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The Effect of Kindling of Different Nuclei in the Left and Right Amygdala on Anxiety in the Rat

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ADAMEC, R. E. AND H. D. MORGAN. *The effect of kindling of different nuclei in the left and right amygdala on anxiety in the rat.* PHYSIOL BEHAV **55**(1) 1-12, 1994.—The effects on rodent anxiety of kindling in the medial or basolateral amygdaloid nuclei in each hemisphere were examined. Anxiety was measured using the hole board and elevated plus maze tests. The animals were kindled in medial or basolateral amygdalas, of either the left or right hemisphere. Controls had electrodes implanted in comparable areas, but were not kindled. Analysis of electrode location showed that some animals were kindled in amygdaloid nuclei other than medial or basolateral amygdala. These animals were labelled outliers. Kindling of the medial/basolateral amygdala in the left hemisphere decreased anxiety for at least 1 week after the last kindled seizure. Right hemisphere medial/basolateral kindling tended to increase anxiety. Outlier-kindled rats were less anxious than their controls regardless of hemisphere 1 week after their last kindled seizure. Clear anxiogenic effects were not likely seen in the right hemisphere in this study because of the electrode locations. The degree of anxiety following kindling was correlated with electrode location in the anterior-posterior plane. More anterior foci in the amygdala were associated with more anxiety. More posterior amygdala foci were associated with less anxiety. These findings point to the importance of kindled focus in the amygdala for behavioral effect. Future research should carefully control the location of kindled foci when investigating effects of amygdala kindling on anxiety and other behaviors.

Anxiety Elevated plus maze Kindling Laterality Medial/basolateral amygdala Medial amygdala Rat

A variety of clinical evidence indicates that limbic epilepsy predisposes human epileptics to anxiety and depression (3,33,51). Moreover, it has been suggested that repeated activation of the limbic system by seizure activity leads to emotional changes in human epileptics (3).

Animal models of epilepsy support the idea that repeated seizure activity in the limbic system changes emotional responding. Henke and Sullivan (32) found that kindling of the centromedial amygdala of the right hemisphere of Wistar rats increases restraint stress-induced stomach ulceration. Adamec (3) then reported that kindling of this area of the amygdala in Wistar rats increases anxiety measured in the elevated plus maze 1 week after the completion of kindling. It was later shown that kindling of the left basolateral amygdala increases anxiety in the elevated plus maze for 2 weeks after kindling (37).

Analogous findings have been reported in cats. Partial kindling of the amygdala or ventral hippocampus permanently increases the fearful response of cats to species characteristic threat (1,2,7). Kindling of the amygdala in cats lastingly decreases the threshold to elicit feline defensive responses by stimulation of the periaqueductal gray (PAG) (35). Partial epilepsy, induced by kainic acid injections into the cat dorsal hippocampus, greatly increases defensive rage in response to touch, and lowers threshold for eliciting defensive response by electrical stimulation of the hypothalamus (31). Moreover, fearful response in felines may model human anxiety states (3,6,8).

Together, these findings suggest that experimental limbic epilepsy increases anxiety interictally, and alters response to stress.

The amygdala seems to be important in interictal increases in anxiety in both animals and humans. Hermann et al. (34) report that anterior temporal lobectomy, which removed the amygdala in human temporal lobe epileptics, reduces anxiety in these patients. This finding is consistent with studies showing that electrical activation of the human amygdala elicits fear (28). In animal studies where the experimental seizure focus was in the dorsal or ventral hippocampus, the behavioral changes appear to be dependent on both spread of seizure activity into the amygdala and on interictal changes of excitability in the amygdala and its efferent pathways (7,9,12,31).

In addition, there is evidence for a functional differentiation of the amygdala in emotional expression in humans and animals. In humans, the medial amygdala is implicated in fear-based uncontrolled aggression, while the lateral amygdala has an opposite, calming action (49). Analogous findings have been reported in the cat and rat. In the cat, Egger and Flynn (23) showed that electrical stimulation of the feline medial amygdala suppressed electrically elicited predatory attack behavior. Stoddard-Apter

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and MacDonnell (50) later showed that medial amygdala stimulation also facilitated electrically elicited defensive behavior in the cat. Adamec (1) found that partial kindling of the medial amygdala lastingly increased fearful response of cats to natural threats. Siegel (48) then showed that repeated seizures in the medial amygdala raised the threshold for electrically eliciting predatory attack, but reduced the threshold for eliciting defensive behavior. Hiyoshi, Matsuda, and Wada (35) recently demonstrated a long-lasting reduction in threshold to electrically elicit defensive behavior from the PAG following medial amygdala kindling in the cat. In contrast, lateral amygdala stimulation and seizures in cats have behavioral effects opposite to medial amygdala stimulation and seizure activity (5,23,48).

A large body of evidence implicates the rodent centromedial amygdala in mediating fearful response to environmental stressors [see (21) for review]. Moreover, kindling of the right medial amygdala is anxiogenic in the elevated plus maze (4,10). In contrast, kindling of the right lateral amygdala is anxiolytic in punished responding tests (55). This finding is consistent with other evidence implicating the lateral amygdala in anxiolytic action of the benzodiazepines (53).

It is curious, however, that right basolateral amygdala kindling is anxiolytic (55), while kindling of the rodent left basolateral amygdala is anxiogenic (37). There are a number of possible explanations. First, different behavioral tests of anxiety were used, i.e., suppression of punished responding (55) as opposed to the elevated plus maze (37). These two tests do not always yield the same patterns of response to the same treatments (11). Second, kindling was done in different hemispheres. It is possible that kindling of the same amygdala nuclei in the left and right hemisphere has different effects on interictal anxiety. There is evidence for hemispheric lateralization of anxiety changes in temporal lobe epileptics (41,42) and nonepileptics suffering from anxiety disorders (45,46). Third, the differences might be due to subtle differences in location of the kindled focus. The studies cited do not give enough detail about the location of the kindling electrodes to decide if differences in electrode placements account for the different behavioral effects. Recent work indicates that small difference in location of electrodes can result in anxiogenic or anxiolytic effects following kindling (4,10).

Taken together, the data suggest surprising cross-species commonality of functioning of the medial amygdala in pathologic anxiety-like states. The data also point to the importance of the amygdala in seizure-induced increases in anxiety that outlast the seizure discharge by 1 week to many months. Nevertheless, given the discrepancies in the literature, there is a need to explore in more detail the role of different amygdala nuclei of both hemispheres in seizure-induced potentiation of anxiety. The present study was designed to map the contribution of different amygdala nuclei in the rat to changes in anxiety produced by amygdala kindling.

METHOD

Subjects

One hundred and forty-four male Wistar rats (Charles River Canada) weighing between 200 and 250 g at the beginning of the experiment were used. Rats were housed individually in transparent plastic cages on racks holding 15 cages in the same holding room. They were maintained on a 12-h light–dark cycle (lights on at 0700 h). Water and rat chow were always available.

Experimental Groups

After adaptation to the laboratory, rats were randomly assigned to one of eight groups, which were a combination of three conditions: medial/basolateral amygdala, left/right hemisphere, and kindling/no kindling. All animals were implanted with stimulating electrodes; however, only half were kindled. Group 1 consisted of animals kindled in the right medial amygdala. Group 2 was kindled in the right basolateral amygdala. Groups 3 and 4 were kindled in the left medial amygdala and the left basolateral amygdala. Groups 5–8 were implanted, but unkindled, controls with electrodes in comparable areas.

