

Stimulation site determines the conditioned effects of kindling in rats: anterior neocortex *versus* amygdala

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Abstract

Rats received 53 stimulations to either the left basolateral amygdala (BA) or left anterior neocortex (AN) in one environment (CS+) and 53 sham stimulations (the stimulation lead was attached but no current was delivered) in another environment (CS–), quasirandomly over 54 days. Confirming a previous report [Barnes, S.J., Pinel, J.P., Francis, L.H. & Wig, G.S. (2001) *Behav. Neurosci.*, **115**, 1065–1072], as BA kindling progressed, the CS+ began to elicit more defensive behaviours (i.e. less activity, more freezing and avoidance of the CS+) than the CS–, and at the end of the experiment, convulsions elicited in the CS+ were more severe than those elicited in the CS–. Like BA kindling, AN kindling led to less activity in the CS+; but unlike BA kindling, AN kindling led to more wet-dog-shakes and less, rather than more, severe convulsions in the CS+. During AN kindling, the mean number of wet-dog-shakes in the CS+ was negatively correlated with the mean convulsion class, suggesting that wet-dog-shakes contribute to the inherent variability of AN kindling. These findings confirm that inherent conditioned effects influence kindled convulsions and interictal behaviour and establish for the first time that the pattern of these conditioned effects is a function of the kindling site.

Introduction

The kindling phenomenon is a widely studied model of epilepsy and neural plasticity. The typical kindling experiment is well suited to the development of conditioned effects. During each of many stimulation trials, the subject is removed from its cage, a stimulation lead is attached, the subject is placed in the stimulation environment and the convulsive current is then delivered. Nevertheless, only a few studies have examined the degree to which the kindling procedure produces conditioned effects (Corcoran *et al.*, 1992; Kline *et al.*, 1997). However, if the kindling procedure does indeed produce conditioned effects, then a characterization of those effects could lead to important new insights into the mechanisms of kindling in particular and of neuroplasticity and epilepsy in general.

We recently demonstrated that a standard kindling protocol produces conditioned effects on both the convulsions and interictal behaviour of rats (Barnes *et al.*, 2001). Rats received 53 stimulations to the basolateral amygdala (BA) 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+. The primary purpose of the present experiment was to determine whether the kindling of structures other than the BA produces conditional effect and, if so, whether such conditional effects are different from those produced by BA kindling.

In the present experiment, we compared the behavioural effects – both ictal and interictal – conditioned to the stimulation environment during kindling of the BA with those conditioned by kindling of the anterior neocortex (AN). The BA was selected as one kindling site so that the experiment would include a replication of the conditioned effects that we had found to be associated with that structure (Barnes *et al.*, 2001). Because of the theoretical significance of this finding and because some investigators had failed to detect conditioned effects associated with amygdalar kindling (Wyler & Heavner, 1979; Myslobodsky *et al.*, 1983), this finding warranted replication. The AN was selected as the other kindling site because the topography of the convulsions elicited by AN kindling differs markedly from the convulsions associated with kindling of the BA or of other limbic sites (Burnham, 1978; Racine, 1975; Pinel, 1981; Seidel & Corcoran, 1986). Our premise was that, because of this difference in topography, a comparison of BA and AN kindling would likely reveal stimulation-site-related differences in the conditioned effects of kindling – if such differences existed. Furthermore, because AN kindled convulsions are highly variable in terms of both their severity (Burnham, 1978) and occurrence (Seidel & Corcoran, 1986) from subject to subject and from stimulation to stimulation in the same subject, we assumed that an analysis of the conditioned effects of AN kindling might provide insight into the source of variability in AN kindled convulsions.

Materials and methods

Subjects

The subjects were 36 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 (Ralston–

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Purina, St Louis, MO, USA) and water under a 12 h light/dark cycle with lights on at 07.30 h. All experimental procedures were approved by the University of British Columbia's Animal Care Committee.

