

BRIEF COMMUNICATIONS

Conditioning of a Flavor Aversion in Rats by Amygdala Kindling

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Rats received 30 stimulations and 30 sham stimulations (the lead was attached to the subjects but no current was delivered) to the left basolateral amygdala in a quasirandom sequence. Stimulations were preceded by the presentation of 1 flavored solution conditional stimulus (CS+); sham stimulations were preceded by the presentation of another flavored solution, CS-. As kindled motor seizures developed, the rats began to consume significantly less of the CS+ than the CS-. Moreover, at the end of the experiment, the rats consumed significantly less of the CS+ than the CS- during a 20-min conditioned flavor preference test in which both solutions were available simultaneously. These findings confirm and extend the recent report that interictal changes in defensive behavior can be conditioned by amygdalar kindling.

Most studies of the kindling phenomenon, first characterized by Goddard, McIntyre, and Leech in 1969, have focused on the development and intensification of the convulsive responses that define it. However, kindling has also been shown to produce a variety of interictal behavioral changes. For example, many studies have shown that kindling is associated with increases in the incidence of interictal emotional behaviors (e.g., Adamec, 1976; Adamec & Young, 2000; Henke & Sullivan, 1985; Kalynchuk, Pinel, & Treit, 1999; Kalynchuk et al., 1998; Nieminen et al., 1992; Pinel, Treit, & Rovner, 1977; Post et al., 1984; Rosen, Hamerman, Sitcoske, Glowa, & Schulkin, 1996).

It has been implicitly assumed that the interictal behavioral manifestations of kindling are unconditional consequences of repeated stimulation; however, it was recently demonstrated that a contextual conditional stimulus (CS) could modulate the interictal behavior of amygdala-kindled rats (Barnes, Pinel, Francis, & Wig, 2001). Barnes et al. stimulated rats in one environment (CS+) and sham stimulated them (i.e., attached the lead but delivered no current) in a similar, but distinctive, environment (CS-). There were two conditional effects on interictal behavior: First, as kindling progressed, more defensive behavior began to occur before the stimulation (or sham stimulation) 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.

The purpose of the present experiment was to assess the generality of the Barnes et al. (2001) finding: to determine whether or not aversions would develop to another class of conditional stimuli experienced before amygdalar stimulations and kindled motor seizures. It is well established that conditioning occurs most readily when the CS is relevant to the unconditional stimulus (US); for example, aversions produced by toxic poisoning can readily be conditioned to flavors but not to lights (see Garcia, Rusiniak, & Brett, 1977). Because seizures and other forms of malaise may accompany the consumption of toxic substances in an animal's natural environment and because the amygdala plays an important role in the perception of flavor (e.g., Bures, Bermudez-Rattoni, & Yamamoto, 1998; Sakai & Yamamoto, 1999), we hypothesized that a flavor may serve as a particularly relevant CS in the production of conditional interictal behaviors by amygdala kindling.

Method

Subjects

The subjects were 6 experimentally naive, male Long-Evans rats (Charles River, St. Constant, Quebec, Canada) that were 10 weeks old at the beginning of the experiment. Each rat was housed individually in a Plexiglas chamber with continuous access to rat chow, but restricted access to water. A 12-hr light-dark cycle was maintained throughout the experiment, with lights on at 7:30 a.m.

Apparatus and Materials

All testing took place in each rat's home chamber (30 cm long × 45 cm high × 45 cm wide). The floor of each chamber was covered with about 2.5 cm of bedding material, and there was one small hole in each end wall through which the spout of a water bottle could be inserted. The two end walls were opaque to prevent the rats from seeing the bottles; the two side walls were transparent to permit observation. The two test solutions were 4% (wt/vol) solutions of vanilla or almond extract. Both test solutions and the rat's daily water were dispensed from graduated cylinders fitted with curved ball-tip drinking spouts. These spouts protruded 3.5 cm through the holes on the end walls, at a height of 11 cm.

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Procedure

Presurgery handling. Before surgery, each rat was handled for 1 min, six times daily, over 4 consecutive days.

Surgery. A single bipolar electrode (MS-303-2, Plastic Products, Roanoke, VA) was implanted in the left basolateral amygdala of each rat under ketamine (100 mg/kg ip) and xylazine (10 mg/kg ip) anesthesia and 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; coordinates were derived from the Paxinos and Watson (1986) stereotaxic atlas. 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. After a postsurgery recovery period of at least 7 days, each rat was handled for 30 s twice daily for 5 days. During postsurgery handling, the stimulation lead was attached to each rat's electrode assembly.