Because of the large total number of subjects, the study was done in stages. Each stage involved 16–40 animals. In each replication, rats were randomly assigned to one of the eight groups.

Handling

All rats were handled in the rat holding room for 3 days before surgery. Handling involved picking up the rat from its home cage with a gloved hand. The rat was gently restrained around the shoulders, while using one arm as a platform on which the rat could rest its feet. When the rat struggled or tried to escape, the grip was tightened to keep the rat still. When the rat was immobile, the grip was relaxed. Rats were held this way for 1 min and then returned to their home cages. Handling continued every other day after surgery, up to the day of adaptation to the kindling apparatus.

Surgical Procedures

Surgery was performed, aseptically, under sodium pentobarbital anaesthesia (60 mg/kg, IP). Twisted bipolar stainless steel stimulating electrodes (0.125 mm in diameter, Plastics One) were implanted in the rats (according to group assignment) using stereotaxic technique.

Coordinates for medial amygdala placement were taken from Adamec (4). They were: 0.6 mm posterior to bregma, 4.0 mm lateral to midline, 8.6 mm ventral to dura, tooth bar elevated 5 mm above the horizontal [according to the atlas of Pellegrino et al. (39)]. Coordinates for basolateral amygdala placement were taken from Witkin et al. (55). They were: 2.2 mm posterior to bregma, 4.7 mm lateral to the midline, 6.7 mm ventral to the dura, skull flat [according to the atlas of Paxinos and Watson (38)].

Wound edges were locally anaesthetized during surgery with lidocaine (2%) infusion. The skull and wound edges were sprayed with antibiotic (Neosporin antibiotic spray) before closing. Electrodes were fixed in place with dental acrylic cement secured to the skull with four stainless steel skull screws. Following surgery, rats were given 10 mg of chloramphenicol, SC. Rats were allowed 1 week of recovery from surgery.

Kindling

Two days before kindling, all rats were adapted to the kindling apparatus. On each day, rats were placed for 10 min in the boxes in which they would be kindled. They were connected to the electrode leads for an additional 10 min. Then they were disconnected and returned to their home cages.

Rats in the kindled groups were stimulated twice per day with at least 3 h between stimulations. The first stimulation occurred between 0900 and 1100 h. The second stimulation was given between 1400 and 1600 h. Stimulation consisted of 400 μA peak-to-peak constant current square wave pulses of 1 ms pulse width delivered in a train at 62.5 pulses per second. Train duration was set to 1 s for the first two stimulations; it was increased to 2 s for the remaining stimulations. Stimulus intensity was kept constant, though in some instances it was increased to as much as 800 μA peak-to-peak in a 3-s train. Stimulus param-

eters were changed in this way if rats failed to show stage 1 seizures after five stimulations. The effect of these manipulations on current passed in the different groups was analyzed as described later. Stimulation was repeated until a rat showed three stage 5 convulsions outlasting the stimulus, as defined by Racine (43). Then kindled rats were left alone in their home cages for 1–4 days to allow other rats in the group to achieve three stage 5 seizures. Then a fourth stage 5 seizure was triggered in all kindled group rats and the rats were left unhandled for 1 week. At the end of the week, the behavior of all rats was tested.

Implanted controls were treated like kindled animals except they were not stimulated.

Behavior Testing

The elevated plus maze test of anxiety was chosen because it is a simple and relatively motivation artifact-free test of rodent anxiety (18). This test has been pharmacologically validated as a model of benzodiazepine-sensitive anxiety (40). The test measures strength of antagonism between exploratory tendency and avoidance of open novel spaces (18).

All rats were tested once in the elevated plus maze and hole board between 0930 and 1700 h. Behavior of the rats was videotaped. Reactions of the rats to the novel hole board apparatus were examined first. The hole board provided independent measures of activity and exploratory tendencies that might influence behavior in the plus maze (24,25). The hole board was a square wooden box, 60 cm on a side, with four sides rising 35 cm above the floor of the box. There were four evenly spaced holes drilled in the floor of the box. The floor of the box was elevated 12 cm above the ground. The holes were drilled at the corners of a square drawn inside the box, with sides 14 cm from the walls of the hole board. The box was painted with flat grey enamel paint. Rats were placed in the center of the hole board and videotaped for 5 min.

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

Several behavioral measures were taken in the hole board test. Activity was measured as time spent in motion and frequency of rearing (24). Exploratory tendency was measured as head dipping [defined as placing the snout or head into a hole in the hole board (25)]. Finally, number of faecal boli left in the hole board were counted.

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

Ratio time was the measure of anxiety in the elevated plus maze. Ratio time was the ratio of the time spent in the open arms divided by the time spent in both arms of the maze. A rat was considered to have entered an arm of the maze when all four feet were within the arm. The smaller the ratio time, the more anxious the rat (24).

Another behavioral measure taken in the plus maze was risk assessment. Risk assessment was measured when the rats poked their heads out into the open arm, but did not enter it. Both time and frequency of this behavior were measured. Then the data were divided by the time spent in the closed arm of the maze. This created ratio risk measures. A ratio was taken because rats cannot engage in risk assessment unless they are initially in the enclosed arm of the maze. Changes in risk assessment could be due to increased opportunity to perform the behavior, rather than an actual increase in risk assessment. Taking this ratio controls for this possibility. Risk assessment was originally defined and investigated as a measure of rodent anxiety by Blanchard and Blanchard (16).

Histology

At the end of the experiment, rats were deeply anaesthetized with Somnotol. A small anodal lesion was made through the electrodes to deposit metallic ions (2.0 mA for 2 s passed between the tips of the electrodes). Rats were then perfused transcardially with 10% formalin and 1% potassium ferrocyanide in phosphate-buffered saline and the brains removed. The potassium ferrocyanide reacts with the metallic ions to produce a blue dot marking the tip of the electrodes. Frozen sections (37 μM) were taken through the electrode tracks, and the tissue was mounted and stained with metachromatic cresyl violet and stained for acetylcholinesterase. Since the basolateral amygdala stains darkly for acetylcholinesterase, the latter stain permitted more precise localization of basolateral amygdala electrodes.

Stereotaxic coordinates of tip location were found with the aid of an image analyzer. The rat brain section being analyzed was normalized to the corresponding atlas section. Normalizing factors were found by dividing the width of the rat section being examined by the width of the same cross section through the atlas. The vertical and lateral position of the electrode tip measured in the section was multiplied by this factor. These normalized coordinates were recorded and plotted on rat atlas sections.

Animals were sorted into groups with electrodes in the same anatomical location, according to Paxinos and Watson (38) and Pellegrino et al. (39) atlases. Rats whose electrodes fell within the medial and basolateral amygdala were called on target rats. Rats with electrodes in neither the medial or basolateral amygdala were labelled outliers.

Statistical Analysis

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

Three-way ANOVA was performed on animals with a medial or basolateral (amygdala) focus of stimulation. The independent variables of this analysis were left/right hemisphere, medial/basolateral nucleus, and kindled/not kindled.

