CONDITIONING OF ICTAL AND INTERICTAL BEHAVIORS IN RATS
BY AMYGDALA KINDLING: CONTEXT AS THE CONDITIONAL STIMULUS

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Abstract

We assessed the ability of contextual conditional stimuli that are normally present during the course of kindling to modulate both motor seizures and interictal behavior. Rats received 53 stimulations to the left basolateral amygdala in one context (CS+) and 53 sham stimulations (The lead was attached but no current was delivered.) in another context (CS-), quasirandomly over 54 days. We observed 3 kinds of conditional effects. First, after several stimulations, less ambulatory activity, more freezing, and less rearing reliably occurred in the CS+ context than in the CS- context. Second, after 45 stimulations, all of the rats chose the CS- context over the CS+ context in a conditioned place-preference test. Third, when the rats were finally stimulated in the CS- context, their motor seizures were less severe in several respects: Latencies were longer, motor seizures were shorter, convulsive patterns were of a lower class, and there were fewer falls.

Table of Contents

Abstract.....	ii
Table of Contents.....	iii
List of Figures.....	iv
Acknowledgements.....	v
Introduction.....	1
Methods.....	2
Subjects.....	2
Apparatus.....	2
Procedure.....	5
Histology.....	8
Statistical Analyses.....	8
Results.....	9
Histology.....	9
Conditioning of Interictal Behaviors.....	12
Conditioning of Motor Seizures.....	17
Discussion.....	22
References.....	25

List of Figures

Figure 1. The two stimulation chambers, and the central chamber.....	4
Figure 2. Histology.....	11
Figure 3. The mean activity, freezing, and rearing of the rats during the prestimulation tests.....	14
Figure 4. The mean time spent by the rats in the CS+ and CS- contexts during the place-preference test.....	16
Figure 5. The mean latency, motor seizure duration, class, and falls when one group of rats was stimulated in the CS- context, and the other group was stimulated in the CS+ context.....	19
Figure 6. The mean latency, motor seizure duration, class, and falls in all rats in the CS- context in relation to the motor seizures elicited by their final stimulation in the CS+ context.....	21

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Conditioning of Ictal and Interictal Behaviors in Rats
by Amygdala Kindling: Context as the Conditional Stimulus

Since its characterization by Goddard, McIntyre, and Leech in 1969, kindling has been widely studied, both as a model of epileptogenesis and as a form of neural plasticity. Yet, little consideration has been given to the role played by the cues regularly associated with the delivery of the kindling stimulations. In the typical kindling experiment, each subject is stimulated many times through an implanted electrode: Each time, the subject is removed from its cage; the stimulation lead is attached; the subject is placed in the stimulation environment; and the current is delivered. Accordingly, there is ample opportunity for kindled animals to learn the predictive relation between antecedent events and the subsequent stimulation and convulsion. Still, it has been implicitly assumed that all of the ictal and interictal consequences of kindling are unconditional effects of the stimulation. The purposes of the present experiment were two fold: (1) to demonstrate the ability of kindled rats to learn the relation between the stimulation context and amygdalar stimulations and (2) to determine the effects of this conditioning on both ictal and interictal behaviors.

There had been several previous attempts to demonstrate the effect of conditional stimuli on kindled animals. Most of these had been attempts to demonstrate that conditional stimuli can elicit kindled seizures, for example, to demonstrate that auditory conditional stimuli can elicit afterdischarges and motor seizures. The results of these studies were either negative (Myslobodsky, Mintz, Lerner, & Mostofsky, 1983; Wyler & Heavner, 1979), nonreplicable, or not convincingly attributable to conditioning because of the lack of critical controls (Janowsky, Laxer, & Rushmer, 1980). In the present experiment, we employed a more sensitive method of

studying the effect of conditional stimuli on kindled seizures: Rather than assessing the ability of conditional stimuli to elicit kindled seizures, we assessed their ability to modulate the seizures elicited by the stimulation. In addition, we did not focus exclusively on seizures; we also assessed the ability of conditional stimuli to influence the interictal behavior of the kindled rats.

