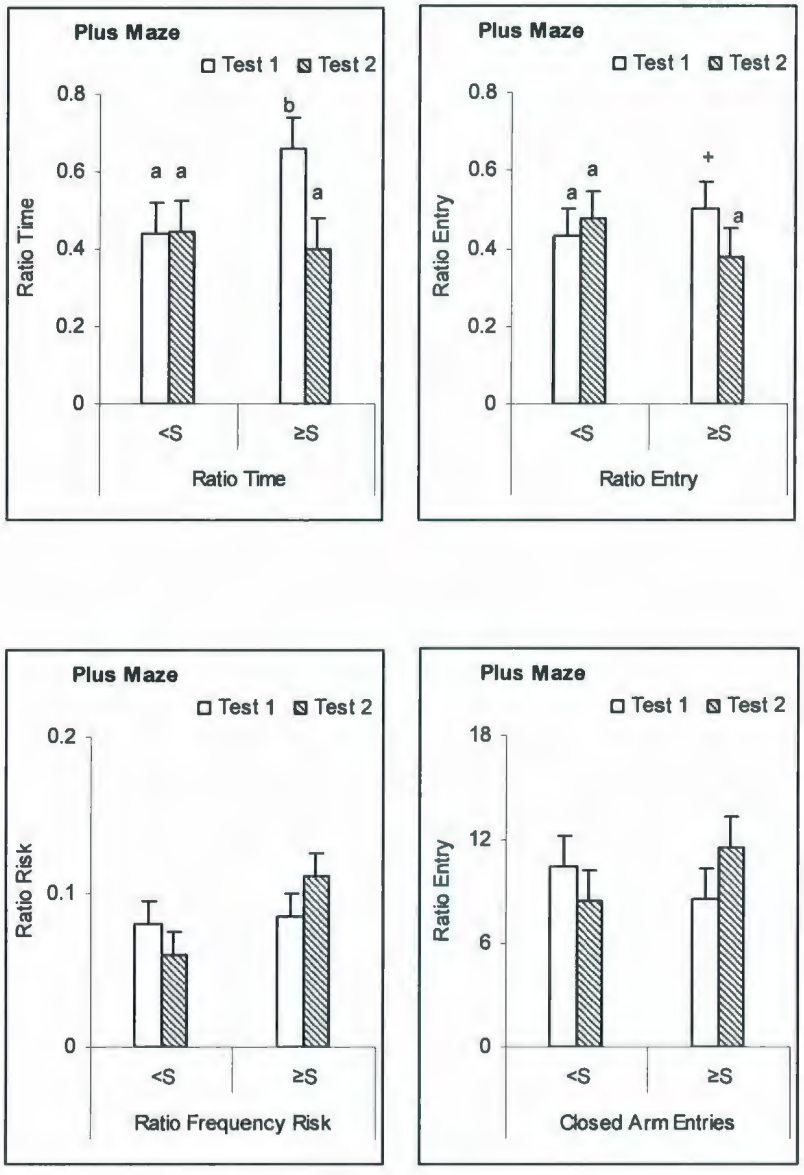


Fig. 3.4 Mean ± SEM of behaviors on tests 1 and 2 in left BLA kindled rats are presented in the figure. The top four figures provide the mean ± SEM for head dips, rears and time active in the hole board and the resistance to capture score. The bottom four figures show the mean ± SEM for ratio time, ratio entry, ratio frequency risk and closed arm entries in the plus maze. The means for tests 1 and 2 are plotted separately for groups below (<S) and at or above (≥S) the split ratio time (approximately .57). For each behavior, unmarked means or means marked with the same letter do not differ, but differ from means marked with a different letter. The test 1 ratio entry mean marked with a + differs from the test 2 mean.

Further analysis using mean contrasts revealed that kindling decreased ratio time ($p < .05$, Fisher's LSD) and tended to decrease ratio entry (one tailed $t\{12\} = 1.41, p < .093$) in Above Mean Split rats. However, kindling had no effect on these measures in Below Mean Split rats (Fig. 3.4). It is noteworthy that tests 1 and 2 ratio times of Below Mean Split rats do not differ from results found for left BLA kindled rats in Adamec and Morgan [19] (mean ± S.E.M. in Adamec and Morgan = .34 ± .05 versus .43 ± .05 in Below Split rats). For these groups, it is possible that they were already at a level to which left BLA kindling would drive them and therefore there was no change. Left BLA kindling was found to have no effect on risk assessment (Fig. 3.4) contrasting the effects found for right BLA kindling (Fig 3.4).

There were no T x S interactions found for measures of activity or exploration in the hole board (head dips, time active) or the plus maze (closed arm entries) (Fig 3.4). There were however, main Mean Split and Day effects and a marginal Test x Mean Split effect for rearing *[*$F\{1,12 \geq 4.87, p < 0.05$ and $F\{1,12 = 3.27, p < 0.096$ for Test x Mean Split]. Further analysis on this interaction using planned comparisons found a similar pattern to that found for ratio time. Kindling appeared to reduce rearing in the hole board in above mean split rats (further information to follow).