Behavior from outliers was analyzed separately. Three-way ANOVA was used. The animals were grouped according to their original medial/basolateral and hemisphere assignments for this analysis. Independent variables were medial/basolateral amygdala, left/right hemisphere, and kindled/not kindled.

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

Finally, the total amount of charge (in micro coulombs, μC) that the animal received during kindling was calculated. Differences between groups of on target and outlier rats were assessed

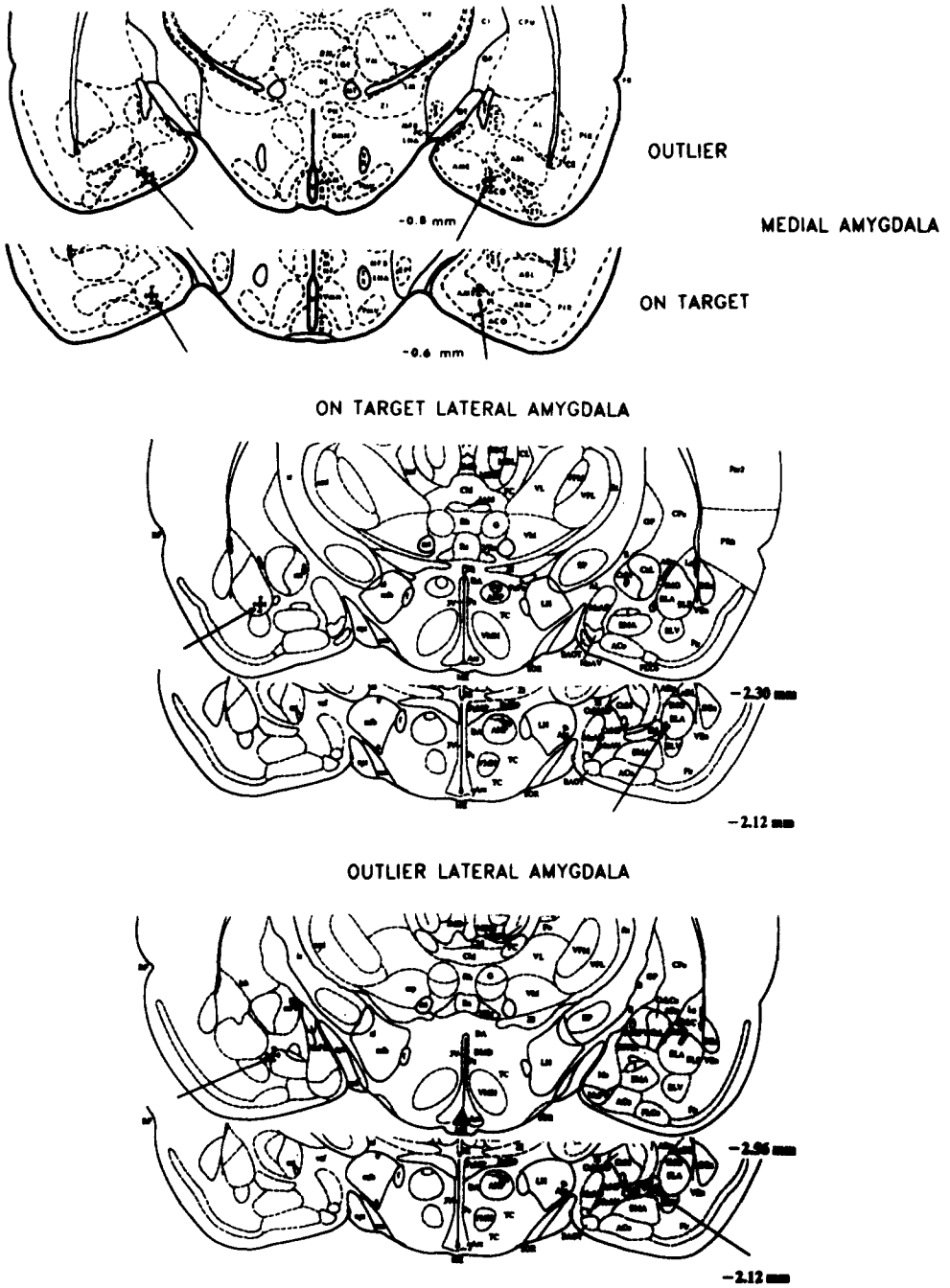

FIG. 1. Mean electrode locations averaged over kindled and control rats ± SEM in the lateral and vertical planes are projected onto rat atlas sections. Sections are taken from the Pellegrino et al. atlas (38) for medial amygdala groups, and from the Paxinos and Watson atlas (39) for basolateral amygdala groups. Locations in the left hemisphere are plotted on the left side of the plates. Right hemisphere placements appear on the right side of the plates. Placements are pointed to by arrows. Electrode locations for medial/basolateral, on target, and outlier groups are plotted separately. Anterior–posterior plane postion (AP, posterior to bregma) is labelled for each section, and is the nearest section to the group average plotted. See Table 1 for the actual mean locations in the AP plane. Anatomical abbreviations are as follows: AAA, anterior amygdala; ABL, lateral basal amygdala; ABM, medial basal amygdala; ACE, central amygdala; ACO, cortical amygdala; AL, lateral amygdala; AME (ME), medial amygdala; BLA, anterior basolateral amygdala; BLP, posterior basolateral amygdala; BLV, ventral basolateral amygdala; BM, basomedial amygdala; BST, bed nucleus of the stria terminalis; BSTIA, bed nucleus of the stria terminalis (intra-amygdaloid division); CLA, claustrum; CE, external capsule; CEL, lateral central amygdala; CELCN, central lateral central amygdala; CEM, medial central amygdala; CEMPV, medial posteroventral central amygdala; CPU, caudate putamen; HPC, hippocampus; IM, intercalated amygdaloid nucleus; MEPD, posterodorsal medial amygdala; MEPV, posteroventral medial amygdala; PIR, piriform cortex; ZT, transition zone.

TABLE 1

COORDINATES OF ELECTRODE PLACEMENTS AND NUMBERS OF ANIMALS IN EACH GROUP

Coordinates of Electrode Placement*

	Hemisphere	
	Left	Right
Posterior to bregma		
On target medial†	0.68 ± 0.06	0.69 ± 0.07
On target basolateral	2.37 ± 0.08	2.13 ± 0.06
Outlier medial	0.85 ± 0.20	0.80 ± 0.16
Outlier basolateral	2.58 ± 0.21	2.19 ± 0.19
Lateral to midline		
On target medial	3.86 ± 0.08	3.89 ± 0.09
On target basolateral	4.68 ± 0.10	4.82 ± 0.08
Outlier medial	3.95 ± 0.13	4.31 ± 0.10
Outlier basolateral	4.42 ± 0.13	4.55 ± 0.12
Ventral to dura		
On target medial	9.57 ± 0.14	9.69 ± 0.10
On target basolateral	8.90 ± 0.11	8.68 ± 0.09
Outlier medial	9.75 ± 0.15	9.70 ± 0.12
Outlier basolateral	8.79 ± 0.15	8.60 ± 0.14

Numbers of Animals in Each Group

	Hemisphere	
	Left	Right
Kindled		
Medial	16 [6]‡	8 [12]
Basolateral	6 [7]	8 [11]
Not kindled		
Medial	11 [9]	8 [13]
Basolateral	7 [8]	13 [7]

* Kindled and control rats are combined, since they do not differ.

