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Conditioned Effects of Kindling Three Different Sites in the Hippocampal Complex of the Rat

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Rats received kindling stimulations to the perirhinal cortex (PRh), ventral hippocampus (VH), or dorsal hippocampus (DH) in 1 environment and an equivalent number of sham stimulations in a 2nd environment. The PRh-kindled rats displayed rapid kindling and a swift emergence of conditioned interictal defensiveness. In contrast, the VH- and DH-kindled rats displayed much slower kindling and slow or no conditioning, respectively. No effects of conditioning on the convulsions, comparable with those associated with amygdala kindling, were observed. These results establish the generality of some of the previously reported kindling-related conditioned effects, confirm the site specificity of some of these effects, and suggest that the convulsions, rather than the stimulations, function as the unconditioned stimuli for the conditioning of interictal behavior.

Keywords: kindling, hippocampus, perirhinal cortex, conditioning, defensive behavior

Although kindling has been the focus of intensive investigation both as a model of epileptogenesis and of neural plasticity, 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 rats to learn the predictive relation between antecedent events and the subsequent stimulation and convulsion.

We recently demonstrated that both the kindled convulsions and interictal behavior of rats are in part a product of conditioned effects. In our first experiment (Barnes, Pinel, Francis, & Wig, 2001), the stimulation environment served as the conditional stimulus (CS). Rats received 53 stimulations to the basolateral amygdala in one environment (CS+) and 53 sham stimulations (the stimulation lead was attached but no current was delivered) in a second environment (CS−), quasirandomly over 54 days. As kindling progressed, the rats became more defensive in the CS+ than in the CS−; they avoided the CS+ in a conditioned place-preference test, and, when they were finally stimulated in the CS−, their convulsions were less severe than in the CS+.

In a second experiment (Barnes, Pinel, Wig, Stuettgen, & Holzel, 2003), we investigated whether the kindling of brain sites with topographically distinct forms of kindled convulsions would consequently produce distinct patterns of conditioned effects. We compared the conditioned effects of anterior neocortical kindling with those of amygdala kindling, because the topography of anterior neocortex-kindled seizures differs markedly from that of seizures associated with amygdala kindling (Burnham, 1978; Pinel & Rovner, 1978; Racine, 1975; Seidel & Corcoran, 1986). In contrast to the conditioned effects of amygdala kindling, anterior neocortex-kindled rats displayed neither enhanced defensiveness in the CS+ nor an aversion to the CS+ in a conditioned place-preference test. However, anterior neocortex kindled rats displayed more wet dog shakes in the CS+ and less, rather than more, severe convulsions in the CS+. These results established that the pattern of conditioned effects of kindling is a function of the kindling site.

The primary purpose of the present experiment was to determine whether the kindling of related brain sites associated with topographically similar kindled convulsions, but vastly different rates of kindling, would produce similar patterns of conditioned effects. Our working premise was that this approach might clarify the nature of the unconditioned stimulus (US) in kindling-related conditioning. Is the US related more to the stimulations or to the convulsive responses?

We compared the behavioral effects—both ictal and interictal—conditioned to the stimulation environment during kindling of three different sites in the hippocampal complex that are known to kindle at vastly different rates (McIntyre, Kelly, & Dufresne, 1999; Racine, Rose, & Burnham, 1977): the perirhinal cortex (PRh), the ventral hippocampus (VH), and the dorsal hippocampus (DH).

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Method

Subjects

The subjects were 54 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 of the rats had continuous access to Purina Rat Chow (Ralston-Purina, St. Louis, MO) and water under a 12:12-hr light–dark cycle with lights on at 7:30 a.m.

Apparatus

The test apparatus was composed of two similar, but discriminable, stimulation chambers—one white and one black—connected by a central chamber that was half white and half black (see Barnes et al., 2001, for an illustration of this apparatus). This entire complex was constructed of Plexiglas and was 150 cm long, 75 cm wide, and 50 cm high. The floor of all three chambers was covered with 2.5 cm of bedding material. During kindling, the central chamber was inaccessible, but during place-preference testing, the central chamber was used as the start box, and the doors to the two stimulation chambers were opened. A video camera was mounted above the apparatus and was used on the test days to record convulsions 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 1.5 min once daily for 5 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, Roanoke, VA) was implanted in the left PRh of 18 rats, in the left VH of another 18 rats, and in the left DH of the remaining 18 rats under ketamine (100 mg/kg, ip) and xylazine (10 mg/kg, ip) anesthesia, following standard stereotaxic protocols. The electrode tip was aimed 3.2 mm posterior, 4.4 mm left, and 7.8 mm (15° angle) ventral to the skull surface at bregma for the PRh rats; it was aimed 5.6 mm posterior, 5.5 mm left, and 7.8 mm ventral to the skull surface at bregma for the VH rats; and it was aimed 3.4 mm posterior, 1.6 mm left, and 4.3 mm ventral to the skull surface at bregma for the DH rats. The incisor bar was set at −3.3 mm, and all 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. After a postsurgery recovery period of between 7 and 14 days, each rat was habituated to the stimulation lead and handled, as before, for 30 s twice daily for 14 consecutive days.

Kindling phase. The day after the final day of postsurgery handling, the PRh, DH, and VH rats were each randomly divided into two equal groups, producing six groups of 9 rats each. The rats in one of the PRh groups, one of the VH groups, and one of the DH groups were stimulated in the white chamber and sham stimulated in the black chamber; the rats in the other three groups were stimulated in the black chamber and sham stimulated in the white chamber. Because the color of the chambers proved to be inconsequential, the two PRh groups were recombined ($n = 18$), the two VH groups were recombined ($n = 18$), and the two DH groups were recombined ($n = 18$) for the purposes of analysis. Most statistical comparisons were within-subjects comparisons between the subject's behavior in their stimulation (CS+) and sham-stimulation (CS−) environments.