Apparatus

The test apparatus comprised two similar, but distinguishable, stimulation chambers – one white and one black – connected by a central chamber which was half white and half black (see Barnes *et al.*, 2001; Fig. 1, 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. Half the central chamber was white and half was black, and 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 employed 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 behaviour.

Procedures

All experimental procedures were administered in the colony room during the light phase of the light/dark cycle, between 08.30 and 18.30 h.

Presurgery handling

Prior to surgery, each rat was handled for 1.5 min each day 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) was implanted in the left BA of 18 rats, and in the left AN of the other 18 rats, under ketamine (100 mg/kg, i.p.) and xylazine (10 mg/kg, i.p.) anaesthesia following standard stereotaxic protocols. The electrode tip was aimed 2.8 mm posterior, 5.0 mm left and 9.0 mm ventral to the skull surface at bregma for the BA rats, and it was aimed 0.5 mm anterior, 4.5 mm left and 1.5 mm ventral to the skull surface at bregma for the AN rats. The incisor bar was set at -3.3 mm, and all coordinates

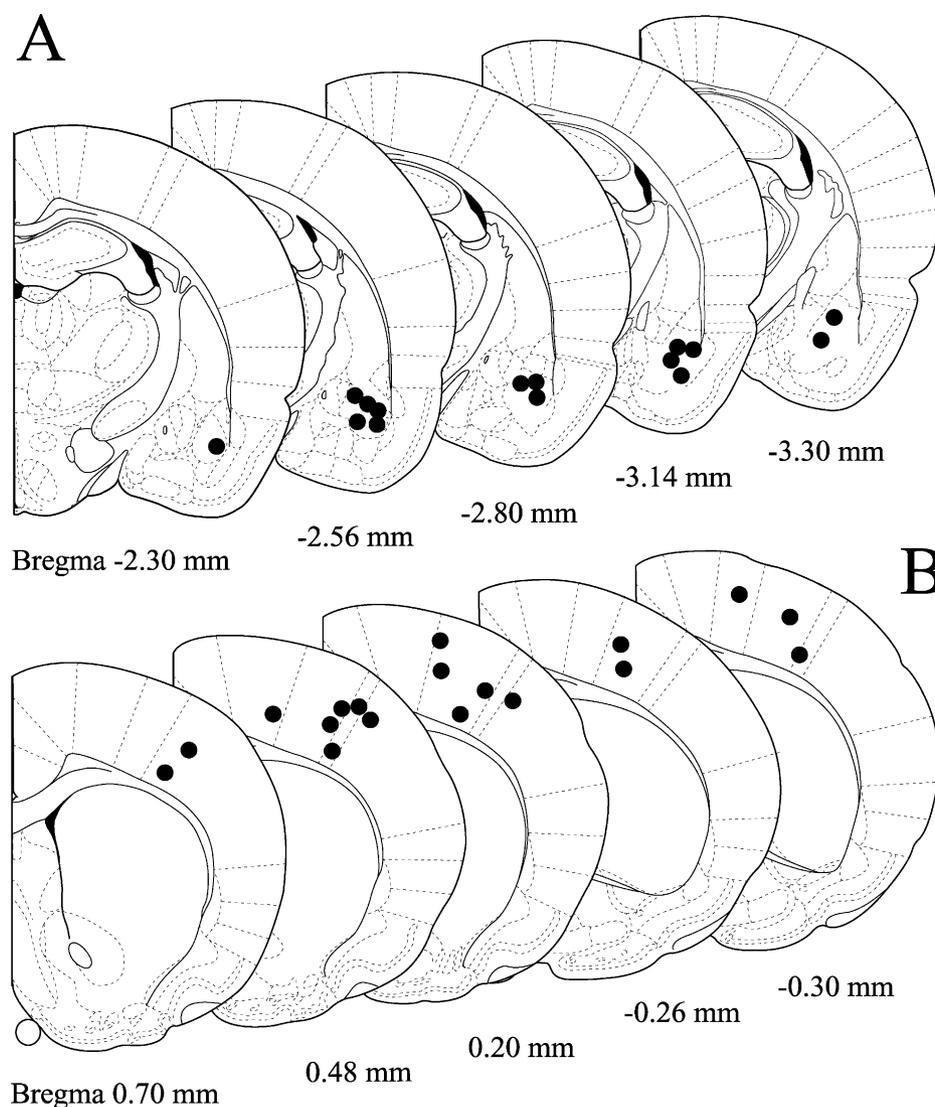


FIG. 1. Histology. The location of the electrode tips in the left basolateral amygdala (BA) of the 15 BA rats (A), and in the left anterior neocortex (AN) of the 18 AN rats (B). Each black dot represents the location of an electrode tip in one of the subjects.

were derived from (Paxinos & Watson, 1986; Seidel & Corcoran, 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 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 BA and the AN rats were each randomly divided into two equal groups, producing four groups (all $n = 9$). The rats in one of the BA and one of the AN groups were stimulated in the white chamber and sham stimulated in the black chamber; the rats in the other groups were stimulated in the black chamber and sham stimulated in the white chamber. Because the colour of the chambers proved to be inconsequential, the two BA groups were recombined ($n = 18$), and the two AN groups were recombined ($n = 18$) for the purposes of analysis. Most statistical comparisons were within-subjects comparisons between the behaviour of the subjects 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 stimulation (1 s, 60 Hz, 400 μ A 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 & Rovner's (1978) extension of Racine's (1972a) widely used 5-class 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; class 8, a running fit 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. EEG 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 subject, and the stimulation button was 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 behaviour of a subject in the CS+ and CS- could be attributed to only the conditional effects of differences between the CS+ and CS- (e.g. the colour, white or black; the location; or the odour).