† Mean ± SEM of coordinates of electrode location in mm. AP and vertical coordinates are references to the Pellegrino et al. (39) atlas for medial and to the Paxinos and Watson (38) atlas for the basolateral on target and outlier groups.

‡ The number of on target animals are listed in each cell and outliers are listed in brackets.

using two-way ANOVA. The independent variables were hemisphere and amygdala nucleus.

RESULTS

Electrode Locations

Mean location of all electrodes appear in Fig. 1 and are listed in Table 1.

Effect of Kindling of the Medial and Basolateral Amygdala on Anxiety in the Plus Maze

Kindling of the medial or basolateral amygdala in the left hemisphere was anxiolytic. Kindling of the medial or basolateral amygdala of the right hemisphere tended to be anxiogenic [kindling × hemisphere interaction, ratio time, $F(1, 63) = 5.85$, $p < 0.02$] (Fig. 2). There were no medial/basolateral differences or interactions. Animals kindled in the left medial or basolateral amygdala spent more time in the open arms than the left hemisphere-implanted controls, $t(82) = 2.05$, $p < 0.02$. In contrast, rats kindled in the right hemisphere tended to spend less time in the open arms than right hemisphere controls, $t(63) = 1.40$, $p < 0.09$.

In addition, controls with electrodes in the right and left hemisphere differed from each other (Fig. 2). Left hemisphere controls were more anxious (smaller ratio time) than right hemisphere controls (Duncan's Test, $p < 0.05$).

Effects of Kindling of the Medial and Basolateral Amygdala on Risk Assessment in the Plus Maze

Kindling affected both ratio risk measures [kindling × hemisphere interaction, log transformation to normalize the data, $F(1, 63) = 4.62, 7.98$, $p < 0.04$ for time and frequency risk, respectively] (Fig. 3). Left hemisphere kindling did not affect ratio time risk. Right hemisphere kindling reduced ratio time risk, $t(63) = 2.09$, $p < 0.04$, and ratio frequency risk, $t(63) = 2.09$, $p < 0.04$. In addition, left hemisphere kindling tended to increase ratio frequency risk, $t(63) = 1.99$, $p < 0.03$ (one-tailed test).

The pattern of changes in risk assessment resemble the changes in ratio time (Fig. 1). This suggests both may be measuring similar phenomena. If true, removal of the effects of ratio risk from ratio time should eliminate the effects of kindling.

To test this possibility, two three-way ANCOVA were done on ratio time in the plus maze using ratio time risk and ratio frequency risk as covariates. The independent variables were: kindling, medial/basolateral placement, and hemisphere.

When ratio time risk was a covariate, the kindling × hemisphere interaction originally observed was preserved, $F(1, 62) = 4.18$, $p < 0.05$. Mean contrasts reveled an anxiolytic effect of left hemisphere kindling like that seen originally [$t(62) = 5.74$, $p < 0.01$; 0.21 ± 0.04 vs. 0.33 ± 0.04 mean ratio time ± SEM for left hemisphere control and kindled groups, respectively]. In contrast, the original trend toward an anxiogenic effect of right hemisphere kindling had disappeared [$t(62) = 0.67$, $p > 0.05$;

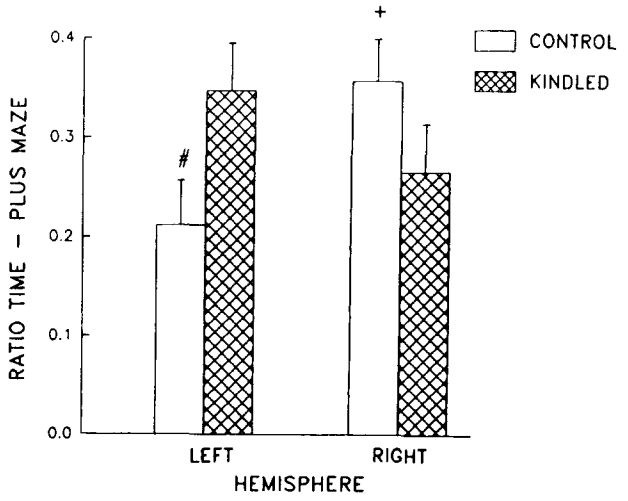

FIG. 2. Plotted is mean ± SEM ratio time in the elevated plus maze for rats with electrodes verified to be within the medial (medial) or basolateral (lateral) amygdala. Means are collapsed over amygdala nucleus and plotted separately for kindled and control animals with electrodes in the right and left hemisphere. Marked control means differ from unmarked kindled means. The right control mean marked with a + tends to differ from the right hemisphere kindled group ($p < 0.09$).

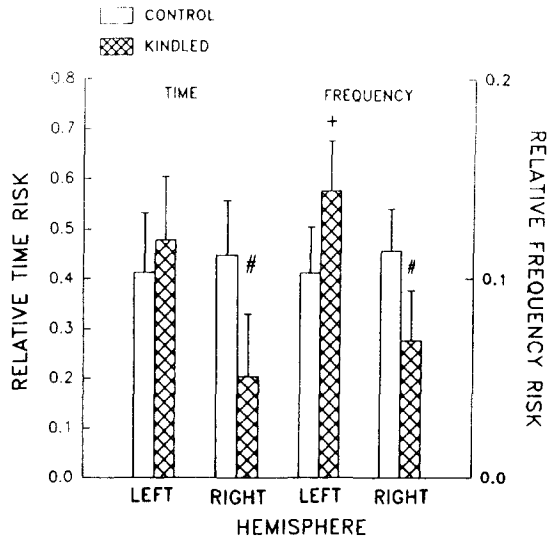

FIG. 3. Plotted are mean ± SEM relative risk measures for rats with electrodes verified to be within the medial (medial) or basolateral (lateral) amygdala. Means are collapsed over medial/basolateral amygdala placements and are plotted separately for hemisphere, and kindled and control groups. Relative time risk is plotted in the leftmost Left/Right hemisphere set of four bar graphs, and is referenced to the left ordinate. Relative frequency risk is plotted in the rightmost Left/Right hemisphere set of four bar graphs, and is referenced to the right ordinate. In the plot of ratio time risk, the mean marked with the # differs from all other means, which do not differ. In the ratio frequency risk plot, the mean marked with a # differs from the right control, and the mean marked with a + tends to differ from the left control. See text for mean contrast data.

0.34 ± 0.03 vs. 0.31 ± 0.04 mean ratio time ± SEM for right hemisphere control and kindled groups, respectively].

When ratio frequency risk was a covariate, the effect of kindling on ratio time disappeared [$F(1, 62) = 2.57$, $p > 0.05$, kindling × hemisphere interaction]. Nor were there any other kindling effects or interactions.

TABLE 2

KINDLING PARAMETERS

Electrode Location	Number of Stimulations to Stage 5 Seizure	Duration (s)	Pause (days)
Medial/lateral	(see Fig. 5)	64.13 ± 4.7	4.21 ± 0.3
Outliers	11.58 ± 0.7	58.74 ± 4.7	3.41 ± 0.3
		Average Current Passed During Kindling (μC)	
Medial/lateral		31.2 ± 4.7	
Outliers		38.6 ± 5.3	

Values are mean ± SEM of kindling parameters for medial/lateral and outlier amygdala rats.