Unlike right BLA Kindling, left BLA kindling had no effect on resistance to capture (Fig. 3.4) nor on the number of boli left in the hole board.

3.3.2.3. *Effects of retesting on control behavior and control and kindled group comparisons on Test 2*

Data from controls were further analyzed to determine whether the Test x Mean Split effects found were in fact due to kindling or were the result of repeated testing in the hole board and plus maze. The analysis for open arm exploration measures revealed no significant Test x Mean Split interactions for ratio time or ratio entry and therefore the effects found for these measures in the current study were due to kindling and not repeated testing.

Planned comparisons (Fisher's LSD tests, $p<.05$) on ratio time showed that rats below the mean split differed from rats above the mean split on Test 1. Other measures such as risk assessment and closed arm entries in the plus maze, and time active, head dips, boli and resistance to capture in the hole board test were stable over tests as they were in kindled rats.

There was a repeated testing effect in controls for rearing in the hole board [$F\{1,23 = 4.39, p < 0.05$; Fig. 3.5, left panel]. Rearing decreased on Test 2 for Above Mean Split rats only (Tukey-Kramer, $p < 0.05$). This pattern is similar to that found in kindled rats and therefore an additional analysis comparing controls and kindled animals was carried out on Test 2. The ANOVA revealed a significant Kindled x Mean Split interaction ($F\{1,35 = 4.13, p < 0.05$: Fig 3.5). As can be observed in the figure, Above Mean Split kindled rats reared more than Above Mean Split Controls. Based on the data, it appears as though kindling may have reduced a decline in rearing that was found in controls, but only in Above Mean Split rats.

3.3.2.4. *Effects of left BLA kindling on body weight*

There was no difference between groups on body weight for Test 1, so the electrode implantation procedure did not affect body weight. An ANOVA comparing groups and mean ratio time split across tests revealed only one main effect of test ($F\{1,33 = 80.28, p < 0.001$). As expected, rats gained weight between tests 1 and 2 (mean ± S.E.M. 335.5 g + 4.3 versus 415.2 g + 7.5 tests 1 and 2 respectively). Therefore left BLA kindling had no effect on body weight.

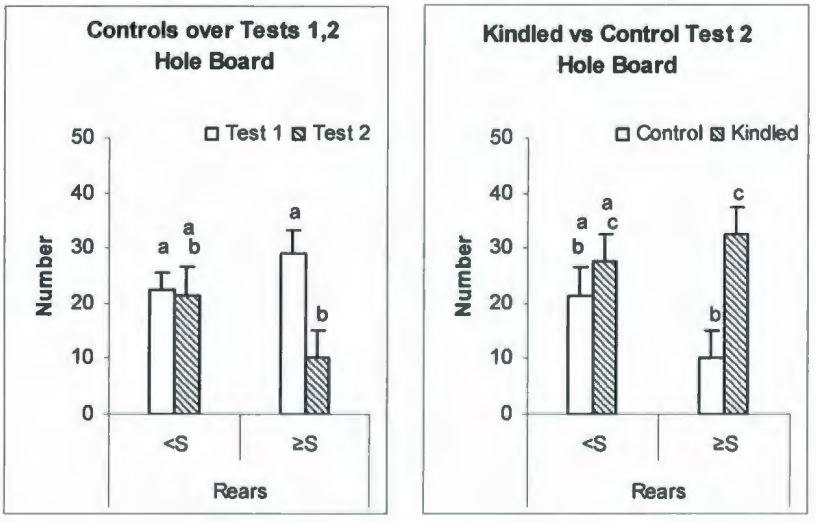


Figure 3.5. The left panel shows mean ± S.E.M. for rearing in the hole board measured on tests 1 and 2 in left BLA control rats (unoperated and implanted controls combined). The right panel shows data for mean ± S.E.M. for rearing measured on Test 2 for controls and left BLA kindled rats. Means are plotted in both histograms for below (<S) and at or above (≥S) Test 1 Split ratio time groups. Unmarked means or means marked with the same letter do not differ, but differ from means marked with a different letter. Means marked with two letters do not differ from means marked with either letter.

3.3.2.5. *Anatomical Considerations*

Electrode tip locations in the left BLA were analyzed to assess whether or not they had an impact on the Mean Split effects in response to kindling. The analysis found

no electrode tip difference in kindled rats below, at or above the ratio time split. Kindled and control electrode placements also did not differ. Therefore there was no effect of electrode placement on behavior differences found in the experiment.

3.3.2.6. Kindling Parameters

ANOVA contrasting Above and Below Mean Split kindled rats found no group differences on the number of stimulations to the first stage five seizure (mean ± S.E.M. 8.8 ± 0.9 versus 10.8 ± 1.1 for Below and Above Mean Split groups respectively) and duration of fourth stage five seizure (mean ± S.E.M. 66.2 ± 9.4 versus 62.2 ± 6.5s for Below and Above Split groups respectively).Therefore kindling parameters did not contribute to the ratio time split effects found.