The objective of the present experiment was not merely to demonstrate that conditioning can occur in kindling experiments, it was to demonstrate that conditional effects are likely an integral, but unrecognized, component of most kindling experiments. That is why we focused on the conditional effects of the stimulation context, employed conventional kindling procedures, and studied the most frequent site of kindling: the amygdala.

Methods

Subjects

The subjects were 16 experimentally naive, male Long-Evans rats (Charles River, St. Constant, Quebec, Canada) that were 10 weeks old at the beginning of the experiment. They were housed in steel hanging cages, in groups before the experiment commenced and individually thereafter. All rats had continuous access to Purina Rat Chow and water under a 12:12-hr light-dark cycle with lights on at 7:30.

Apparatus

The test apparatus comprised two similar, but discriminable, stimulation chambers—one white and one black—connected by a central chamber. This entire complex, drawn to scale in Figure 1, was constructed of plastic-coated wood and was 50 cm long, 80 cm wide, and 25 cm high. Notice in Figure 1 that half the central chamber was white and half was black and that the floor of all three chambers was covered with about 2.5 cm of bedding material. During kindling, the central chamber was inaccessible; but during place-preference testing, the central chamber

Figure 1. The two stimulation chambers: one white and one black—connected by a central chamber. During kindling, the central chamber was inaccessible; but during place-preference testing, the central chamber was employed as the start box, and the doors into the two stimulation chambers were open.

was employed as the start box, and the doors into the two stimulation chambers were opened. A Panasonic VHS camcorder was mounted above the apparatus; it was used on periodic test days to record motor seizures and interictal behavior.

Procedure

All experimental procedures were administered in the colony room during the light phase of the light-dark cycle, between 8:30 and 18:30.

Presurgery Handling. Prior to surgery, each rat was handled for 1 min on 24 occasions, over 4 consecutive days. Each time, the rat was removed from its home cage, held, and lightly stroked.

Surgery. A single bipolar electrode (Plastic Products Company, MS-303-2) was implanted in the left basolateral amygdala of each rat under sodium pentobarbital anesthesia (65 mg/kg, i.p.) using standard stereotaxic procedures. The electrode tip was aimed at a site 2.8 mm posterior, 5.0 mm left, and 9.0 mm ventral to the skull surface at bregma, with the incisor bar set at -3.3 mm--coordinates were derived from Paxinos and Watson (1986). The electrode was secured to the skull with four stainless steel screws and dental acrylic. Powdered tetracycline was sprinkled on the incision to reduce the risk of infection.

Postsurgery Handling. Following a postsurgery recovery period of between 7 and 14 days, each rat was handled, as before, twice per day for 5 min on 2 consecutive days.

Kindling Phase. The day after the final day of postsurgery handling, the rats were randomly divided into two groups. The rats in one group (n=8) were stimulated in the white chamber and sham stimulated in the black chamber; the rats in the other group (n=8) were stimulated in the black chamber and sham stimulated in the white chamber. Because there were no systematic differences between the behaviors of these two groups, they were combined for all

analyses; and all comparisons were within-subjects comparisons between their behavior in their stimulation (CS+) and sham-stimulation (CS-) environments.

On each stimulation trial, the rat was removed from its cage; carried to the apparatus in the same room; attached to the stimulation lead, and placed facing the same corner of the CS+ context. The rat was then allowed to move freely around the CS+ context for 30 s, during which time the experimenter stood immobile in front of the chamber. After the 30 s was complete, the experimenter pressed the button on the stimulator to deliver a brief amygdalar stimulation (1 s, 60 Hz, 400 μ A). After stimulation offset, the stimulation lead was promptly removed, and the rat remained in the CS+ context for an additional 150 s before it was returned to its home cage. Each convulsive response was rated according to Pinel and Rovner's extension (1978) of Racine's (1972) widely used five-class scale (class 1: facial movements only; class 2: facial movements and head nodding; class 3: facial movements, head nodding, and forelimb clonus; class 4: facial movements, head nodding, forelimb clonus, and rearing; class 5: facial movements, head nodding, forelimb clonus, rearing, and falling once; class 6: a class 5 with multiple rearing and falling episodes; class 7: a class 6 with running fits; class 8: a running fit with periods of tonus). In addition, both the latency to the onset of the motor seizure and the motor seizure duration were recorded; and if a class 5 seizure or greater occurred, the number of times the rat fell during the course of the seizure was also recorded.