Effects of Kindling of the Medial and Basolateral Amygdala on Behavior in the Hole Board

Animals kindled in the left basolateral amygdala head dipped more than all other groups, which did not differ [kindling by medial/basolateral placement × hemisphere interaction, $F(1, 63) = 4.23$, $p < 0.05$; Duncan's test, $p < 0.05$] (Fig. 4).

Analysis of Electrode Locations in Rats With Electrodes in the Medial and Basolateral Amygdala

Three-way ANOVA (with the same independent variables as above) was used to examine electrode placements in the three planes. Since medial and basolateral placement groups were compared, AP and vertical coordinates of basolateral amygdala groups [after Paxinos and Watson (38)] were adjusted trigonometrically to the coordinate system of the medial placements [Pellegrino et al. (39)]. The adjustment was to add 0.82 mm to the basolateral AP coordinate, and to multiply the vertical coordinate of basolateral animals by 0.948.

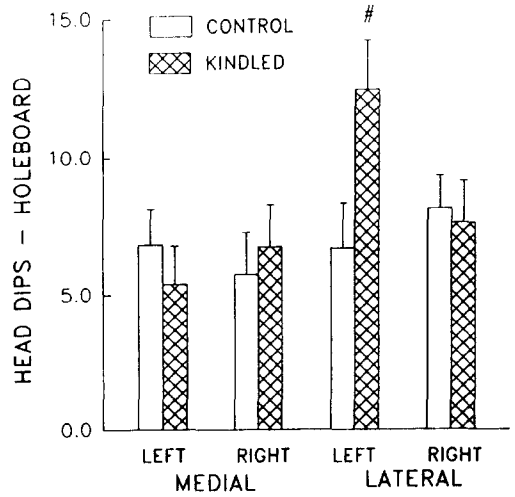

FIG. 4. Plotted is mean ± SEM frequency of head dipping observed in the hole board for rats with electrodes within the medial (medial) or basolateral (lateral) amygdala. The left lateral kindled mean marked with the # is larger than the all the other groups, which do not differ from each other (Duncan's test, $p < 0.05$).

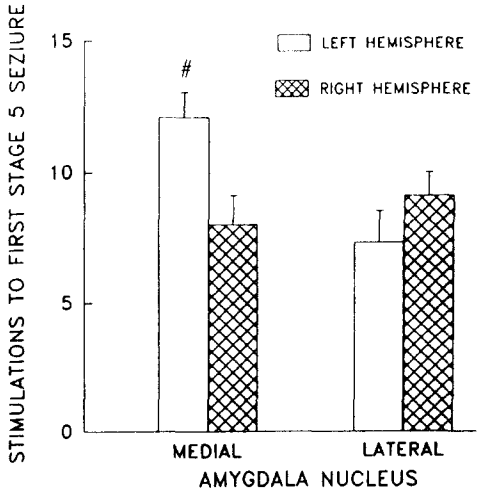

FIG. 5. Plotted are mean ± SEM of number of stimulations to the first stage 5 seizure for rats stimulated with electrodes within the medial (medial) or basolateral (lateral) amygdala. The left medial mean marked with a # is larger than all other groups, which do not differ from each other.

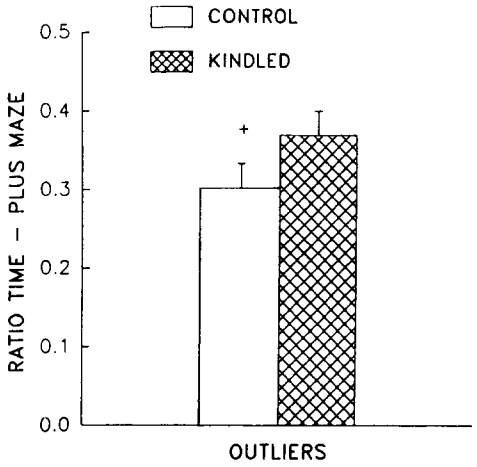

FIG. 6. Plotted is mean ± SEM for ratio time observed in the plus maze for outlier rats collapsed over medial/basolateral and hemisphere groupings. Means are for kindled and not kindled (control) animals. The mean for control animals marked with a + tends to be smaller than the mean for kindled animals.

Kindled rats did not differ from their implanted controls. Medial and basolateral amygdala groups differed in three planes. These differences were due to the different coordinates used.

In the AP plane, basolateral amygdala group placements were more posterior than medial amygdala group placements [medial/lateral effect, $F(1, 63) = 126.39$, $p < 0.01$; 1.43 ± 0.05 mm vs. 0.69 ± 0.05 mm, posterior to bregma, basolateral and medial animals, respectively].

In the lateral plane, basolateral amygdala group placements were more lateral to the midline than medial amygdala group placements [medial/lateral effect, $F(1, 63) = 105.83$, $p < 0.01$; 4.75 ± 0.06 mm vs. 3.88 ± 0.05 mm, basolateral and medial animals, respectively].

In the vertical plane, medial amygdala group placements were more ventral to the dura than basolateral amygdala group placements [medial/lateral effect, $F(1, 63) = 194.97$, $p < 0.01$; 8.34 ± 0.07 mm vs. 9.62 ± 0.06 mm, coordinates ventral to the dura for basolateral and medial placements, respectively].

Analysis of Kindling Parameters in Rats With Electrodes in the Medial and Basolateral Amygdala

Two-way ANOVAs were performed on kindling parameters (Table 2). Independent variables were medial/basolateral placements and hemisphere. The three parameters analyzed in stimulated animals were: the number of stimulations to the first stage 5 seizure, duration of the fourth stage 5 seizure, and current passed during kindling. A three-way ANOVA with kindling added as the third independent variable was used on the pause between the third and fourth stage 5 seizure.

Left medial animals required more stimulations to the first stage 5 seizure than all other groups, which did not differ [hemisphere × medial/basolateral interaction, $F(1, 27) = 7.27$, $p < 0.02$, Duncan's test, $p < 0.05$] (Fig. 5). There were no differences in either the duration of the last seizure or pause data.

Because there was a difference in number of stimulations to kindle, current passed during kindling was divided by the number of stimulations to yield average current passed per train. Kindled groups did not differ in average current passed (Table 2).

Effects of Kindling on Behavior of Rats With Electrodes in Neither the Medial or Basolateral Amygdala (Outliers)

Kindled outlier animals tended to be less anxious than controls [kindling effect only, $F(1, 63) = 3.18$, $p < 0.08$, or $t(63) = 1.78$, $p < 0.04$ comparing kindled and controls, square root transformation to normalize the data] (Fig. 6). Ratio time of kindled rats was greater than controls (one-tailed test).

Analysis of Kindling Parameters of Outliers

The different groups of outlier animals did not differ with respect to any kindling parameter (Table 2). Data in Table 2 are averaged over all outlier groups.

Effects of Kindling on Other Behavioral Measures

Kindling in the medial/basolateral amygdala or in outliers had no effect on several other behaviors in the plus maze and hole board (Table 3). These were: boli produced in the hole board and plus maze, activity (time active) in the hole board, and activity (total arm entries) in the plus maze. Kindling in outliers did not affect head dipping in the hole board or ratio risk measures.