3.3.2.7. Comparison of left and right BLA kindled rats on kindling parameters and anatomical location of electrodes

An ANOVA was completed comparing the parameters of kindling (number of seizures to the first stage five and duration of fourth stage five seizure) for rats kindled in the right BLA (experiment 3.1) to the same parameters for left BLA kindled rats (experiment 3.2). The analysis contrasting above and below splits used and side of kindling found no effects or interactions for either kindling parameter. Therefore hemispheric differences in kindling in the two experiments did not contribute to the differential kindling effects.

A similar ANOVA comparing right and left BLA kindled rats with respect to

electrode placement also found no main or interaction effects in any plane (Table 6). Thus, kindling of different locations in the left and right BLA did not contribute to hemispheric differences found in the two experiments.

Table 7: Table of electrode locations (means ±S.E.M.) in the right and left basolateral amygdala in the present study.

Hemisphere	Anterior-posterior[a] plane	Lateral[b] plane	Vertical plane
Right	2.15 ± .07	4.92 ±.07	8.25 ± .09
Left	2.36 ± .08	4.88 ±.08	8.33 ± .10

[a] Data (mm), posterior to bregma.
[b] Lateral position (mm) is lateral to midline, vertical (mm) is below the dura.

4.0 Discussion

4.1 Experiment 1 - Differences in location of kindling leading to differing behavioral outcomes

4.1.1 Effects of kindling on anxiety

Overall, the data confirmed that changes in anxiety in kindled rats measured using ratio time and ratio frequency risk assessment were not due to changes in activity levels or exploratory behavior.

Kindling of the posterior central amygdala generally increased anxiety-like behavior, observed 1 week after the last seizure as a decrease in ratio time. Kindling in this area also resulted in a decrease in risk assessment, a result often accompanying an increase in anxiety-like behavior [19, 20, 23]. In certain cases, an increase in risk assessment has been cited as a measure of increased anxiety-like behavior [29, 83, 90].

However, the strong link between a decrease in risk assessment and the decrease in ratio time has led to an interpretation in this laboratory that the decrease in risk assessment represents an anxiogenic response. The factor analysis and the analysis of covariance carried out in this study support these interpretations for central nucleus kindled rats showing a positive relationship between ratio risk assessment and ratio time. However, for nucleus basalis kindled rats, the changes in ratio frequency risk assessment were found to be independent of ratio time.

The factor analysis findings for central nucleus kindled rats along with the positive relationship found between ratio time and ratio frequency risk assessment also suggest that a shared neural substrate is affected by kindling. This factor analysis result whereby risk assessment and ratio time share the same "anxiety" factor is consistent with previous findings in this laboratory [17].

Factor analysis for rats in the kindled nucleus basalis group showed a different result with open-arm avoidance and risk assessment falling on two different factors. These animals also showed an increase in ratio frequency risk assessment making it difficult to determine if the effect was anxiogenic or anxiolytic. This could be further studied by testing the effects of an anxiolytic compound such as diazepam on animals kindled in the same location.

Other studies have shown open-arm avoidance and risk assessment loading on different factors including a study using hooded rats [17]. Studies using Wistar rats, as was the case for this experiment, have reported that the measures loaded on the same factors [17, 18]. The main difference in this case is the nucleus basalis group, with results

that suggest that kindling in this area results in a disassociation of ratio time from risk assessment. This effect would only be possible if the nucleus basalis is part of circuitry which can impact these two measures separately.

Recent data support the idea that these two measures may be altered separately. In this laboratory, multiple studies have found that exposure to a cat causes a decrease in both ratio time and ratio frequency risk assessment up to three weeks after exposure [17, 20]. This effect can be blocked for both measures by a systemic injection of NMDA or CCKB receptor blockers prior to the exposure to the cat [15, 23]. However, when NMDA blockers are directly cannulated into the lateral amygdala prior to the exposure to a cat, the change in ratio frequency risk assessment is blocked but the decrease in ratio time still occurs when measured 1 week after the exposure [16].

Therefore, the findings suggest that these two behaviors are likely measuring different but related neurobehavioral effects, particularly given the similarity in the circumstances which generate the response, i.e. conditions of potential threat [29, 30].

4.1.2 The location within the central amygdala and the effects of kindling on anxiety

The results of this experiment, further support findings from previous studies indicating the importance of hemisphere and AP plane location of kindled foci on kindling induced behavioral outcomes [12,19]. Previous studies of kindling in the medial and cortical amygdala nuclei revealed a graded effect of AP plane position of kindled foci on the behavioral outcome, with a linear correlation between the change in risk assessment and the location of the kindled site in the AP plane. More specifically,

kindling in the anterior foci were anxiogenic while kindling of the more posterior nuclei were anxiolytic. A similar correlation was found in the current study. However, the graded effects were in the reverse direction with kindling of the more anterior foci decreasing anxiety while kindling of the more posterior foci were anxiogenic. In the current study, it was primarily risk assessment that changed.

A question then for consideration is how amygdala foci that are merely 0.5 mm apart are able to produce different behavioral outcomes. Other studies including Adamec and Morgan [19] and Adamec and McKay [18] have found behavioral differences in other closely located amygdala nuclei. This is further supported by findings by Watson et al. [101] who used [^{14}C]2-deoxyglucose autoradiography to measure the spread of stimulation from an electrode within the rat amygdala and found a rapid 70-90% reduction in stimulation within a sphere of 0.3mm radius.