The sham-stimulation trials were identical to the stimulation trials except that they occurred in the CS- context and the distal end of the stimulation lead was not connected to the stimulator—even the stimulation button was pressed. Accordingly, any differences that developed in the behavior of a subject in the CS+ and CS- contexts could be attributed only to

the conditional effects of differences between the CS+ and CS- contexts (e.g., the color, white or black; the location; or the odor).

The order of stimulation and sham-stimulation trials was quasirandom and was determined according to the following three restrictions: (1) There were 45 stimulations and 45 sham stimulations. (2) No more than three stimulations or sham stimulations ever occurred consecutively. (3) Every fourth day (e.g., day 1, day 5, day 9, etc.), which were prestimulation test days, always comprised one stimulation and one sham-stimulation trial in counterbalanced sequence.

The purpose of the prestimulation tests was to compare the subject's behavior in the 30 s prior to the stimulations and sham stimulations. Three behaviors were quantified: (1) a measure of general activity—the number of boundary lines of a 3x3 square grid (placed in front of the monitor) that were crossed by the tip of a rat's nose; (2) a measure of freezing—the total duration of time during which a rat made no observable movements, other than those associated with breathing, for at least 5 consecutive s; and (3) a measure of rearing—the number of times that a rat lifted both its forepaws off the floor. All three measures were quantified from the videotapes by the same experimenter. Because each stimulation or sham-stimulation period was recorded in its entirety, it was not possible to score the tapes blind. Thus, a sample of the tapes was scored by a second experimenter for the purpose of calculating Pearson's r reliability quotients: general activity=.95; freezing=.93; rearing=.93.

Conditioned Place-Preference Test. On day 46, the day after the final two trials of the kindling phase, all rats were tested for their relative preference of the CS+ and CS- contexts. Each rat was placed in the central chamber of the apparatus and allowed to move freely among the three chambers for 5 min. The test was videotaped, and the time spent in the CS+ and CS-

contexts was subsequently derived from the tapes. A rat was considered to be in a chamber only if all four of its paws were totally inside it.

Conditioning Maintenance Trials. Because of the possibility that the conditioned place-preference test may have partially extinguished any conditional effects, the discrimination training procedure was reinstated on day 47 for an additional 8 days. Days 50 and 54 were test days.

Switch Tests. On day 55, the day following the final conditioning maintenance trial, the rats were randomly divided into two groups. One group was stimulated in the CS- context on day 55, whereas the other group was stimulated in the CS+ context on day 55. This second group was then stimulated in the CS- context later on day 55. In this final phase of the experiment, the experimenter recording the ictal data was blind to which chamber had previously served as the CS+.

Histology.

At the conclusion of the experiment, all the rats were killed with CO₂ according to the Canada Council on Animal Care guidelines. Their brains were removed and preserved in formalin for at least 1 month. They were then frozen and sliced along the coronal plane through the amygdala. Each slice was 35 μ m thick, and every fourth slice was mounted on a slide and stained with cresyl violet. The position of each electrode tip was estimated from the stained slides using a light microscope and the Paxinos and Watson stereotaxic atlas (1986).

Statistical Analyses.

Because this experiment was similar to two separate pilot studies, directional (one-tailed) tests were employed. Three different kinds of analyses were conducted. First, the activity, freezing, and rearing data were analyzed using 2-way repeated-measures ANOVAs, with context

and day as the within-subjects factors; significant interactions were followed up with one-tailed dependent-samples t-tests. Multiple t-tests were chosen because these three measures show marked heterogeneity of variance across days. Second, the place-preference data were analyzed with a single one-tailed dependent-samples t-test. Third, the seizure data from the switch tests were analyzed with a multivariate Hotelling's T^2 —followed by one-tailed independent-samples t-tests. As a confirmation of this latter effect, the seizures elicited in all the rats by the final stimulation in the CS+ context were compared with the seizures subsequently elicited by stimulation in the CS- context using one-tailed dependent-samples t-tests.