Comparisons With Previous Findings

Previous work in this laboratory has shown that right medial amygdala kindling is anxiogenic in the elevated plus maze (4). In the present study there was only a trend. Previous work has also shown the importance of electrode location for behavioral outcome of kindling (10). So it was of interest to compare elec-

TABLE 3

BEHAVIORAL MEASURES UNCHANGED BY KINDLING

	Kindled	Control
Time active in the hole board		
Medial/lateral	294.47 ± 2.08	293.13 ± 1.95
Outliers	294.74 ± 0.83	296.91 ± 0.82
Head dipping in the hole board		
Medial/lateral*	—	—
Outliers	7.01 ± 0.64	7.58 ± 0.63
Ratio frequency of risk assessment in the plus maze		
Medial/lateral†	0.11 ± 0.01	0.11 ± 0.09
Outliers	0.13 ± 0.01	0.11 ± 0.01
Ratio time spent in risk assessment in the plus maze		
Medial/lateral	0.37 ± 0.07	0.43 ± 0.07
Outliers	0.41 ± 0.07	0.39 ± 0.07
Total entries into all arms of the plus maze		
Medial/lateral	13.04 ± 0.57	14.53 ± 0.61
Outliers	15.19 ± 0.62	14.13 ± 0.61

	Kindled		Control	
	Hole Board	Plus Maze	Hole Board	Plus Maze
Number of boli				
Medial/lateral	0.19 ± 0.15	0.19 ± 0.12	0.50 ± 0.14	0.42 ± 0.11
Outliers	0.64 ± 0.25	0.02 ± 0.10	0.71 ± 0.24	0.24 ± 0.10

Values are mean ± SEM.
* See Fig. 4 for head dipping data.
† See Fig. 3 for risk data.

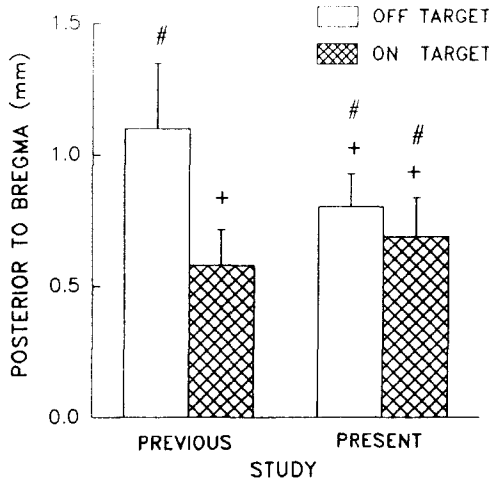

FIG. 7. Plotted are mean ± SEM anterior–posterior plane electrode coordinates in mm posterior to bregma for rats with electrodes aimed at the right medial amygdala in the present study and in a previous study (10). Means are collapsed over kindled and control groups and are plotted separately for the present and previous studies and for off target and on target groups. Means marked similarly do not differ, but differ from means marked with a different symbol. Means marked with two symbols fall between means marked with either symbol.

trode locations in right medial amygdala-kindled rats in the present and past studies.

A three-way ANOVA compared electrode locations from the previous study in this laboratory (10) with those of the present study. The independent variables considered were study (previous/present), kindled/not kindled, and on target/off target. Target sites in the previous study were defined as whether the electrode was in the right medial amygdala nucleus (on target) or not (off target). In the present study, data were taken from the right medial amygdala (on target) and right medial (outlier) rats.

There were differences between the two studies in location of kindling electrodes in their AP position [study × target interaction, $F(1, 56) = 4.28$, $p < 0.05$, log transform to normalize the data] (Fig. 7]. Electrodes of previous study on target animals were more anterior than previous study off target animals. Present study on and off target animals did not differ, and fell between the previous study on and off target animals (Duncan's test, $p < 0.05$).

There was a study × target interaction for lateral coordinates, $F(1, 57) = 8.71$, $p < 0.01$. Previous study off target electrodes were more medial than the present study off target electrodes (3.76 ± 0.17 mm vs. 4.34 ± 0.09 mm, previous vs. present study lateral coordinates, respectively; Duncan's test, $p < 0.05$). Previous and present study on target animals did not differ in the lateral plane.

The two studies also differed in the location of electrodes in the vertical plane. Electrodes of animals in the previous study were not as deep as those of rats in the present study [main study effect for the vertical plane, $F(1, 64) = 11.32$, $p < 0.01$; 9.15 ± 0.11 vs. 9.70 ± 0.09, below the dura for previous and present studies, respectively].

Correlations Between Kindling Electrode Location and Relative Anxiety

The relationship between location of kindling electrode and anxiety produced by kindling in the present study was examined by correlating electrode location and a score of relative anxiety. Relative anxiety was found by dividing ratio time of kindled rats by the average ratio time of the appropriate control group. For example, each right medial-kindled rat's ratio time score was divided by the mean ratio time of right medial not kindled rats. This was done because in some cases control groups differed from each other in anxiety. Correlations were done separately for left and right hemispheres.

Since both medial and basolateral placement groups were included in the correlations in a given hemisphere, AP and vertical coordinates of basolateral amygdala groups [after Paxinos and Watson (38)] were adjusted trigonometrically to the coordinate system of the medial placements [Pellegrino et al. (39)]. The adjustment was to add 0.82 mm to the basolateral AP coordinate, and to multiply the vertical coordinate of basolateral animals by 0.948.

Relative anxiety correlated with electrode location in the AP plane in the right hemisphere ($r = 0.509$, $p < 0.01$). More posterior kindled foci were associated with less anxiety (greater ratio time scores). In addition, deeper kindled foci in the right or left hemispheres produced more anxiety (lower ratio time scores) following kindling ($r = -0.876$, $p < 0.01$; $r = -0.362$, $p < 0.05$, right and left hemispheres, respectively) (Table 4). There was no correlation of AP plane with relative anxiety in the left hemisphere, however.

DISCUSSION

The Effect of Kindling on Anxiety

Kindling of the medial and basolateral amygdalas has similar effects on anxiety. The nature of that effect depends on the hemisphere, however. Kindling of the left medial or left basolateral amygdala was anxiolytic. Right medial or right basolateral amygdala kindling tended to be anxiogenic. Kindling of other than the medial or basolateral amygdala (outlier areas) tended to be anxiolytic regardless of hemisphere.

These effects may be considered selective to anxiety if changes in ratio time produced by kindling are independent of changes in exploratory tendency and activity. Kindling influenced exploration in the hole board, however. Left basolateral amygdala-kindled animals head dipped more than all other groups (which were equal). Head dipping is considered a measure of exploratory behavior (25). Increased exploratory behavior could explain the anxiolytic-like effects in the plus maze by increasing exploration of the open arms. However, if exploration of the plus maze were increased, one also would expect more entries into the arms of

TABLE 4

CORRELATIONS OF ELECTRODE COORDINATES AND RELATIVE ANXIETY (RANX)

	AP*	Lateral	Vertical
Right hemisphere			
RANX	0.509	0.050	−0.876
$p <$	0.001	NS	0.001
Left hemisphere			
RANX	0.053	0.062	−0.362
$p <$	NS	NS	0.05 (one-tailed)

* AP: anterior–posterior coordinates (in mm posterior to bregma); lateral: lateral coordinates (in mm from midline), vertical: vertical coordinates (in mm below the dura). AP and vertical coordinates were expressed relative to the Pellegrino atlas. NS = not significant.

the maze in kindled rats. This was not observed. Also, the pattern of results for head dipping is different from ratio time. Therefore, head dipping and the ratio time measure of anxiety are independent. Independence of head dipping and ratio time has been reported in previous studies of kindling and anxiety in rats (4,10).