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 h. The order of stimulation and sham-stimulation trials was quasirandom and was determined according to the following three restrictions: (i) there were 45 stimulations and 45 sham stimulations; (ii) no more than three stimulations or sham stimulations ever occurred consecutively; (iii) and every fourth

day (e.g. day 1, day 5, day 9, etc.) was a pre-administration-test day; those days always comprised one stimulation and one sham-stimulation trial in counterbalanced sequence.

The purpose of these pre-administration tests was to compare the subjects' behaviour in the 30 s prior to the stimulations in the CS+ and sham stimulations in the CS-. Accordingly all pre-administration tests were videotaped in their entirety. Three behaviours were quantified from the videotapes: (i) 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; (ii) 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; and (iii) rearing – the number of times that a rat lifted both its forepaws off the floor. All three measures were quantified by the same experimenter. 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 pre-administration test day, and Pearson- r inter-rater reliability quotients were calculated (activity = 0.98; freezing = 0.92; rearing = 0.94). Though these three behavioural measures are correlated with one another, we have demonstrated using multiple regression analyses that each measure contains nonredundant information (Barnes *et al.*, 2001).

Unexpectedly, on day 29, we noticed that several rats displayed wet-dog-shakes in the 30-s prestimulation period. A wet-dog-shake is a burst of rapid back-and-forth rotations of the upper torso and head – a movement similar to that made by a wet dog (Bedard & Pycocock, 1977). Because a wet-dog-shake has such a clear onset and offset, after day 29, thereafter the experimenter regularly recorded their incidence during the 30 periods prior to each stimulation in the CS+ or sham stimulation in the CS-. In contrast, the activity, freezing and rearing data were recorded only on pre-administration test days.

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- 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 blind to 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.

Conditioning maintenance trials

Because of the possibility that the conditioned place-preference test partially extinguished any conditional effects, the discrimination training procedure was reinstated on day 47 for an additional 8 days. All procedures during this phase were identical to those of the kindling phase.