Results

The rats learned the relation between amygdalar stimulations or sham stimulations and their respective conditional contexts; and this conditioning affected both their ictal and interictal behavior. Three kinds of conditional effects were observed. First, after several stimulations, the rats behaved differently in the CS+ context than in the CS- context. Second, after 45 stimulations, all of the rats chose the CS- context in a conditioned place-preference test. Third, when the rats were stimulated for the first time in the CS- context, their motor seizures were less severe.

Histology.

One rat did not develop behavioral seizures during discrimination training and was thus eliminated from the experiment. Figure 2 illustrates the location of the electrode tips in the basolateral amygdala in the remaining 15 rats. All electrodes rested within the boundaries of the basolateral nucleus of the amygdala.

Figure 2. The location of the electrode tips in the basolateral amygdala of the 15 rats.

Conditioning of Interictal Behaviors.

The effects of the stimulation and sham stimulation contexts on the interictal behavior of the rats during the course of kindling are illustrated in Figure 3. All three interictal behaviors that were analyzed—ambulatory activity, freezing, and rearing—were significantly affected by the context.

Activity. Figure 3A illustrates the mean number of line crossings in the CS+ and CS- contexts during the prestimulation tests, which occurred prior to every fourth stimulation. Notice that after the first few tests the rats became less active in the CS+ context than in the CS- context, $F(13,182)=2.70$, $p<.005$. Significantly less activity occurred in the CS+ context than in the CS- context on days 17, 25, 33, and all subsequent tests, $p<.05$.

Freezing. Figure 3B illustrates the mean duration of freezing in the CS+ and CS- contexts during the prestimulation tests. Notice that after the first few tests the rats displayed more freezing in the CS+ context than in the CS- context, $F(13, 182)=2.31$, $p<.01$. Significantly more freezing occurred in the CS+ context than in the CS- context on days 25, 29, 41, and all subsequent tests, $p<.05$.

Rearing. Figure 3C illustrates the mean number of times the subjects reared in the CS+ and CS- contexts during the prestimulation tests. Notice that after the first few tests, the rats reared less in the CS+ context than in the CS- context, $F(13,182)=2.36$, $p<.01$. Significantly less rearing occurred in the CS+ than in the CS- context on days 21, 25, 37, 45, 50, and 54, $p<.05$.

Conditioned Place-Preference. Figure 4 shows the total amount of time spent in the CS+ and CS- contexts during the 5-min conditioned place-preference test. The rats spent significantly less time in the CS+ context than in the CS- context, $t(14)=7.61$, $p<.000001$. In fact, all 15 rats spent

Figure 3. (A) The mean number of line crossings, (B) the mean duration of freezing, and (C) the mean number of rears in the CS+ and CS- contexts during the prestimulation tests.

Figure 4. The mean amount of time spent by the rats in the CS+ and CS- contexts during the 5-min place-preference test.

less time in the CS+ context; 2 did not enter the CS+ at all; 14 of the 15 chose to enter the CS- context first, $\chi^2(1)=11.27$, $p<.001$.

Conditioning of Motor Seizures

Between-groups comparisons. Figure 5 illustrates the means of the four measures of the severity of the motor seizures that were elicited on Day 55, when one group of rats was stimulated for the first time in the CS- context while the other group of rats was stimulated as usual in the CS+ context. Overall, the motor seizures elicited in the CS- context were less severe, $T^2=26.381$, $F(4,10)=5.073$, $p<.05$. Indeed, each of the four measures of motor seizure severity differed significantly in the two contexts: The motor seizures in the CS- context were associated with shorter durations, $t(13)=2.07$, $p<.05$; lower classes, $t(13)=3.84$, $p<.005$; fewer falls, $t(13)=3.52$, $p<.005$; and longer latencies, $t(13)=2.65$, $p<.01$.

Within-groups comparisons. Figure 6 illustrates the means of the four measures of the severity of the motor seizures elicited in all rats in the CS- context in relation to the motor seizures elicited by their final stimulation in the CS+ context. The results of the within-groups comparisons confirmed those of the between-groups comparisons in every respect; that is, each of the four measures indicated that the motor seizures elicited in the CS- context were less severe than those that had been elicited in the CS+ context in the same subjects. The motor seizures in the CS- context were associated with shorter durations, $t(14)=4.00$, $p<.001$; lower classes, $t(14)=2.68$, $p<.01$; fewer falls, $t(14)=2.65$, $p<.01$; and longer latencies, $t(14)=2.78$, $p<.01$. Interestingly, one rat failed to respond with any motor seizure when stimulated in the CS- context—despite previously having 16 consecutive motor seizures in the CS+ context.