Changes in activity cannot account for the effects of kindling on behavior in the plus maze. Kindling was without effect on rearing or time active in the hole board. Rearing is considered a measure of activity (25).

These data show that kindling effects on the ratio time measure of anxiety are not due to changes in activity or exploratory tendency. Rather they reflect a selective change in anxiety-like behavior measured by ratio time.

A body of evidence suggests that enhancement of normal limbic system functioning is responsible for interictal behavioral changes following kindling (3). If kindling does enhance the normal function of the focus, then the present findings suggest the following. The left medial/basolateral amygdalas mediate anxiolytic processes. The right, more anterior, medial/basolateral amygdalas mediate anxiogenic processes. Outlier nuclei of the amygdala may mediate anxiolytic processes.

However, these conclusions must be seen within the context of the interaction of kindling with effects of electrode damage on amygdala nucleus function.

Effects of Electrode Damage on Baseline Anxiety and Interaction With Kindling

Electrode damage to the amygdala per se may have affected anxiety. Differences in anxiety were found between on target control rats with electrodes in the medial/basolateral amygdalas of the left and right hemispheres. Left hemisphere control rats were more anxious than right hemisphere controls (Fig. 2). Therefore, electrode damage in the left hemisphere may be anxiogenic. Electrode damage in the right hemisphere could also have been anxiolytic. Without an unoperated control, it cannot be said for certain which is true.

It is unlikely that right hemisphere electrode damage was anxiolytic, however. Placing electrodes in the anterior right medial amygdala does not affect anxiety, whereas more posterior medial amygdala electrode damage is anxiogenic (10). The electrodes in question in the present study fell between the anterior and posterior medial placements in the previous study (10). Therefore, an anxiolytic effect of electrode damage in the right hemisphere in this study is not likely. It is more likely that the anxiety levels of right hemisphere on target control rats was unaffected by electrode damage.

Assuming that anxiety levels of right hemisphere control rats were unaffected by electrode damage, then the greater anxiety of left hemisphere control rats may be due to electrode damage. If true, then kindling of the left hemisphere reverses the anxiogenic effect of electrode damage. This also would explain why the left hemisphere-kindled rats are not less anxious than the right hemisphere controls (Fig. 2). This interpretation is also consistent with the view that the normal function of cell groups or fibers of passage in the left medial/basolateral amygdala is anxiolytic, and that kindling enhances that function. The right medial/basolateral amygdala data suggest a different hypothesis. Since damage to this area does not likely affect anxiety levels, the trends toward changes in behavior produced by kindling of these areas may be due to changes distal to the kindled focus. Right medial/basolateral kindling may selectively alter particular output pathways. The same may apply to outlier rats. Outlier controls do not differ from right medial/basolateral controls in ratio time (see Figs. 2 and 6). So kindling of outliers, which tends to be anxiolytic, likely does not reverse electrode damage. Rather, activation of outputs from outlier areas may promote anxiolytic processes. The issue of selective alteration of output pathways by kindling is discussed further below.

A tentative picture emerges from these data. Depending on the area of the amygdala, electrode damage either has no effect on baseline anxiety (right medial/basolateral, outlier) or it is anxiogenic (left medial/basolateral). When there are effects of electrode damage, kindling may restore lost function by enhancing normal function of undamaged amygdala tissue (left medial/basolateral amygdala). Kindling may also produce its behavioral effects by altering pathways efferent to the kindled focus (right medial/basolateral amygdala and outlier areas).

These conclusions are based on the assumption that electrode damage in right medial control rats did not affect anxiety level. This study should be replicated with unoperated controls to test these hypotheses.

Possible Mechanisms of Altered Amygdala Excitability Following Kindling

The enhancement of amygdala functioning by kindling suggests some increase in amygdala neural excitability mediates behavioral changes. Kindling may change behavior in some cases by altering GABA functioning in the amygdala. Basolateral amygdala kindling lastingly decreases GABAergic inhibitory postsynaptic potentials contralateral to the kindling site (27). Loss of inhibition in the basolateral amygdala is accompanied by an increase in NMDA-dependent excitability in that nucleus. Reduced inhibition and increased excitability of amygdala cells in different nuclei could mediate some or all of the changes in anxiety following kindling.

Comparisons of the Effects of Kindling of Different Foci on Anxiety in This and Previous Studies

The present findings are both consistent and inconsistent with previous work. Adamec (4) and Henke and Sullivan (32) found that kindling the right medial amygdala produced anxiogenic effects and increased susceptibility to stress ulcers. The tendency for kindling of the right medial amygdala to be anxiogenic in this study is consistent with these findings.

Adamec and McKay (10) found that kindling in the more posterior medial amygdala in the vicinity of the cortical nucleus in the right hemisphere was anxiolytic. This finding was replicated in part in this study in the right hemisphere, and extended to the left hemisphere. Kindling in right and left outlier rats tended to be anxiolytic. That is, the effect on ratio time was only significant with a one-tailed probability. On the other hand, the location of electrodes of right medial outlier rats (see Fig. 1) is nearly identical to a site the kindling of which was recently shown to be anxiolytic in the plus maze (10). Since kindling of comparable amygdala nuclei had similar effects, it is likely that outlier kindling was anxiolytic in the present study.

Despite these consistencies, there are several inconsistencies. Nieminen et al. (37) reported an anxiogenic effect of left basolateral amygdala kindling. In contrast, kindling of the left basolateral amygdala was anxiolytic in the present study. Also, Witkin et al. (55) observed that right hemisphere basolateral amygdala kindling was anxiolytic. This report is inconsistent with the present findings that left (and not right) basolateral amygdala kindling is anxiolytic.

Electrode placement may be an important factor in these differences in results. The findings of the present study suggest that electrode placement is linearly related to the effects of kindling on anxiety in the AP and vertical planes. Therefore, there

may be very localized anatomical areas where kindling has either anxiolytic effects, anxiogenic effects, or no effects on anxiety.

In fact, location of the kindled focus may explain the effects of kindling of the right medial amygdala in the present study. Electrodes in the present study fell between two amygdala areas that, when kindled, either increase or decrease anxiety in the plus maze (4,10). Moreover, there was a correlation between anxiety and AP location of the kindled focus in the Adamec and McKay (10) study ($r = 0.506$, $p < 0.05$). A similar correlation was seen in the present study ($r = 0.509$, $p < 0.05$). Therefore, the lack of significant effect on anxiety in the present study might represent a partial cancellation of both anxiogenic and anxiolytic effects of kindling in these animals.

Therefore, the differences in the effects of kindling of the right medial amygdala on anxiety between this study and Adamec (4) and Adamec and McKay (10) may be due to the precise location of the stimulating electrode. If so, it is possible that the discrepancies between this study and Witkin et al. (55) also could be due to differences in location of kindled focus. The target site within the amygdala of the Witkin et al. (55) study is near the intra-amygdaloid zone. This is an outlier anxiolytic site in the present study. Unfortunately, Witkin et al. (55) did not report the exact location of their electrodes.