These results have been combined to develop a hypothesis to explain the different behavioral effects [12]. This hypothesis involves long-term potentiation (LTP) which occurs when electrical or chemical stimulation leads to a strengthening of synaptic signals that lasts for an extended period of time. When strengthened or "potentiated", these synapses require very little stimulation to become activated. In this hypothesis, Adamec suggests that kindling first initiates LTP in specific efferents in a small sphere directly under the electrode tip. Once a seizure is triggered, the excitation is spread to other parts of the amygdala causing LTP in other efferent pathways, but the already potentiated efferent pathways are also reinforced. The hypothesis thus accounts for the spread of potentiation to other areas of the amygdala and suggests that the outcome is a lasting

behavioral change predicated on the reinforced LTP efferents activated by the electrode.

This hypothesis may also explain the different behavioral changes found in the current study. Chemical neuroanatomical studies show high concentrations of CRF peptide and mRNA containing cells in the posterior central amygdala foci [51, 64]. In addition, CRF is found to increase in these areas following social stress [52, 67] an effect accompanied by an increase in anxiety in the elevated plus maze [52]. It is possible that kindling may increase the release of CRF in this area in response to the stress of the plus maze. This idea is, in fact, supported by Stenzel-Poore et al. [94] who found that transgenic mice with CRF overproduction are more anxious in the plus maze than controls, and a CRF receptor antagonist (i.c.v) normalizes the transgenic mouse anxiety. Furthermore, amygdala kindling has been found to increase the expression of CRF mRNA [93].

LTP of central amygdala CRF efferents may also contribute to the behavioral response, in particular, the CRF projections to the bed nucleus of the stria terminalis and periacqueductal gray [82, 48]. Activation of the bed nucleus leads to behavioral changes that resemble those produced by stress [32], and PAG excitability has been identified as an important component of plus maze anxiety [48, 91, 92].

The anterior central nucleus foci overlap with the origins of catecholamine projections to the ventral tegmental area, substantia nigra, and locus coeruleus [100]. In addition, Ray et al. [81] found that infusion of a beta noradrenergic (NE) agonist into the anterior central amygdala protected against cold restraint ulcers, a result expected from an area that plays a role in reducing anxiety.

The lack of a behavioral effect found in the midcentral nucleus may be due to an overlap between the posterior anxiogenic and anterior anxiolytic systems. If a 0.3 mm sphere of activation is assumed, there would be an overlap between the midcentral nucleus (mean AP plane location of -2.44 mm ± 0.3 mm) and these systems.

4.1.3 Role of the nucleus basalis

With respect to the nucleus basalis, there is some uncertainty regarding its role in rodent anxiety. Previous studies have suggested lesions in this area produce both "anxiolytic" effects, observed as severe deficits in passive avoidance of drinking which might suggest reduced fearfulness [68], and anxiogenic effects, reported as increased "anxiety" in agonist social encounters [69]. The plus maze is believed to tap into unconditioned defensive response to species-characteristic threats, similar to the effects measured in social encounters. If we follow this logic, it appears as though damage in this area of the brain has an anxiogenic effect, suggesting the normal function of the nucleus basalis may be to reduce anxiety-like behavior when facing these types of threats. Kindling in this case may enhance this normal function and may explain the anxiolytic response found with respect to risk assessment in this study. Further study is required in this area.

The effects of nucleus basalis kindling on behavior can also be explained using the LTP hypothesis of Adamec [12]. Kindling, in this case, may have potentiated GABAergic inhibitory projections to amygdala nuclei [65, 66, 95]. This potentiation within amygdala areas that facilitate anxiety, could result in the observed anxiolytic

effects.

4.1.4 Effects of electrode damage on baseline anxiety

There is evidence from this laboratory, that implanting electrodes into both the left BLA [19] and the right anterior cortical nucleus [18] is anxiogenic with respect to ratio time. This effect was found to be reversed by kindling. In the current study, posterior central nucleus implanted controls were found to exhibit more risk assessment than mid and anterior central nucleus and nucleus basalis implanted controls. Furthermore, there was a significant linear correlation found in implanted control groups between AP plane and the level of risk assessment. This result may mean that the damage caused by implantation in the posterior central nucleus leads to increased risk assessment, with kindling reversing the effect. In contrast, the damage caused by implantation in the mid central nucleus, anterior central nucleus and nucleus basalis may reduce risk assessment with kindling of the anterior central amygdala and the nucleus basalis reversing the effect. Unfortunately, this suggestion cannot be tested using data from the current study due to the lack of an unoperated control group.

4.1.5 Differences in kindling parameters found in different brain areas

One of the two kindling parameters measured varied by brain area. Animals kindled in the nucleus basalis required a significantly higher number of stimulations to reach the first stage five seizure than did animals kindled in the mid or anterior central amygdala. When comparing by brain location, animals kindled in the central amygdala

areas were the same and required the least amount of stimulations to reach a stage five seizure. Animals kindled in the posterior central amygdala required more stimulations to reach a stage five seizure than those in the central amygdala groups but less than those included in the nucleus basalis group. This pattern was found to be unrelated to behavioral effects and therefore did not play a role in the kindling differences.