Habituation. Habituation to the deprivation schedule and test situation was performed for 5 consecutive days. On each habituation day, each rat was attached to a lead from 8:30 a.m. to 4:30 p.m. while in its home chamber; the lead was connected to a commutator so that it was less likely to become tangled. Access to water was restricted. Each rat was given access to water for 5 min at 9:30 a.m. and again at 1:30 p.m. through a single water bottle. The position of the bottle was alternated between the two ends of the chamber, counterbalanced among subjects, to deter the development of position preferences. In addition, each rat was given access to water simultaneously, from two bottles mounted at opposite sides of their chambers, for 1 hr between 3:30 and 4:30 p.m. Starting on the final habituation day, each rat was provided with 49 hr continuous access to water, from 3:30 p.m. until 4:30 p.m. 2 days later. The bottles were then removed in preparation for the commencement of conditioning.

Kindling and conditional discrimination training. Immediately after the habituation phase, the rats were randomly divided into two groups. During the conditioning phase of the experiment, the rats in one group ($n = 3$) were always stimulated after presentation of the vanilla solution (CS+) and sham stimulated after presentation of the almond solution (CS-); the rats in the other group ($n = 3$) were always stimulated after presentation of the almond solution (CS+) and sham stimulated after presentation of the vanilla solution (CS-). Because there were no systematic differences between the behaviors of these two groups, they were combined for all analyses, and statistical comparisons were performed within subjects. However, it should be noted that the small number of rats in these two groups may have precluded the observation of such differences.

Each stimulation trial entailed attaching the stimulation lead to the rat and then presenting the CS+ solution from one bottle for 5 min. Then, the experimenter pressed the button on the stimulator to deliver a brief amygdalar stimulation (1-s, 60-Hz sine wave, 400 μ A rms). After stimulation offset, both the stimulation lead and bottle were promptly removed. There were two measures of consumption: the volume of solution that was consumed and the amount of time spent drinking. Each elicited convulsion was rated according to Pinel and Rovner's (1978) extension 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: Class 5 with multiple rearing and falling episodes; Class 7: Class 6 with running fits; Class 8: a running fit with periods of tonus).

The sham-stimulation trials were identical to the stimulation trials except that they occurred after consumption of the CS-, rather than the CS+, solution, and the distal end of the stimulation lead was not connected to the stimulator—the stimulation button was even pressed. Thus, any differences that developed in the behavior of a subject after consumption of the CS+ and CS- solutions could be attributed only to the conditional effects of the differences between the CS+ and CS- solutions (e.g., the flavor).

All conditional discrimination-training trials were conducted on weekdays, with two sessions per day, one at 9:30 a.m. and the other at 1:30 p.m. The water-deprivation schedule was the same as that used during habituation trials; it resulted in the immediate consumption of test solutions upon presentation. The order of stimulation and sham-stimulation trials was quasirandom and was determined according to the following three restrictions: (a) There were 30 stimulations and 30 sham stimulations. (b) No more than three stimulations or sham stimulations ever occurred consecutively. (c) Every 4th day (e.g., Day 1, Day 5, Day 9, etc.) always comprised one stimulation and one sham-stimulation trial in counterbalanced sequence. In addition, both the end wall of the bottle presentation and the session time were pseudorandomly ordered to reduce the likelihood of end or time preferences. Each weekend, the rats were allowed free access to water from two different bottles, from Friday at 3:30 p.m. until Sunday at 4:30 p.m., at which time the water bottles were removed in preparation for Monday's training trial.

Conditioned preference test. The day after the final discrimination-training trial, each rat's relative preference for the CS+ and CS- solutions was assessed. During the preference test, both the CS+ and CS- solutions were simultaneously available, one at each end of each rat's home chamber. The preference test began at approximately 10:00 a.m. and lasted for 20 min. Because the preference test was the first time that two different test solutions were made simultaneously available to the rats, before the test, all rats were first individually presented with each flavored solution (CS+ and CS-), one from each end of the chamber, until they had sampled each solution for 5 s. When the second bottle was removed, both bottles were immediately reintroduced in their original respective positions. Both the end and order of presentation of the CS+ and CS- solutions were counterbalanced.