Figure 5. The mean (A) latency to motor seizure, (B) motor seizure duration, (C) motor seizure class, and (D) number of falls elicited on Day 55, when one group of rats was stimulated for the first time in the CS- context while the other group was stimulated as usual in the CS+ context.

CS+ CS-
Context

CS+ CS-
Context

Figure 6. The mean (A) latency to motor seizure, (B) motor seizure duration, (C) motor seizure class, and (D) number of falls elicited in all rats in the CS- context in relation to the motor seizures elicited by their final stimulation in the CS+ context.

CS+ CS-
Context

CS+ CS-
Context

Discussion

There were three major findings. First, after several stimulations, less ambulation, more freezing, and less rearing reliably occurred in the CS+ context than in the CS- context. Second, after 45 stimulations, all of the rats chose the CS- context over the CS+ context in a conditioned place preference test. Third, when the rats were finally stimulated in the CS- context, their motor seizures were less severe in several respects: latencies were longer, motor seizures were shorter, convulsive patterns were of a lower class, and there were fewer falls. These three findings clearly demonstrate that conditional effects can modulate both the ictal and interictal manifestations of amygdalar kindling. Moreover, because we employed conventional kindling procedures and assessed the conditional effects of the stimulation environment, these findings suggest conditioning is likely an integral, albeit unrecognized, component of many kindling experiments.

The pattern of conditional interictal behaviors observed in the present experiment—less ambulatory activity, more freezing, less rearing, and an avoidance of the CS+ context—are indicative of increased defensiveness. This pattern may be attributable to the fact that the amygdala appears to play a role in fear and defensiveness (e.g., Walker, Cassella, Lee, De Lima, & Davis, 1997). Alternatively, it is possible that the kindled convulsions are aversive and that the observation of conditional defensive behaviors will prove to be associated with other kindling sites, uninvolved in fear and defensiveness.

The present results are comparable to demonstrations of the situational specificity of drug tolerance. Just as subjects have been shown to learn the relationship between the injection environment and drug effects, the rats in the present experiment learned the relationship between the stimulation environment and seizures. There is, however, one major difference in the two

phenomena. When subjects receive a series of drug administrations in the same environment, the environment begins to elicit conditioned compensatory responses that offset the drug effects, thus contributing to the development of tolerance (Kim, Siegel, & Patenall, 1999). In contrast, in the present experiment these conditioned effects seemed to potentiate rather than counteract the effects of the unconditional stimulus: Motor seizures elicited in the usual stimulation environment were more severe than those elicited in the usual sham stimulation environment. We might explain this apparent discrepancy by considering the motor seizure as an adaptive response to the stimulation.

Although elicited motor seizures in rats are not directly comparable to spontaneous motor seizures in human patients, the present findings raise the possibility that conditioning plays a significant role in the manifestations of both the ictal and interictal symptoms of human epilepsy. Indeed, Mostofsky & Balaschak (1977) have suggested that environmental stimuli may be a major source of potentiation or attenuation of seizure disorders. In support of this hypothesis, reports exist of epileptics having a high frequency of seizures in certain locations and a low frequency of seizures in others (e.g., Henner, 1962; Temkin & Davis, 1984).

It has been implicitly assumed in virtually all previous studies of kindling that fluctuations in motor seizure latency, duration, and class are attributable entirely to unconditional effects of the brain stimulations. Similarly, in those studies of the interictal sequelae of kindling (e.g., Adamec & Morgan, 1994; Cammisuli et al., 1997; Helfer, Derensart, Marescaux, & Depaulis, 1996; Kalynchuk, Pinel, & Treit, 1999), it has been implicitly assumed that these are unconditional effects of the stimulations. Clearly, the reliability, magnitude, and systematic nature of the current findings challenge these widely held assumptions. However, whether conditional effects are associated with the kindling of structures other than the amygdala, or

whether stimuli other than the context can serve as conditional stimuli in kindling experiments awaits to be established.

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