Switch tests

Prior to testing on day 55, the day following the final conditioning maintenance trial, each of the two groups of rats (i.e. the BA and AN rats) was randomly divided into two equal groups (all $n = 9$). Then, the rats in one of the BA groups and one of the AN groups were stimulated in the CS-, whereas the rats in the other two groups were stimulated in the CS+. The latter two groups of rats were then stimulated in the CS- 4 h later on day 55; this schedule adhered to the pseudorandom two-trial-per-day schedule established during the kindling phase. The experimenter who scored the convulsions was blind to which chamber had previously served as the CS+ for each rat.

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 sectioned along the coronal plane through the amygdala. 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 stereotaxic atlas (1986).

Data transformations

All time-series data underwent two separate transformations prior to analysis. First, they were smoothed using the Hanning procedure, with a running mean of three, recommended for time-series data by Tukey (1977). Next, these smoothed data were blocked into four blocks. For the activity, freezing and rearing data, each of the four blocks consisted of three consecutive pre-administration-test days. In contrast, because wet-dog-shakes were not recorded until day 30 and were then recorded on every stimulation and sham-stimulation trial thereafter, they were blocked in a different manner. Each of the four blocks of wet-dog-shake data consisted of four consecutive stimulation sessions or four consecutive sham-stimulation sessions over the last 16 days of the experiment (i.e. days 30–45 inclusive). 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

Most of the analyses for the BA group (i.e. except for the time-series data) were one-tailed tests because that group replicated a previous experiment (Barnes *et al.*, 2001). Four different kinds of analyses were conducted to assess the statistical significance of the between-group and within-group differences. First, the activity, freezing, rearing and wet-dog-shake time-series data were analysed using planned orthogonal contrasts (POCs) between the CS+ and CS- for each separate block (i.e. blocks 1–4). Because POCs use the within-cell error term from an omnibus ANOVA (in these analyses this value was obtained from a 3-way between-within-ANOVA with block and CS as within-subject factors, and group as a between-subjects factor (i.e. BA *versus* AN); Keppel & Zedeck, 1989, p. 362; Winer *et al.*, 1991, pp. 342–343 and p. 526), they are mathematically independent and mutually exclusive, thus removing the need for corrections often employed when performing multiple comparisons (Miller, 1966; p. 35). Second, the place-preference data were analysed separately for each group (BA or AN) using dependent-samples *t*-tests. Third, the measures of the convulsion severity from the between-subjects switch test were analysed separately for each group (BA or AN) using independent-samples *t*-tests. Fourth, to confirm the results of this latter analysis, the statistical significance of the differences in the severity of the convulsions elicited in both groups of rats (AN or BA) by the final stimulation in the CS+ *versus* those elicited by the stimulation in the CS- (the within-subjects switch test) was assessed using dependent-samples *t*-tests.

Correlational analyses

Shortly after the systematic recording of wet-dog-shakes began (i.e. on day 30), it was noted that those rats tending to display the least number of wet-dog-shakes in the CS+ also seemed to have the most severe convulsions and that on those days when a particular rat displayed wet-dog-shakes, its convulsions were often less severe than on those days when it did not display wet-dog-shakes. Accordingly, two sorts of correlational analyses were performed. First, to assess the possibility

that a significant relationship existed between the number of wet-dog-shakes and between-subject differences in the severity of convulsions, two Pearson's *r*-values were calculated for the BA group and two were calculated for the AN group. The correlation was calculated between the mean number of wet-dog-shakes and either the mean convulsion class or duration of the convulsions displayed by each rat over the four blocks (from the time the wet-dog-shakes began to be systematically recorded on day 30 to the end of the kindling phase on day 45). Second, to assess the possibility that a significant relationship existed between the number of wet-dog-shakes and within-subject differences in the relative severity of convulsions, two dependent-samples *t*-tests were performed for the BA group and two were performed for the AN group. First, the median number of wet-dog-shakes that was displayed by each group of rats (i.e. BA or AN) over all four blocks was calculated. Then, this median value was used to divide the motor seizure class and duration data of each animal into two separate data sets for the dependent-samples *t*-test: one data set for those blocks when a rat's number of wet-dog-shakes exceeded the group median, and a second data set for those blocks when a rat's number of wet-dog-shakes did not. Finally, dependent-samples *t*-tests were performed on these sets for the BA rats and the AN rats.