On each stimulation trial, each 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+ chamber. The rat was then allowed to move freely around the CS+ for exactly 30 s, during which time the experimenter stood immobile in front of the chamber. After the 30 s, the experimenter pressed the button on the stimulator to deliver a brief stimulation (1 s, 60 Hz, 400 μA peak-to-peak, square wave). After stimulation offset, the stimulation lead was promptly removed and the rat remained in the CS+ for an additional 120 s before it was returned to its home cage. Each convulsive response was rated according to Pinel and Rovner's (1978) extension of Racine's (1972b) widely used scale of limbic convulsion severity (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; and Class 8: a convulsion with periods of tonus). In addition, both the latency to the onset of the convulsion and the convulsion duration were recorded, and if a Class 5 convulsion or greater occurred, the number of times the rat fell during the course of the convulsion was also recorded. Electroencephalographic activity was not recorded.

The sham-stimulation trials were identical to the stimulation trials except that they occurred in the CS− and no stimulation was delivered. The stimulation lead was attached to each rat and the stimulation button was even pressed, but the rats received no stimulation because the distal end of the stimulation lead was not connected to the stimulator. Accordingly, any differences that developed in the behavior of a rat in the CS+ and CS− environments could be attributed only to the conditional effects of differences between the CS+ and CS− (e.g., the location, the white or black color, or the odor).

There were two sessions each day; thus, on any given day a rat received either two sham stimulations, two stimulations, or one stimulation and one sham stimulation. The interval between the two sessions on a given day was between 2 and 6 hr. The order of stimulation and sham-stimulation trials was quasirandom and was determined according to the following two restrictions: (a) no more than three stimulations or sham stimulations ever occurred consecutively, and (b) every fourth day (e.g., Day 1, Day 5, Day 9) was a preadministration test day, which always comprised one stimulation and one sham-stimulation trial in counterbalanced sequence.

The purpose of the preadministration (i.e., before administration of a stimulation or a sham stimulation) tests was to compare each rat's behavior in the 30 s prior to the stimulations in the CS+ with its behavior prior to the sham stimulations in the CS−. All of the preadministration tests were videotaped in their entirety. Two behaviors were quantified from the videotapes: (a) general activity—the number of boundary lines of a 4×5 square grid (placed in front of the video monitor) that were crossed by the tip of a rat's nose, and (b) freezing—the total duration of time during which a rat made no observable movements for at least 2 s, other than those associated with breathing. Both measures were quantified by the same experimenter, but because each stimulation or sham-stimulation period was recorded in its entirety, including the responses to the stimulation or sham stimulation, it was not possible to score the tapes blind. Accordingly, a second experimenter quantified these measures from a single preadministration test day and Pearson r interrater reliability quotients were calculated (activity = .92; freezing = .90). Although these two behavioral measures are correlated with one another, we have demonstrated, using multiple regression analyses, that each measure contains nonredundant information (Barnes et al., 2001).

Testing phase. On the day after the final day of the kindling phase, all of the rats were tested for their relative preference of the CS+ and CS− environments. 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− was subsequently derived from the tape by an experimenter who was unaware of which environment had previously served as the CS+ for each rat. A rat was

considered to be in a chamber only if all four of its paws were totally inside it.

Because of the possibility that the conditioned place-preference test partially extinguished any conditional effects, the discrimination training procedure was reinstated for an additional 8 days prior to further testing. All procedures during this phase were identical to those of the kindling phase. The day after the final trial of these conditioning maintenance trials, each of the three groups of rats (i.e., PRh, VH, and DH) was randomly divided into two equal groups (all *n*s = 9). Then, the rats in one of the PRh groups, one of the VH groups, and one of the DH groups were stimulated in the CS−, whereas the rats in the other three groups were stimulated in the CS+. The latter three groups of rats were then stimulated in the CS− 4 hr later; this schedule adhered to the pseudorandom two-trial-per-day schedule established during the kindling phase. The experimenter who scored the convulsions observed during this final phase was unaware of which chamber had previously served as the CS+ for each rat.

Conditioning and testing schedule. As expected (McIntyre, Kelly, & Armstrong, 1993; Sato, Yamada, Morimoto, Uemura, & Kuroda, 1998), PRh kindling progressed much more rapidly than VH and DH kindling. However, by the conclusion of the behavioral testing protocol, the three groups were significantly, but not equivalently, kindled. After comparing conditioned effects in the three groups after 53 stimulations, we attempted to bring the VH and DH rats up to a level of kindling comparable with that in the PRh rats by repeating the behavioral testing protocol for the VH and DH rats, but not the PRh rats. Accordingly, a second testing phase was administered after another 53 stimulations and 53 sham stimulations, at which point all three groups were significantly, but not equivalently, kindled.

Histology

At the conclusion of the experiment, all of the rats were killed with CO_2 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 sectioned along the coronal plane. Each section was 35 μm thick, and every fourth section 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 (1986) stereotaxic atlas.

Blocking of Time-Series Data

The activity and freezing time-series data for the PRh rats were blocked into four blocks, each block consisting of three consecutive preadministration test days. The activity and freezing time-series data for the VH and DH rats were blocked into eight blocks; each block consisted of three consecutive preadministration test days. Blocking of time-series data is recommended to reduce the probability of Type I errors that increase with multiple comparisons between means (Tukey, 1977).