Histology

At the conclusion of the experiment, all the rats were killed with carbon dioxide in accordance with Canada Council on Animal Care guidelines. Their brains were removed, preserved in Formalin, and then sectioned along the coronal plane through the amygdala. The position of each electrode tip was estimated with a light microscope and the Paxinos and Watson (1986) stereotaxic atlas.

Statistical Analyses

Because the two dependent measures, volume consumed and time spent drinking, were highly correlated (Pearson's $r = .89$), only those analyses based on the volume consumed are reported here. Three kinds of analyses were conducted. First, the volume of each solution (CS+ or CS-) consumed during the training trials was collapsed into 6 blocks of 5 days each and analyzed with a two-way repeated measures analysis of variance, with CS and block as within-subjects factors; significant interactions were followed by simple main effects analyses using the Bonferroni level of significance, $p < .01$. Second, the results of the conditioned taste preference test were analyzed with a single one-tailed dependent samples t test. Third, a Pearson's correlation coefficient was calculated between the number of days it took each rat to reach a criterion of three consecutive Class 5 or higher motor seizures and the number of days it took each rat to reach a criterion of three learning ratios of .10 or higher (these learning ratios were calculated by dividing the difference between the volumes of the CS- and CS+ consumed on a particular day by the sum of those two volumes).

Results

One of the 6 original subjects was removed from the experiment because it did not develop motor seizures. The tips of all five

electrodes in the remaining subjects were positioned well within the boundaries of the basolateral nucleus of the amygdala.

Figure 1 illustrates the progressive development of the kindled seizures (Panel A) and the mean volume of the CS+ flavor and CS- flavor consumed (Panel B) in each of the six blocks. During kindling, all of the rats displayed many motor seizures of Class 5 or greater ($M = 10.6$), and all but 1 of the rats displayed Class 6 motor seizures ($M = 4.4$). Notice in Figure 1 that after the first few trials the rats consumed less of the CS+ flavor than the CS- flavor: CS \times Day, $F(5, 20) = 2.090$, $p < .02$. Significantly less consumption of the CS+ flavor than the CS- flavor occurred during Block 4 (Trials 16–20), $F(1, 20) = 12.402$, $p < .01$; Block 5 (Trials 21–25), $F(1, 20) = 11.495$, $p < .01$; Block 6 (Trials 26–30), $F(1, 20) = 19.717$, $p < .01$; but not during Block 1 (Trials 1–5), $F(1, 20) = 0.155$, $p < .70$; Block 2 (Trials 6–10), $F(1, 20) = 0.069$, $p < .71$; or Block 3 (Trials 11–15), $F(1, 20) = 3.376$, $p < .09$.

Figure 2 illustrates the mean volume of the CS+ and CS- solutions consumed during the 20-min conditioned flavor preference test ($M = 8.00$ vs. 20.40 , respectively). The rats consumed significantly less of the CS+ flavor than the CS- flavor, $t(4) = 2.204$, $p < .05$.

There was a significant correlation between the number of days that it took for each subject to display three consecutive Class 5 or greater motor seizures ($M = 17$ days) and the number of days that it took each subject to display three consecutive learning ratios of .10 or greater ($M = 23.4$ days), $r = .901$, $t(4) = 3.607$, $p < .03$.

Discussion

The purpose of the present experiment was to determine whether an aversion could be conditioned to flavors presented before the administration of amygdalar stimulations and kindled motor seizures. Two findings indicate that such a flavor aversion

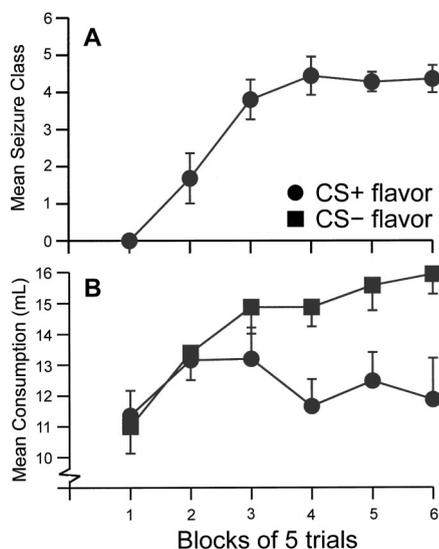


Figure 1. The (A) mean motor seizure class and (B) mean consumption of the conditional stimulus (CS+ and CS-) flavors during the course of the experiment. The data were blocked into six blocks of five trials each. Error bars indicate the standard error of the mean.