These findings are consistent with previous studies of LeGal La Salle [62] who also found that amygdala kindling occurred faster in the central nucleus.

4.2 Experiment 2 - Relation of baseline behavior to response to medial amygdala kindling

4.2.1 Stability of behavior on retest

In the introduction, studies were reviewed which indicated that retesting animals in the elevated plus maze at various intervals within one week reliably resulted in increased open-arm avoidance. This effect was measured as a decrease in ratio time. However, decreased ratio time on retest appears to be eliminated by retesting hooded and Wistar rats with intervals of 3 weeks in the same maze placed in a novel room (unpublished). Both factors (time and novelty of the room) were required to prevent the decrease in ratio time. The results suggested that the amount of time that has passed along with the novelty of the new test room appear to be enough to promote renewed exploration of the open arms of the maze. The current study replicated these findings and provided additional information regarding the effects of electrode implantation. Overall the level of open-arm exploration (ratio time) did not change from Test 1 to Test 2 in both handled and implanted animals. This is an important result that confirms that damage

caused by the placement of the electrode does not have an effect on the level of open-arm exploration during retesting. There was also a lack of change between tests for time active in the hole board and total and closed-arm entries in the plus-maze for these groups of animals.

There was a change in exploratory and vertical activity (head dipping and rearing) from Test 1 to Test 2 for handled and implanted controls (Figure 9). The amount of change between tests was equal for both groups; however implanted controls did rear more than handled controls on both tests.

Head dipping did decrease on Test 2 in both control groups. Given that head dipping is a measure of exploratory activity, this change may suggest a level of habituation to the hole board on Test 2 despite the novel room. This result, when considered in relation to the lack of change in ratio time, appears to confirm the independence of hole-board exploration from open-arm exploration, a result found in this laboratory and in other studies [17].

The decrease in head dipping for both control groups may have influenced a rise in rearing activity on Test 2 (Figure 9). Increased rearing in implanted controls may have also resulted from electrode placement, whereby electrode damage in the medial amygdala may have impaired the ability of this region to promote immobility. This conclusion is supported by a study by Rodgers and File [84] that showed the presence of opioid receptors in the medial nucleus of the amygdala which mediate increased immobility in the hole board.

4.2.2 Baseline and kindling impacts on anxiety

Kindling of the right anterior medial amygdala leads to an increase in open-arm avoidance, measured as a decrease in ratio time. This effect lasted at least one week post kindling and was not due to changes in exploratory activity, as these measures were unchanged by kindling. Also the changes in ratio time were not due to electrode location (implanted controls and kindled animals did not differ) or implantation (no differences between implanted and handled controls). Thus, the changes in open-arm avoidance appear to be anxiogenic changes that are attributable to right anterior medial amygdala kindling.

Of particular importance is the relation of baseline open-arm avoidance to the changes in behavior. Rats that showed more anxiety-like behavior (below the median on ratio time) in the first plus-maze test did not show a kindling-induced change in behavior on the second test. However, rats that exhibited less anxiety-like behavior (above the median for ratio time) on Test 1 showed more anxiety-like behavior on Test 2 after kindling. This was measured as a decrease in ratio time. Therefore, the change in open-arm avoidance resulting from kindling is dependent on the baseline level of animals as determined in Test 1. Further analysis confirmed that the low level of ratio times for the below the median groups were not due to a floor effect (t-tests against a constant of 0, all $p < 0.05$).

Previous research from this laboratory suggests that behavioral changes in rats following kindling are due to an enhancement of normal limbic system functioning [6]. If that theory is applied to the current study, a possible interpretation may be that kindling

leads to an enhancement of medial amygdala activity which in turn results in an anxiogenic behavioral response in the elevated plus-maze. There are a number of studies and subsequent theories which support this interpretation. The first theory relates to the function of GABA transmission in this area of the brain. Amygdala kindling has been found to interfere with normal GABA functioning in the basolateral and medial amygdala [31, 79, 99]. Other studies have shown evidence of an inhibitory effect of GABA on amygdala cells [77, 78]. Therefore, following kindling, activation of the medial amygdala by the plus maze exposure with limited inhibitory GABA function could result in increased excitability of the medial amygdala, which has been linked to anxiety-like behavior in other models of rodent anxiety [40, 47, 88, 92]. Indeed, reports of increased c-fos immunoreactivity in the medial amygdala of rats exposed to the elevated plus maze [39, 92] and i.c.v. GABA transmission enhancers in medial amygdala decreasing open arm avoidance in the elevated plus maze [103], together support the view that medial amygdala kindling may lead to a failure of GABA transmission, increased medial amygdala excitability and anxiety.