Nieminen et al.'s (37) report that kindling the left basolateral amygdala is anxiogenic is more puzzling, since we find the opposite. Nieminen et al. (37) did not specify the exact location of their electrodes either. They may have found an anxiogenic area in the left amygdala that was not detected in the present study.

Such anatomical specificity of kindling effects on behavior could be due to different efferent pathways engaged by stimulating different foci. For example, there are different efferent pathways from the anterior and posterior medial amygdalas (30). Anterior (but not the posterior) medial amygdala projects to the olfactory bulb, the intermediate part of the posterior bed nucleus of the stria terminalis, the basolateral part of the medial preoptic area, and the core of the ventromedial hypothalamus (VMH). The posterior region of the medial nucleus projects to the medial parts of all of these areas except the VMH. The posterior region projects to the shell around the VMH (30).

Kindling of different foci within the amygdala might induce long-term potentiation (LTP) in different efferent pathways. Long-term potentiation in these pathways could mediate the interictal behavioral changes. For example, kindling in rodents induces LTP in amygdala efferents to the medial hypothalamus (44). Moreover, LTP of these efferents is closely correlated with increased anxiety following partial kindling in cats (3,7,12,14).

Contribution of Current Spread to Behavioral Effects of Kindling

The localizability of effects of kindling on behavior is somewhat surprising. Diameter of the stimulating electrodes used (0.125 mm) is not trivial with respect to the size of the structures stimulated. Nevertheless, localization of stimulus effect is supported by an extensive study by Watson et al. (54). Watson et al. used [^{14}C]2-deoxyglucose autoradiography to visualize spread of excitation from the tip of a stimulating electrode within the rat medial and basolateral amygdala. Electrode and stimulus parameters used by Watson et al. (54) were nearly identical to those used in the present study. Watson et al. (54) found that excitation decreased rapidly within fractions of a millimetre from the centre of the electrode tip. There was a 90% reduction within a sphere of 0.3 mm radius in the medial amygdala, and 70% reduction within a sphere of similar radius in the basolateral amygdala.

Finally, Watson et al. (54) found little overlap in efferent areas activated by basolateral and medial amygdala stimulation. This finding further supports the view that localized focal and efferent effects of stimulation do occur.

Kindling Parameters and Effects of Kindling on Behavior

Rats kindled in the left medial amygdala took more stimulations to reach the first stage 5 seizure than all other on target kindled groups, which did not differ. This difference was not reflected in average current passed, however, or in any other kindling parameter. Moreover, the difference in number of stimulations to first stage 5 seizure does not parallel any behavioral difference.

In addition, outlier groups did not differ in kindling parameters. Therefore, variation in kindling parameters did not contribute to any of the group differences in behavior following kindling.

The Relationship Between Plus Maze Measures of Anxiety Changed by Kindling and Risk Assessment

Blanchard's group has suggested that risk assessment behavior is a measure of anxiety (15,16). Decreases in risk assessment have been seen in situations promoting fearfulness. Risk assessment is decreased after the presentation of a cat (15,16) and increased by diazepam (15). Adamec and Shallow (11) report decreased risk assessment associated with increased anxiety in the elevated plus maze 1–21 days following exposure to a cat. In contrast, Rodgers and Cole (47) report increased risk assessment (as measured here) in the elevated plus maze in mice just exposed to defeat in conspecific conflict. At the same time anxiety is increased (ratio time is decreased).

The present study provides another opportunity to examine how risk assessment is changed by manipulations that alter anxiety measured by a conventional test of animal anxiety (plus maze). Since kindling in this study increases and decreases anxiety, it is of interest to determine if risk assessment changes in a parallel fashion. If risk assessment and ratio time are measuring the same thing, then risk assessment should change in parallel with ratio time.

In this study, decreased risk assessment is associated with increased anxiety (decreased ratio time). The ratio frequency risk measure more closely paralleled the ratio time data than did the ratio time risk measure, however. Ratio frequency risk tended to rise in on target rats rendered less anxious by left amygdala kindling. In addition, ratio frequency risk decreased in on target rats tending to be more anxious following right amygdala kindling. Moreover, ratio time and ratio frequency risk share much of the same variance, since effects of kindling on ratio time were eliminated when ratio frequency risk was used as a covariate in ANCOVA.

In contrast, effects of kindling on ratio time remained after ratio time risk was removed by ANCOVA. Interestingly, the anxiolytic effect of on target left hemisphere kindling remained, but the anxiogenic trend of right hemisphere kindling was eliminated.

Together, the data suggest that ratio frequency risk is measuring processes very similar to those measured by ratio time. In contrast, ratio time risk may be measuring only anxiogenic-like effects produced by right hemisphere kindling.

This conclusion is tempered somewhat by the effects of outlier kindling on ratio time and ratio risk measures. Though ratio time was increased in outlier-kindled rats, neither ratio risk measure changed. This suggests, at least with respect to anxiolytic effects, that ratio risk measures do not reflect processes totally

common to those measured by ratio time. This conclusion is supported by the fact that even though there was no main effect of on target kindling on ratio time when ratio frequency risk was a covariate, the anxiolytic effect of kindling in the left hemisphere tended to remain [$t(62) = 1.66$, $p < 0.05$, one-tailed test; 0.22 ± 0.03 vs. 0.30 ± 0.04, ratio time left control vs. left kindled on target rats). In addition, Rodgers and Cole (47) found increased risk assessment associated with decreased ratio time in the plus maze in stressed mice.

Together, these findings suggest risk assessment does not covary in a simple fashion with ratio time. How risk changes in relationship to ratio time may depend on how anxiety is modified. When the modifier is kindling, or interspecies threat, the variation is direct. When the modifier is conspecific stress, the variation is inverse, as seen by Rodger and Cole (47). The latter example may also be species specific, since Rodgers and Cole studied mice.

Implications for Human Epilepsy

Kindling of the medial/basolateral amygdala changes the anxious state of the rat. These changes are stable for at least 1 week in this study and previous studies (4,10), and 2 weeks in Nieminen et al. (37). It has been suggested that kindling-induced changes in anxiety in animals models aspects of anxiety in the human epileptic (3). This study adds further evidence to the link between limbic epilepsy, which involves the amygdala, and changes in anxiety.

A most interesting finding in this report is that kindling had differential effects in the two hemispheres. There are few studies showing hemispheric functional lateralization in rats (22,26,36). The present findings suggest asymmetrical representation of emotion in rats that is similar to humans.

The pattern of results in this study fits well with studies of the lateralization of emotion in humans (17,20). An anxiogenic trend was noted for right hemisphere kindling, but not left. This parallels the observation in humans that the right hemisphere (and not the left) is involved in depressive and unpleasant affects (19,52). The anxiolytic effects of left hemisphere kindling are consistent with the view that the left hemisphere is specialized for positive affects (20). Of course, it must be recalled that outlier placements in both hemispheres tended to be anxiolytic, so a strict hemispheric separation of positive and negative affect modulation by kindling in the rat is not supported by the data.

In summary, the behavioral changes associated with kindling may model behavioral changes associated with human epilepsy. This study suggests, further, that precise location of the limbic focus is critical for the nature of the behavioral change induced by kindling. Similar findings have been reported in the cat (1,7,9). It would be of interest to determine if the same is true in human epilepsy.

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