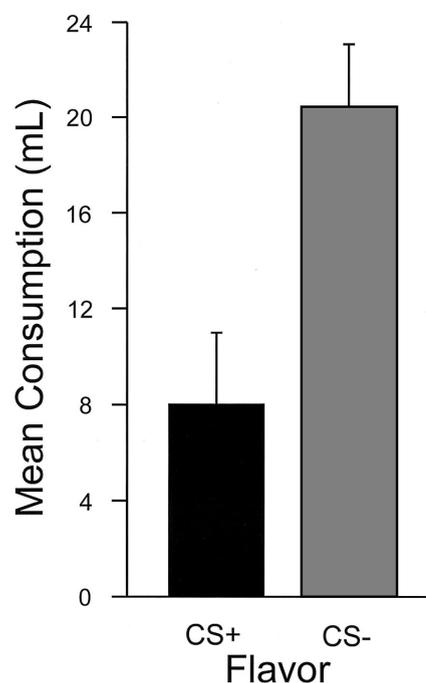


Figure 2. The mean consumption of conditional stimulus (CS+ and CS-) flavors during the 20-min taste-preference test. Error bars indicate the standard error of the mean.

was conditioned. First, after about 13 stimulations, all of the rats began to consistently consume less of the CS+ flavor than the CS- flavor (see Figure 1). Second, during the two-bottle conditioned taste preference test that was administered at the end of the experiment, the rats consumed significantly less of the CS+ flavor than the CS- flavor (see Figure 2).

The rationale for this experiment was based in part on the premise that flavors would serve as particularly relevant conditional stimuli in the development of conditional aversions produced by amygdala kindling. Although this experiment was not conducted to directly test this hypothesis, some relevant information can be gleaned by comparing the present results with those of Barnes et al. (2001), who used the stimulation context as the CS in a comparable study. For example, statistically significant differences between the effects of the CS+ and CS- began to emerge after 13–15 stimulations in both experiments (see Figure 1). However, after 30 stimulations the mean learning ratio in the present experiment was .249 (see Figure 2), whereas after an equivalent number of stimulations (i.e., 29) in the Barnes et al. experiment, the mean learning ratio was only .141 (unpublished data). Thus, although methodological differences between the present experiment and the Barnes et al. experiment preclude unequivocal conclusions based on a comparison of their results, there is some evidence that flavor served as a more relevant CS in the conditioning of interictal defensive behaviors by amygdala kindling.

From the present results, it is not possible to determine whether the brain stimulations, the motor seizures, or both served as the US. Another possibility is that only those motor seizures above a certain class function as effective USs. The significant correlation between the rate at which subjects kindled and the rate at which the

conditioned flavor aversion developed suggests that generalized motor seizures may have played a role in the aversive conditioning: Those subjects that were the first to reliably display generalized motor seizures tended to be the first to reliably display conditioned flavor aversions. However, there are other equally tenable interpretations of this correlation.

Paradoxically, numerous studies have reported that amygdala-kindled seizures produce anterograde and retrograde amnesia (for a review, see Hannesson & Corcoran, 2000). The results of the present study, along with those of Barnes et al. (2001), demonstrate that amygdala-kindled rats are able to learn and remember CSs that have been repeatedly presented before the stimulations and motor seizures. Apparently, the amnesia produced by amygdala-kindled motor seizures is not so complete as to preclude the development of memory for stimuli presented before each of a series of motor seizures. Thus, the conditional effects of kindling might complicate the interpretation of the results of those experiments that employ kindling as an amnesic agent to study the mechanisms of learning and memory. For example, the present results seem to contradict previous demonstrations that kindled seizures disrupt the acquisition of flavor and odor aversions (Mikulka & Freeman, 1984; Peele & Gilbert, 1992).

The changes in interictal emotional behaviors that accompany amygdala kindling have been well documented (e.g., Adamec & Morgan, 1994; Cammisuli et al., 1997; Helfer, Derensart, Marescaux, & Depaulis, 1996; Kalynchuk et al., 1999). These reports are of potential importance because they provide a means of modeling the interictal psychopathology commonly experienced by persons with temporal lobe epilepsy (e.g., Bear & Fadjo, 1977). Together with the results of Barnes et al. (2001), the present findings clearly demonstrate that the alterations in interictal behaviors that accompany amygdala kindling can be influenced by conditional stimuli.

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