Results

Both the BA-kindled and AN-kindled rats learned the relation between the stimulations or sham stimulations and their respective conditional contexts, and this conditioning affected both their convulsions and interictal behaviour. However, the nature of these conditioned effects was markedly different in the two kindled groups.

Histology

Of the original 18 BA rats, three were eliminated from the main analysis – one for having a defective electrode that precluded the development of convulsions and two because the tips of their electrodes lay outside the amygdala. Figure 1A illustrates the location of the electrode tips in the left amygdala of the 15 BA rats that successfully completed the experiment. Of these 15 BA rats, 11 rats had their electrode tips in the basolateral amygdala; one rat had its electrode tip in the lateral amygdala; one rat had its electrode tip in the central amygdala, and two rats had their electrode tips on the border between the basolateral and lateral amygdala. Because no systematic differences were observed between the behaviour of the 11 BA rats with electrode tips in the basolateral amygdala and the behaviour of the four BA rats with electrode tips in the lateral or central nuclei of the amygdala, the data of all 15 BA rats were subjected to analysis. Figure 1B illustrates the location of the electrode tips in the 18 AN rats. Of those 18 AN rats, 16 rats had their electrode tips in the somatosensory cortex, and two rats had their electrode tips in the motor cortex. Because no systematic differences were observed between the behaviour of the 16 AN rats with electrode tips in the somatosensory cortex and the behaviour of the two AN rats with electrode tips in the motor cortex, the data of all 18 AN rats were subjected to analysis.

Kindling

As previously reported (Miller, 1966; Burnham, 1978), the topography of the convulsions that were elicited by stimulations of the AN differed markedly from those elicited by stimulations of the BA. In the BA rats, the first stimulations elicited no convulsive responses, but with repeated stimulations facial clonic convulsions developed, and these clonic convulsions became progressively more generalized until they involved the entire body and loss of equilibrium. In other words, the

development of the BA convulsions was virtually always characterized by a progression through the classic limbic convulsion classes (i.e. classes 1–6). Moreover, the first few convulsions of the BA rats tended to have relatively long latencies, which became shorter as kindling progressed, until stimulation and convulsion onset were virtually synchronous. After about 20 stimulations, all 15 of the BA rats consistently displayed convulsions culminating in a loss of equilibrium (i.e. of a class 5 or higher) and lasting more than 40 s.

AN kindling produced convulsions that were topographically distinct from BA convulsions, and the topography of the AN convulsions displayed much greater between- and within-subjects variability (Seidel & Corcoran, 1986). The first stimulation elicited convulsions in 5 of the 18 AN rats, and these and all subsequent AN convulsions were very brief (usually less than 10 s) in comparison to the convulsions of the BA rats (Della Paschoa *et al.*, 1997). In addition to their brevity, all AN convulsions began coincidentally with the stimulation (i.e. there was no apparent latency) and always involved an initial brief clonic response (less than 5 s in duration) that consisted of either jaw clonus

(i.e. a class 1 convulsion), head bobbing with or without jaw clonus (i.e. a class 2 convulsion), or forelimb clonus with or without jaw clonus and/or head bobbing (i.e. a class 3 convulsion). This early clonic response was often accompanied by, or followed by, a mild tonic twisting of the head and sometimes the entire upper torso. AN convulsions differed markedly between subjects during each stimulation session; some of the AN rats would display a clonic-tonic response to the stimulation, whereas other AN rats would display only the clonic response or no observable response whatsoever. In addition to this between-subject variability, there was also substantial day-to-day variation in the convulsions of individual AN rats in the length of the convulsions, the nature of the clonic response (i.e. of different classes) or the presence or absence of tonus (Racine, 1975; Burnham, 1978). Often if a convulsion was elicited by a stimulation, many of the AN rats would not respond to the next stimulation, an effect termed 'days off' by Seidel & Corcoran (1986). Towards the end of the kindling phase, two of the 15 AN rats began to display convulsions that were similar in topography to those of the BA rats (Burnham, 1978).