Planned Statistical Analyses

Four different kinds of analyses were conducted to assess the statistical significance of the between-group and within-group differences. First, the activity and freezing data were analyzed separately for each group (PRh, VH, or DH) using planned orthogonal contrasts (POCs) between the CS+ and CS− for each separate block (i.e., Blocks 1 to 4 for the PRh rats, and Blocks 1 to 8 for the VH and DH rats). POCs use the within-cell error term from an omnibus analysis of variance (ANOVA). In these analyses, this value was obtained from a two-way within-subjects ANOVA with block and CS as within-subject factors (Keppel & Zedeck, 1989, p. 362; Winer, Brown, & Michels, 1991, pp. 342–343, 526). Because multiple ANOVAs were used for the analysis of the activity and freezing data, the *p* value required for a rejection of the null hypothesis was calculated using the Bonferroni correction: $p < .025$. Second, the place-preference data were analyzed using a between-within ANOVA, with group (PRh, VH, or DH) as the between-subjects factor and CS (CS+ or CS−) as the within-subjects factor; simple-main-effects analyses were used to investigate significant interactions. Third, the four measures of the convulsion severity from the between-subjects switch test were analyzed using two-way ANOVAs, with test-stimulation location (CS+ or CS−) and group (PRh, VH, or DH) as between-subjects factors; simple-main-effects analyses were used to investigate significant interactions. Fourth, to confirm the results of these latter analyses, the statistical significance of the differences in the severity of the convulsions elicited in each of the three groups of rats (PRh, VH, or DH) by the final stimulation in the CS+ versus those elicited by the stimulation in the CS− was assessed using between-within ANOVAs, with group (PRh, VH, or DH) as the between-subjects factor and CS (CS+ or CS−) as the within-subjects factor. Because multiple ANOVAs were used for the analysis of the convulsion-severity data, the *p* value required for a rejection of the null hypothesis was calculated using the Bonferroni correction: $p < .0125$.

Results

The rate of kindling was related to both the rate of conditioning and the magnitude of the conditioned effects. The PRh rats kindled quickly and displayed robust conditioning, the VH rats kindled more slowly and displayed statistically significant conditioned effects only toward the end of the experiment, and the DH rats kindled most slowly and did not demonstrate any statistically significant conditioned effects. Unlike amygdalar and anterior neocortex kindling (Barnes et al., 2003), kindling at any one of three sites within the hippocampal complex did not produce conditioned effects that influenced the convulsions themselves. Conditioned effects influenced only the interictal behavior.

Histology

Figure 1 illustrates the location of the electrode tips in each of the three groups. First, it shows the location of the electrode tips in the left PRh of the 18 PRh rats that completed the experiment. The electrodes of all 18 PRh rats lay within the boundaries of the PRh. Specifically, the electrodes of 13 of the PRh rats terminated in Layers 4–6 of PRh, whereas the electrodes of the remaining 7 PRh rats terminated in Layers 2–3. There were no obvious differences in the behavior of these two PRh subgroups.

Second, Figure 1 also illustrates the location of the electrode tips in the left VH of 16 of the 18 VH rats that completed the experiment. The electrode tips of these 16 VH rats all lay within the boundaries of the VH. Specifically, the electrodes of 6 of the VH rats terminated in the CA1 subfield of the hippocampus, another 7 of the VH rats had electrodes terminating in the CA3 subfield of the hippocampus, and the remaining 3 VH rats had electrodes terminating in the ventral aspect of the dentate gyrus. There were no obvious differences in the behavior of these three VH subgroups. The electrode placements of the other 2 VH rats that completed the experiment could not be determined because their brains were damaged during the slicing procedure. However, their data were included with the other VH rats for statistical analysis because the behavior of these 2 VH rats did not differ in any obvious fashion from that of the other 16 VH rats.

Finally, Figure 1 illustrates the location of the electrode tips in the left DH of the 15 DH rats that completed the experiment. The other 3 DH rats developed severe infections around their electrode