The impact of amygdala excitability on other parts of the brain involved in generating the anxiety response should also be considered. Previous studies have suggested that long-lasting LTP of amygdala projections to other parts of the brain involved in anxiety-like behavior is required for behavioral changes [5,7,10,12,24,26,88]. Furthermore, kindling in rodents has been found to lead to LTP of amygdala projections to the medial hypothalamus [76] which may play a role in determining anxiety in the plus maze [89]. The efferent LTP theory is also relevant given the median split findings from

the current study, in particular the lack of behavioral change in animals that fell below the median split. It may be that, in these animals, amygdala projections are already potentiated to a maximum level and therefore no additional change or subsequent behavioral difference is possible. While animals above the median split may have less potentiation already occurring, and therefore have more potential for LTP and subsequent behavioral change.

4.2.3 Effects of electrode implantation on anxiety

There was no behavioral effect of electrode implantation in the right medial amygdala in this study. These results clarify a previous study from this laboratory. Adamec and Morgan [19] found that right medial amygdala-implanted controls showed less anxiety than left medial amygdala implanted controls (lower ratio time). However, without handled controls they were unable to pinpoint the source of this difference. Data in this study confirms that implantation did not have an effect in the right hemisphere, and therefore the difference may be due to an anxiolytic effect caused by implantation in the left medial amygdala.

4.2.4 Risk assessment and the impact of baseline and kindling

Results from the current study indicate that kindling did not have an effect on either the time or frequency of risk assessment. There was also no median split grouping found when using ratio time as the median measure. These results differed from previous work in this laboratory [19].

The analysis was repeated using frequency of risk assessment as the median split criterion variable. This assessment did result in a median split grouping of rats into high and low baseline risk assessment for Test 1. However, further assessment to determine the effects of kindling on Test 2 data showed no effects for kindling or median split. This result may be due to the instability of risk assessment on retest. However, an additional hypothesis may warrant consideration. There was an increase in risk assessment for electrode implanted animals on Test 2 (Figure 5). If we assume that the same increase in risk assessment happened in kindled rats, this increase may have covered up the expected decrease that would have been anticipated post kindling. However, further analysis of covariance which removed the potential rise in risk assessment that may have been attributed to electrode implantation again showed no effects of kindling and no baseline effects.

This lack of change in risk assessment and the inability to test the effects using a median split prohibits any conclusions with respect to the impact of kindling on risk assessment. The data as presented appear to show no impact, a result which contradicts previous work in this laboratory [19]. The differences however, may be explained by looking at electrode placement. When comparing electrode placements in this study to the previous study (Table 4) it is apparent that electrode placements in this study are more lateral and dorsal than those in Adamec and Morgan [19]. While the differences are small, the placements in the current study may be in locations that do not influence risk assessment. Small changes in electrode placement leading to differing behavioral outcomes have been documented in a past review [12].

4.2.5 Relation of baseline and kindling to arm entries

Total and closed arm entries were increased by kindling. The total arm entry increase was accounted for by increases in entries into the closed arm. When rats were divided into groups with arm entries below and above a cutoff point, then the effects of kindling were revealed. The cutoff was not the median of arm entries but rather a value below the median.

The increase in closed-arm entries is consistent with what would be expected with an enhancement of the normal functioning of the medial amygdala – an increase in anxiety-like behavior and a decrease in open arm exploratory behavior [84]. The current data also suggest that, in certain animals, kindling may facilitate activity and exploration in the plus maze. Because immobility functions of the medial amygdala appear to involve activation of opioid receptors [84], kindling may induce some dysfunction in this system in rats with reduced levels of exploratory tendencies in the plus maze. A reduction in release of an endogenous opioid ligand could account for the increased activity seen in the present study.

4.2.6 Effects of kindling on body weight

In this study animals that were kindled showed higher weight gains than animals that were not kindled. Research has shown that there are primarily two neuropeptides that are changed following kindling – TRH and neuropeptide Y. Amygdala TRH has been found to suppress feeding [97,98] while neuropeptide Y may facilitate feeding [49]. There is a temporary increase of these neuropeptides in the amygdala following kindling and

seizures [28,38,56,60,87]. In this case, kindling may be leading to the development of more neuropeptide Y, which in turn may have caused a temporary increase in feeding and subsequently the increased weight gain of the kindled animals.

4.3 Experiment 3: Relation of baseline anxiety and electrode location to the response to kindling in the right and left basolateral amygdala

The results found in this study add further support to the conclusion that baseline anxiety level interacts with kindling to lead to a lasting behavioral outcome. In right BLA kindled animals, rats exhibiting more anxiety-like behavior at baseline became less anxious, while rats that exhibited less anxiety-like behavior at baseline became more anxious. The results in left BLA kindled rats were different. Rats exhibiting less anxiety-like behavior at baseline did become more anxious, while rats at mid levels of baseline anxiety appeared to show no effect. These results for left BLA kindled rats contrast previous findings where left BLA kindling was found to be anxiolytic.

4.3.1. The relation of baseline anxiety to behavioral changes in right BLA kindled rats.

The fact that baseline anxiety levels do play a role in determining the behavioral outcomes found in this study is consistent with findings from other studies in the laboratory. In Experiment 2, less anxious Wistar rats showing high levels of open arm exploration at baseline, showed a reduced level of open arm exploration (increase in anxiety-like behavior) following kindling of the right medial amygdala. Animals already showing more anxiety-like behavior at baseline did not show any behavioral change

following kindling.