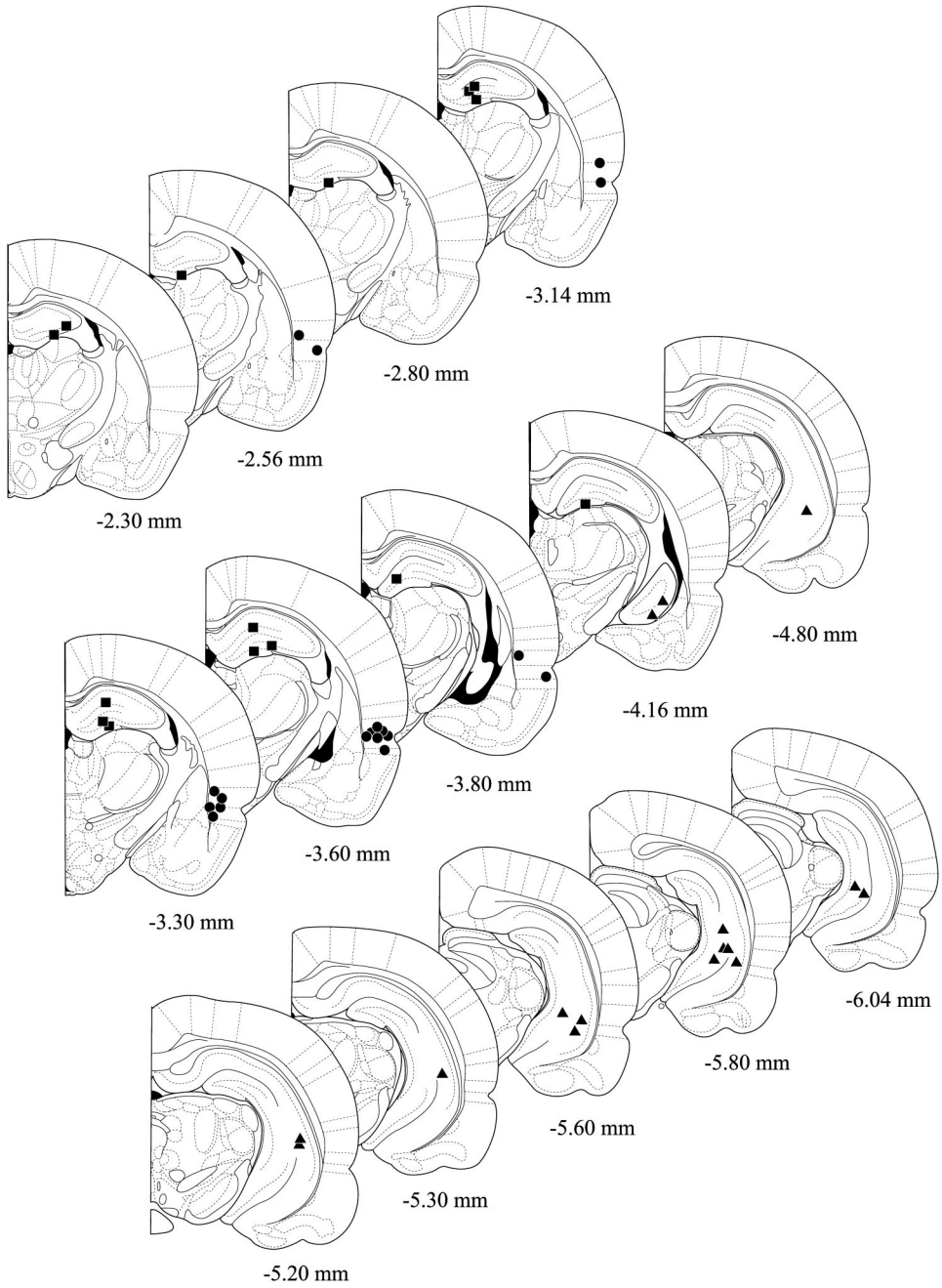

Figure 1. Histology: The location of the electrode tips in the left perirhinal cortex (PRh) of the 18 PRh rats that completed the experiment (black circles), in the left ventral hippocampus (VH) of 16 of the 18 VH rats that completed the experiment (black triangles), and in the left dorsal hippocampus (DH) of the 15 DH rats that completed the experiment (black squares). Each circle, triangle, or square represents the location of an electrode tip in one of the rats. Distances are measured from bregma.

assemblies and did not complete the experiment. Of the 15 DH rats completing the experiment, the electrodes of 2 DH rats terminated in the CA1 subfield of the hippocampus, the electrode of 1 DH rat terminated in the CA3 subfield of the hippocampus, and the electrodes of the other 12 DH rats terminated within the boundaries of the dentate gyrus. There were no obvious differences in the behavior of these three DH subgroups.

Kindling

Figure 2 illustrates the mean class of the convulsions displayed by the PRh, VH, and DH rats in response to stimulation in the CS+ on the preadministration test days, which occurred prior to every fourth stimulation. As previously reported, although the rate of kindling in the PRh, VH, and DH rats differed markedly, the

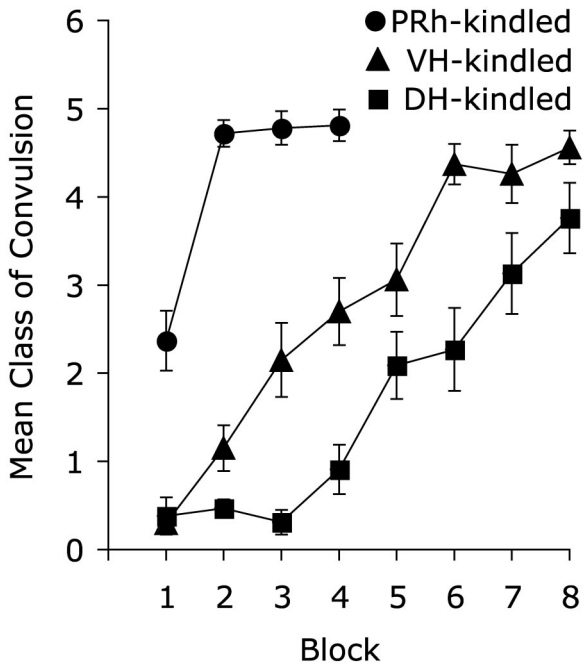

Figure 2. Kindling-phase convulsions: The mean class of the convulsions displayed by the perirhinal cortex (PRh)-kindled rats during each of the first four blocks of test days of the kindling phase, and by the ventral hippocampus (VH)- and dorsal hippocampus (DH)-kindled rats during each of the eight blocks of test days of the kindling phase. Error bars represent the standard error of the mean.

topography of the convulsions elicited by stimulations of the PRh, VH, and DH did not (McIntyre et al., 1993; Sato et al., 1998). In the PRh rats, the first few stimulations elicited no convulsive responses, but after several stimulations there was an abrupt emergence of fully generalized convulsions (i.e., Class 5 or higher). The PRh rats required a mean of 12.9 stimulations before they displayed three convulsions of Class 5 or greater, a commonly used kindling criterion; and they displayed a mean of 20.6 Class 5 or greater convulsions in response to the 53 stimulations of the first series.

In contrast, the VH and DH rats displayed much slower kindling. The first few stimulations elicited no convulsive response, but with repeated stimulations facial clonic convulsions developed, and these clonic convulsions gradually became more generalized until they involved the entire body and the loss of equilibrium. In other words, the development of the VH- and DH-kindled convulsions was virtually always characterized by a slow progression through the classic limbic convulsion classes (i.e., 1 to 6). The VH rats required a mean of 48.1 stimulations before they displayed three convulsions of Class 5 or greater. In contrast, only 12 of the 15 DH rats reached this latter criterion. Assigning these 3 rats the maximum score of 106 (the total number of stimulations administered to the DH and VH rats in the experiment), the DH rats required a mean of 74.1 stimulations before they displayed three convulsions with a class of 5 or greater. During the first series of 53 stimulations, the VH and DH rats displayed a mean of 6.2 and 0.8 Class 5 or greater convulsions, respectively, and during the second series of 53 stimulations, the VH and DH rats displayed a mean of 24.9 and 14.3 Class 5 or greater convulsions, respectively.

Conditioning of Interictal Behaviors

The effects of the CS+ and CS− environments on the ambulatory activity and freezing recorded during the preadministration tests are illustrated in Figure 3. Overall, the PRh rats displayed significantly less ambulatory activity and more freezing in the CS+ than in the CS−. In contrast, the VH and DH rats did not display any significant differences in their activity and freezing in the two environments during the first phase of the experiment (i.e., Blocks 1–4); however, after receiving further stimulations, the VH rats, but not the DH rats, displayed significantly more freezing in the CS+ than in the CS−.

Activity. Figures 3A, 3B, and 3C illustrate the mean number of line crossings by the PRh, DH, and VH rats, respectively, in the CS+ and CS− during the preadministration tests, which occurred prior to every fourth stimulation. The PRh rats were significantly less active in the CS+ than in the CS− during Block 2 (Days 13–21), $F(1, 51) = 7.87$, $p = .0071$, Block 3 (Days 25–33), $F(1, 51) = 11.44$, $p = .0014$, and Block 4 (Days 37–45), $F(1, 51) = 43.41$, $p = .00000002$, but not during Block 1 (Days 1–9), $F(1, 51) = .20$, $p = .66$. In contrast, the VH and DH rats displayed no significant differences in their activity in the two CS environments, $ps > .026$.

Freezing. Figures 3D, 3E, and 3F illustrate the mean percentage of freezing of the PRh, DH, and VH rats, respectively, in the CS+ and CS− during the preadministration tests. The PRh rats displayed significantly more freezing in the CS+ than in the CS− during Block 4, $F(1, 24) = 9.15$, $p = .0051$, but not during Block 1, $F(1, 24) = .10$, $p = .75$, Block 2, $F(1, 24) = 0.39$, $p = .54$, and Block 3, $F(1, 24) = 2.63$, $p = .12$. Similarly, the VH rats displayed significantly more freezing in the CS+ than in the CS− during Block 7 (Days 80–88), $F(1, 50) = 7.81$, $p = .0074$, and Block 8 (Days 92–100), $F(1, 50) = 7.68$, $p = .0078$, but not during Block 1, $F(1, 50) = 0.073$, $p = .79$, Block 2, $F(1, 50) = 0.027$, $p = .87$, Block 3, $F(1, 50) = 0.0096$, $p = .92$, Block 4, $F(1, 50) = 0.10$, $p = .75$, Block 5 (Days 56–64), $F(1, 50) = 0.69$, $p = .41$, and Block 6 (Days 68–76), $F(1, 50) = 1.29$, $p = .24$. In contrast, there were no significant differences in the freezing of the DH rats in the two environments during any of the 8 blocks, $ps > .089$.

Conditioned Place-Preference Test

The left half of Figure 4 shows the total amount of time that the PRh, DH, and VH rats spent in the CS+ and CS− during the first conditioned place-preference test, which was administered after 45 stimulations. During this test, the PRh, VH, and DH rats displayed differences in their relative preference for the CS− environment, $F(2, 48) = 8.281$, $p = .001$. The PRh rats spent significantly less time in the CS+ than in the CS−, $F(1, 48) = 44.82$, $p = .00000002$. In fact, 17 of the 18 PRh rats spent less time in the CS+, and 9 did not enter the CS+ at all. The VH rats also spent significantly less time in the CS+ than in the CS−, $F(1, 48) = 4.53$, $p = .038$; 13 of the 18 VH rats spent less time in the CS+, and all of them entered the CS+. In contrast, the amount of time the DH rats spent in the CS+ did not differ significantly from the amount of time they spent in the CS−, $F(1, 48) = 1.17$, $p = .29$.

The right half of Figure 4 shows the total amount of time that the VH and DH rats spent in the CS+ and CS− during the second conditioned place-preference test, which was administered after 98