There are two differences between these two studies. The first is the target nuclei. Previous research has shown that the amygdala nuclei kindled is important in determining the outcomes caused by kindling [12]. In the current study the target nucleus was the basolateral nucleus while the previous study was focused on the medial amygdala nucleus. The second difference relates to the baseline ratio times used to determine below and above median split groups. The values used in Experiment 2 were lower than the same measures used in the current experiment. Overall, there appear to be bidirectional behavioral changes in animals kindled in the right BLA, compared to unidirectional changes in animals kindled in the right medial amygdala.

There are also consistencies between the results found in the current study and those found in Adamec and Morgan [19]. The current study found both anxiogenic and anxiolytic effects of kindling in the right BLA. Adamec and Morgan [19] also found anxiogenic effects of right BLA kindling. While a detailed baseline assessment (i.e., median split) is not available for Adamec and Morgan, further support for the influence of baseline anxiety levels could be found by revisiting control data for that study to determine if control levels of open-arm exploration could predict an anxiogenic effect. Other influential factors such as electrode location and kindling parameters would have to be considered as well.

Upon review it was found that kindling parameters for right BLA kindled rats did not differ for these studies. There was one difference with respect to electrode location. Electrodes in Adamec and Morgan [19] were lower on the vertical plane (deeper) than

those in the current study ($t\{22\} = 2.30$, $p<.04$), placing them closer to the ventral basolateral amygdala. This may explain some of the differences found between the studies. With respect to control data in the Adamec and Morgan Study, the ratio time for right BLA control rats was .36 ±.04 (mean ± S.E.M). In comparison the ratio time for right BLA kindled rats was .28 ± .03 (mean ± S.E.M). As reported in that study, the data represented a tendency toward a decline in ratio time or an increase in anxiety-like behavior in these rats. Of interest is the fact the neither of these times are statistically different than the Test 1 ratio times for below mean split animals in the current study (mean ± S.E.M., 32 ± .04). Had there been an ability to split the animals into low and high baseline groups, there may have been an increase in ratio time found for these groups as was seen in the current study. Further research on the right BLA, using groups with various baselines of anxiety-like behavior, is required to draw firm conclusions regarding the role of baseline anxiety and kindling location in behavioral outcomes.

4.3.2. The impact of baseline anxiety on behavioral changes in left BLA kindled rats.

The increase in anxiety-like behavior that resulted in above split rats following left BLA kindling in the current study differed from the decrease in anxiety-like behavior observed following left BLA kindling in Adamec and Morgan [19]. It is important to note however, that controls in left BLA kindled rats in Adamec and Morgan had low ratio times (.21) which could explain why left BLA kindling in that study resulted in an increase in ratio time (an anxiolytic effect).

Results from this study confirmed that baseline anxiety is important across

hemispheres as evidenced by no significant difference between kindling electrode locations or kindling parameters. When comparing the current study to Adamec and Morgan [19] there was one slight difference found. As with the right BLA, the left BLA electrodes in the Adamec and Morgan study were lower on the vertical plane (deeper) than left BLA electrodes in the current study ($t\{11\} = 3.93$, $p<.01$). Given this slight electrode location difference and the differential findings between studies, it is difficult to generalize the findings of the current study to those in Adamec and Morgan. Further research on the left BLA, using groups with various baselines of anxiety-like behavior, is required to draw concrete conclusions regarding the role of baseline anxiety and kindling location in subsequent behavioral outcomes.

4.3.3 Stability of plus maze behavior on retest

Many studies have reported an increase in open arm avoidance in the elevated plus maze when retesting occurs within one week of the original test [44, 46, 85]. This is typically measured as a decrease in ratio time, i.e. less time spent in the open arms. Previous research from this laboratory has shown that this change can be prevented if the retest occurs at an interval of three weeks and in a different room [Experiment 2, 27]. The current study replicated these findings, as there was no increase in open arm avoidance observed upon retest. In fact, in Experiment 3.1 there was actually an increase in open arm exploration in rats for Test 2 over that which was seen in Test 1 in Below Split rats. Of importance is the fact that effects of kindling on anxiety-like behavior were confirmed through a comparison of controls to kindled animals on Test 2.

Based on the above results, further investigation was carried out on increased open arm exploration across tests in Experiment 3.1 to determine if there might be a baseline effect. The first focus for further assessment was to look at the differences in control groups. The below split control animals in Experiment 3.1, that increased their ratio times on Test 2, had mean baseline ratio times of 0.24. This score is significantly less than the baseline ratio time mean score for control rats below the split in Experiment 2 which did not rise on retest [(0.40), $t\{32\} = 2.35$, $p<.03$]. The findings suggest that animals with Test 1 ratio times around 0.4-0.6 show stability on retest, whereas animals with Test 1 ratio times of 0.24 or below show an increase in open arm exploration. These findings are consistent with the theory that retest reductions in ratio time with short inter-test intervals may be demonstrating habituation of exploratory motivation on retest which can be prevented by increasing the interval and retesting in a novel room [Experiment 2]. The increased open arm exploration levels of rats with low initial baseline ratio times suggest that these animals are also habituating to the fear provoking properties of the maze. Thus, a novel room combined with habituation of fear may be leading to the increase in open arm exploration on retest.