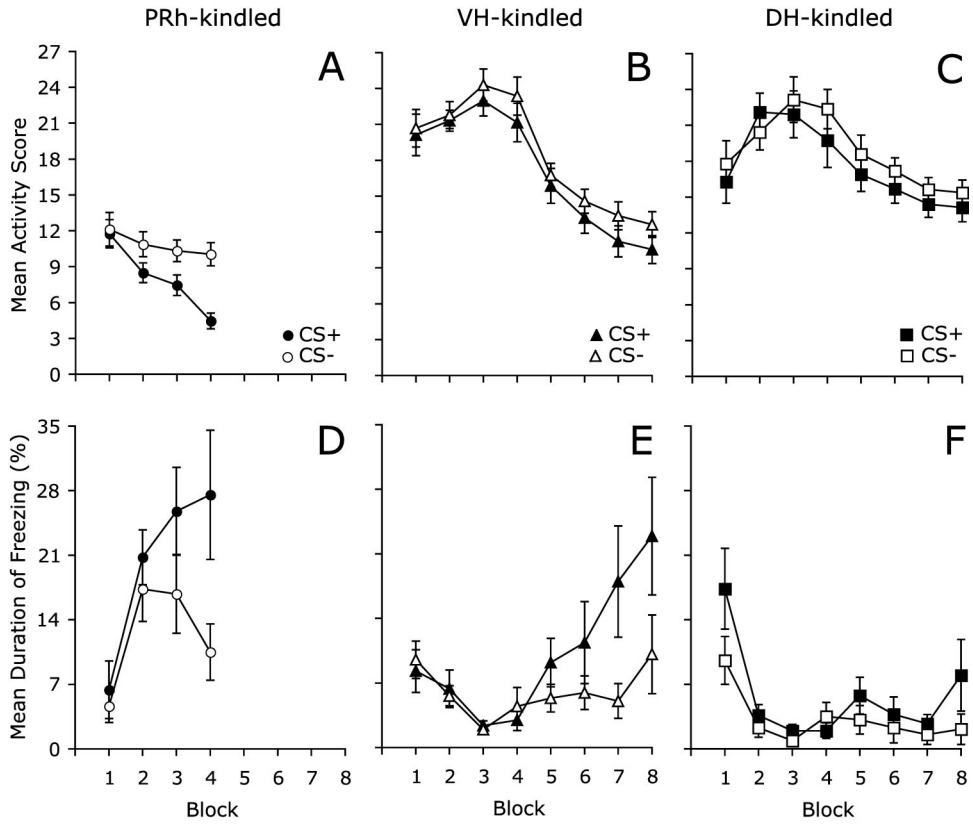

Figure 3. Conditioning of interictal behaviors: The mean ambulatory activity (A) and freezing (D) displayed by the perirhinal cortex (PRh)-kindled rats in their conditioned stimulus (CS)+ and CS− environments during each of the first four blocks of test days of the kindling phase. The mean ambulatory activity (B) and freezing (E) displayed by the ventral hippocampus (VH)-kindled rats in their CS+ and CS− environments during each of the four blocks of test days of the kindling phase. The mean ambulatory activity (C) and freezing (F) displayed by the dorsal hippocampus (DH)-kindled rats in their CS+ and CS− environments during each of the four blocks of test days of the kindling phase. Error bars represent the standard error of the mean.

stimulations. During this test, the VH and DH rats displayed differences in their relative preference for the CS− environment, $F(1, 31) = 5.17$, $p = .030$. The VH rats spent significantly less time in the CS+ than in the CS−, $F(1, 31) = 14.82$, $p = .00055$. In fact, 14 of the 18 VH rats spent less time in the CS+, and 4 did not enter the CS+ at all. In contrast, the amount of time that the DH rats spent in the CS+ did not differ significantly from the amount of time they spent in the CS−, $F(1, 31) = 0.189$, $p = .67$.

Conditioning of Convulsions

After 53 stimulations, one half of the PRh, VH, and DH rats were stimulated for the first time in the CS−, whereas the other half were stimulated as usual in the CS+. There were no significant differences in the four measures (i.e., latency, duration, convulsion class, and falls) of severity of the convulsions elicited in the two environments, ps $> .13$. After 106 stimulations, one half of the VH and DH rats were stimulated for the second time in the CS−, whereas the other half were stimulated as usual in the CS+. Again, there were no significant differences in the four measures (i.e., latency, duration, convulsion class, and falls) of severity of the convulsions elicited in the two environments, ps $> .13$.

Discussion

The present experiment compared the effects conditioned to the stimulation environment during the kindling of each of three different sites within the hippocampal complex: PRh, VH, and DH. There were three major findings. First, unlike amygdala and anterior neocortex kindling (see Barnes et al., 2003), kindling at any one of the three sites within the hippocampal complex did not produce conditioned effects that significantly influenced the convulsions themselves: Conditioned effects influenced only the interictal behavior. Second, the rate of kindling was related to the rate and magnitude of conditioning: The PRh rats kindled quickly and displayed robust conditioning, the VH rats kindled more slowly and displayed significant conditioned effects only toward the end of the experiment, and the DH rats kindled most slowly and had not demonstrated any significant conditioned effects by the end of the experiment. Third, the direction of the conditioned effects observed on the interictal behavior of the PRh and VH rats was the same: Both groups displayed more freezing in the CS+ than in CS−, and they both avoided the CS+ in the conditioned place-preference test.

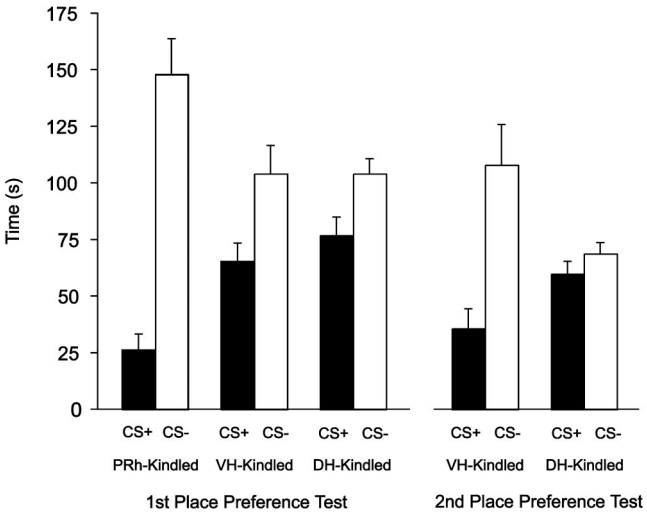

Figure 4. Conditioned place-preference test: The mean amount of time spent by the perirhinal cortex (PRh)-kindled rats, the ventral hippocampus (VH)-kindled rats, and the dorsal hippocampus (DH)-kindled rats in the CS+ and CS− during the 5-min place-preference test on Day 46 (left side). The mean amount of time spent by the VH- and DH-kindled rats in the CS+ and CS− during their second 5-min place-preference test on Day 100 (right side). Error bars represent the standard error of the mean.

On the basis of, in part, demonstrations of amygdala-kindling-induced increases in defensive behavior (e.g., Adamec, 1990; Helfer, Deransart, Marescaux, & Depaulis, 1996; Kalynchuk, Pinel, & Treit, 1999; Nieminen et al., 1992; but see Ebert & Koch, 1996; Witkin, Lee, & Walczak, 1988), the pattern of conditioned effects produced by amygdala kindling (i.e., less activity and more freezing in the CS+ environment, and an avoidance of the CS+ in a conditioned place-preference test) has been characterized as defensive (Barnes et al., 2001). Two equally tenable interpretations could account for the development of these conditioned defensive behaviors associated with amygdala kindling. The first is that they are a specific consequence of the amygdala's well-established role in fear and defensive behavior (e.g., Davis, 1998; Fanselow & Gale, 2003; Ledoux, 2003; Maren, 2001). The second is that they are a general consequence of the aversiveness of amygdala-kindled convulsions. The fact that the pattern of conditioned effects displayed by anterior neocortex-kindled rats is not indicative of increased defensiveness seems to support the first alternative: Anterior neocortex-kindled rats displayed no freezing and did not avoid the CS+ environment during a conditioned place preference test (Barnes et al., 2003). However, the fact that the PRh- and VH-kindled rats in the present experiment displayed a pattern of conditioned effects that seemed defensive in nature supports the second alternative, but with one modification: It seems that only convulsions of a "limbic" topography, such as those observed in amygdala, PRh, VH, and DH kindling but not in anterior neocortex kindling, can serve as an aversive unconditioned stimulus in the conditioning of interictal defensive behaviors in kindled rats. In light of the latter interpretation, it is particularly interesting that Hannesson et al. (2005) have recently shown that PRh kindling produces increases in defensive behavior comparable with those seen after amygdala kindling.

In contrast to the hippocampal-kindled convulsions in the present experiment, amygdala-kindled convulsions have been found to be less severe in the CS− than in the CS+. One possible explanation for this effect is that it is an indirect consequence of the effects of the CS+ on interictal behavior: Amygdala-kindled rats were less active and froze more in the CS+ than in the CS−. It is possible that these differences in interictal activity could differentially affect the subsequent convulsions. The results of the present experiment suggest otherwise. There was no effect of the CS+ on the convulsions of the PRh and VH rats, despite it having an effect on their interictal behavior, even more pronounced than it was in the amygdala-kindled rats (e.g., see Figure 2A and 2B in Barnes et al., 2003).

In their study on the conditioning of flavor aversions by amygdala kindling, Wig, Barnes, and Pinel (2002) reported a large positive correlation (i.e., $r = .90$) between kindling rate and the rate at which rats learned to discriminate between two flavors, one that always preceded an amygdalar stimulation and another that always preceded sham stimulation. Similarly, Barnes et al. (2001) noted that the emergence of significant conditioned effects of the stimulation environment on the interictal behavior of amygdala-kindled rats roughly coincided with the emergence of Class 5 or greater convulsions. Likewise, in the present experiment, kindling rate in the PRh-, VH-, and DH-kindled rats was related to the rate and magnitude of conditioning. The fact that the same relationship has been observed when kindling from several sites suggests that the generalized convulsions, rather than the stimulations or focal convulsions, serve as the US in the conditioning of interictal defensive behavior by kindling. However, a conclusive determination of the nature of the US in kindling experiments will be difficult if not impossible, because the two most obvious ways of separating the effects of the stimulation from the effects of the resulting convulsion seem to be unfeasible. First, using subthreshold stimulation intensities, which do not elicit convulsions, would produce only a temporary separation because such subthreshold stimulations rapidly lower the convulsion threshold (Pinel, Skelton, & Mucha, 1976; Racine, 1972a). Second, any pharmacological procedure for keeping stimulated rats from experiencing convulsions would confound any comparisons with those rats experiencing both stimulations and convulsions.

The lack of an effect of the stimulation environment on the interictal behavior of the DH-kindled rats in this experiment is surprising in light of the importance of the DH in spatial learning and memory (e.g., Pothuizen, Zhang, Jongen-Relo, Feldon, & Yee, 2004) and contextual fear conditioning (e.g., Lee & Kesner, 2004). One potential explanation lies in the conclusion reached by Hannesson and Corcoran (2000) about the amnestic effects of kindled convulsions. They concluded that kindling-related memory impairments are specific to the mnemonic functions of the kindling site. For example, DH-kindled convulsions seem to specifically disrupt spatial learning and memory while leaving other sorts of learning and memory intact (Hannesson et al., 2001). Moreover, DH kindling can have retrograde effects on spatial tasks learned several days prior to kindling (Laurent-Demir & Jaffard, 1997; Leung & Shen, 1991) with as few as five DH afterdischarges (Laurent-Demir & Jaffard, 1997). This might be why no conditioned effects were observed on the interictal behavior of the DH-kindled rats in the present experiment. The DH stimulations may have impaired their ability to discriminate between the two

environments. Alternatively, the lack of conditioned effects in the DH-kindled rats might simply reflect the fact that they experienced fewer fully generalized convulsions (i.e., of a Class 5 or greater). They experienced a mean of only 14.3 fully generalized convulsions, whereas the PRh- and VH-kindled rats experienced means of 20.6 and 24.9, respectively.

The lack of a conditioned effect on the convulsions of the PRh-, VH-, and DH-kindled rats was unanticipated given that the convulsions of both amygdala- and anterior neocortex-kindled rats are significantly influenced by a contextual CS+ (Barnes et al., 2001, 2003). One potential explanation may be that the nature of the CS is a determinant of the effects of conditioning on convulsions. For example, an environmental CS may be effective in conditioning some types of convulsions, such as those kindled from the amygdala and anterior neocortex, but not others, such as those kindled from the PRh, VH, and DH. According to this view, other types of CSs, such as discrete light or tone stimuli, could have effects on PRh-, VH-, or DH-kindled convulsions but not on amygdala- or anterior neocortex-kindled convulsions.

References

Adamec, R. E. (1990). Does kindling model anything clinically relevant? *Biological Psychiatry, 27,* 249–279.

Barnes, S. J., Pinel, J. P., Francis, L. H., & Wig, G. S. (2001). Conditioning of ictal and interictal behaviors in rats by amygdala kindling: Context as the conditional stimulus. *Behavioral Neuroscience, 115,* 1065–1072.

Barnes, S. J., Pinel, J. P., Wig, G. S., Stuettgen, M. C., & Holzel, C. H. (2003). Stimulation site determines the conditioned effects of kindling in rats: Anterior neocortex versus amygdala. *European Journal of Neuroscience, 17,* 1671–1679.