4.3.4 *Differential nature of the effects of kindling*

The current study confirmed previous findings whereby changes in open-arm exploration in the plus maze are not explained by changes in overall activity or exploration in either the hole board or plus maze [21, 22, 19]. There were changes in activity (rearing) found in the hole board, however these were found to either be a test effect (right BLA kindling) or a diminished reduction in activity normally induced by a retest (left BLA, above split animals). In left BLA animals kindling actually led to an increase in activity relative to controls, yet ratio time was reduced by kindling. This further supports the notion that activity level is not a determining factor in ratio time.

4.3.5 *Effects of kindling on resistance to capture*

Kindling decreased resistance to capture in Above Split right BLA kindled rats. This decrease occurred in animals that also exhibited an increase in anxiety-like behavior in the plus maze. If these animals are more anxious, then the decreased resistance to capture may be a demonstration of defensive immobility. The fact that left BLA kindled rats did not have a change in resistance to capture on Test 2 may be partially explained by the low resistance to capture score overall for these animals. The levels found for left BLA kindled rats started at a level comparable to the level where right BLA kindled rats decreased to (see figures 3.2 and 3.4). The results found in this study for both left and right BLA differ from increased resistance to capture scores found in previous studies using hooded rats [57, 58]. However, these studies differed in several ways including strain of rat used and kindling procedure which may account for the differential results.

4.3.6 Relation of baseline anxiety and kindling to risk

Risk assessment increased in all groups of right BLA kindled animals regardless of baseline anxiety level. Kindling in the left BLA did not have any effect on risk assessment. Of particular importance is the fact that kindling in both hemispheres led to different changes or lack of changes in risk assessment than it did for ratio time.

These findings are consistent with other studies which have reported that ratio time and risk assessment are changed by kindling and stress independently of one another Experiment 1,16]. The findings are also consistent with previous results from this laboratory which suggest changes in ratio time and risk assessment are controlled by separate neural substrates [16].

The determination of substrates impacted by kindling which moderate changes in risk assessment is difficult. Varying results have been found. However, the hemisphere and amygdala nucleus kindled appear to be important determinants in predicting change. Kindling of the right anterior central amygdala was found to increase risk assessment without an effect on ratio time [Experiment 1]. Kindling of posterior right central amygdala decreases both risk assessment and ratio time, and kindling of the mid central amygdala did not have an effect on risk assessment or ratio time [Experiment 1]. The results of the current study, while targeting the same nuclei, differed from the results found in Adamec and Morgan [19]. However, as previously mentioned this may be explained by the differences in electrode locations.

5.0 Conclusion

5.1 Experiment 1

Limbic system epilepsy is commonly accompanied by increased anxiety in humans [5]. Additional studies have found that these anxiety increases in epilepsy, as well as those found in other anxiety disorders, involve the amygdala [55, 66]. Previous studies from this laboratory and others [5,7,12,18,19,53,59] involving cats and rats have found that kindling of the amygdala leads to long-term changes in anxiety-like behavior. The results from Experiment 1 support the use of amygdala kindling as a model for affective changes in epilepsy, and also go further in extending the findings to additional areas of the amygdala. In general, kindling of more anterior foci of the central nucleus were found to decrease anxiety-like behavior, kindling of more posterior foci was anxiogenic and kindling of the nucleus basalis was found to be anxiolytic.

These findings also offer additional considerations with respect to the role of the behavioral measure in determining or predicting a behavioral outcome. Different behavioral measures assessed in the plus maze changed differently depending on the amygdala area kindled and therefore the measure itself, not just the electrode location, is important when considering behavioral outcomes. While the link between open arm measures in the plus maze and human anxiety cannot be stated for certain, present findings suggest that the location of the limbic epileptic focus in humans may be important to the affective change experienced. Similar findings have been reported in the cat [7, 9].

5.2 Experiment 2

The results in Experiment 2 added further evidence concerning the importance of the nucleus kindled in effects on risk assessment. This study went further to suggest that baseline anxiety levels of the animals, that is open-arm exploration and closed arm entries, interact with kindling to lead to the final behavioral outcomes. This impact of baseline anxiety-like behavior may in fact explain some of the inconsistencies found in previous kindling studies [5,24].

5.3 Experiment 3

The results of Experiment 3 confirmed the importance of baseline anxiety level on how kindling changes behavior in the elevated plus maze. The study also showed that kindling in the right BLA can lead to different behavioral effects depending on the baseline anxiety measured in the elevated plus maze. While the same results were not as evident in the left BLA, they may in fact be similar when current and past study results are taken into account. Further studies in the BLA and other amygdala nuclei will assist in further defining the contributions of both electrode location and baseline anxiety in the behavioral response to kindling in rats.

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