Burnham, W. M. (1978). Cortical and limbic kindling: Similarities and differences. In K. E. Livinston & O. Horykiwicz (Eds.), *Limbic mechanisms: The continuing evolution of the limbic system concept* (pp. 507–519). New York: Plenum Press.

Davis, M. (1998). Anatomic and physiologic substrates of emotion in an animal model. *Journal of Clinical Neurophysiology, 15,* 378–387.

Ebert, U., & Koch, M. (1996). Amygdala kindling does not change emotional responding as measured by the acoustic startle response in the rat. *Brain Research, 733,* 193–202.

Fanselow, M. S., & Gale, G. D. (2003). The amygdala, fear, and memory. *Annals of the New York Academy of Sciences, 985,* 125–134.

Hannesson, D. K., & Corcoran, M. E. (2000). The mnemonic effects of kindling. *Neuroscience and Biobehavioral Reviews, 24,* 725–751.

Hannesson, D. K., Howland, J., Pollock, M., Mohapel, P., Wallace, A. E., & Corcoran, M. E. (2001). Dorsal hippocampal kindling produces a selective and enduring disruption of hippocampally mediated behavior. *Journal of Neuroscience, 21,* 4443–4450.

Hannesson, D. K., Howland, J., Pollock, M., Mohapel, P., Wallace, A. E., & Corcoran, M. E. (2005). Anterior perirhinal cortex kindling produces long-lasting effects on anxiety and object recognition memory. *European Journal of Neuroscience, 21,* 1081–1090.

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

Kalynchuk, L. E., Pinel, J. P., & Treit, D. (1999). Characterization of the defensive nature of kindling-induced emotionality. *Behavioral Neuroscience, 113,* 766–775.

Keppel, G., & Zedeck, S. (1989). *Data analysis for research designs: Analysis of variance and multiple regression/correlation approaches.* New York: W. H. Freeman.

Laurent-Demir, C., & Jaffard, R. (1997). Temporally extended retrograde amnesia for spatial information resulting afterdischarges induced by electrical stimulation of the dorsal hippocampus in mice. *Psychobiology, 25,* 133–140.

LeDoux, J. (2003). The emotional brain, fear, and the amygdala. *Cellular and Molecular Neurobiology, 23,* 727–738.

Lee, I., & Kesner, R. P. (2004). Differential contributions of dorsal hippocampal subregions to memory acquisition and retrieval in contextual fear-conditioning. *Hippocampus, 14,* 301–310.

Leung, L. S., & Shen, B. (1991). Hippocampal CAl evoked response and radial 8-arm maze performance after hippocampal kindling. *Brain Research, 555,* 353–357.

Maren, S. (2001). Neurobiology of Pavlovian fear conditioning. *Annual Review of Neuroscience, 24,* 897–931.

McIntyre, D. C., Kelly, M. E., & Armstrong, J. N. (1993). Kindling in the perirhinal cortex. *Brain Research, 615,* 1–6.

McIntyre, D. C., Kelly, M. E., & Dufresne, C. (1999). Fast and slow amygdala kindling rat strains: Comparison of amygdala, hippocampal, piriform and perirhinal cortex kindling. *Epilepsy Research, 35,* 197–209.

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

Paxinos, G., & Watson, C. (1986). *The rat brain in stereotaxic coordinates* (2nd ed.). San Diego, CA: Academic Press.

Pinel, J. P. J., & Rovner, L. I. (1978). Experimental epileptogenesis: Kindling-induced epilepsy in rats. *Experimental Neurology, 58,* 190–202.

Pinel, J. P. J., Skelton, R., & Mucha, R. F. (1976). Kindling-related changes in afterdischarge "thresholds." *Epilepsia, 17,* 197–206.

Pothuizen, H. H., Zhang, W. N., Jongen-Relo, A. L., Feldon, J., & Yee, B. K. (2004). Dissociation of function between the dorsal and the ventral hippocampus in spatial learning abilities of the rat: A within-subject, within-task comparison of reference and working spatial memory. *European Journal of Neuroscience, 19,* 705–712.

Racine, R. J. (1972a). Modification of seizure activity by electrical stimulation: I. Afterdischarge threshold. *Electroencephalography and Clinical Neurophysiology, 32,* 269–279.

Racine, R. J. (1972b). Modification of seizure activity by electrical stimulation: II. Motor seizure. *Electroencephalography and Clinical Neurophysiology, 32,* 281–294.

Racine, R. J. (1975). Modification of seizure activity by electrical stimulation: Cortical areas. *Electroencephalography and Clinical Neurophysiology, 39,* 1–12.

Racine, R., Rose, P. A., & Burnham, W. M. (1977). Afterdischarge thresholds and kindling rates in dorsal and ventral hippocampus and dentate gyrus. *Canadian Journal of Neurological Sciences, 4,* 273–278.

Sato, T., Yamada, N., Morimoto, K., Uemura, S., & Kuroda, S. (1998). A behavioral and immunohistochemical study on the development of perirhinal cortical kindling: A comparison with other types of limbic kindling. *Brain Research, 811,* 122–132.

Seidel, W. T., & Corcoran, M. E. (1986). Relations between amygdaloid and anterior neocortical kindling. *Brain Research, 385,* 375–378.

Tukey, J. W. (1977). *Exploratory data analysis.* Reading, MA: Addison–Wesley.

Wig, G. S., Barnes, S. J., & Pinel, J. P. (2002). Conditioning of a flavor aversion in rats by amygdala kindling. *Behavioral Neuroscience, 116,* 347–350.

Winer, B. J., Brown, D. R., & Michels, K. M. (1991). *Statistical principles in experimental design* (3rd ed.). New York: McGraw–Hill.

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

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