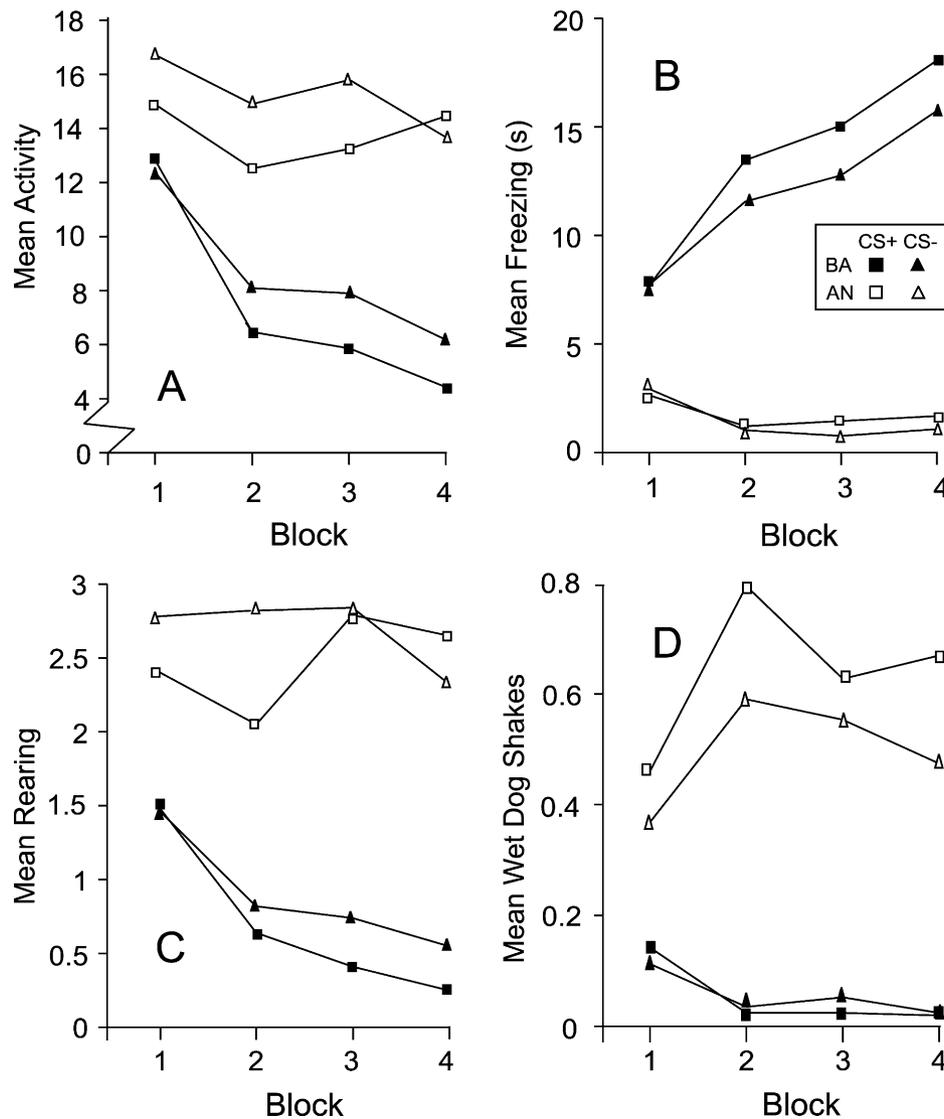


FIG. 2. Conditioning of interictal behaviours. The (A) mean ambulatory activity (B) freezing (C) rearing and (D) wet-dog-shakes recorded during each of the four blocks of three preadministration test days each; preadministration tests occurred every 4 days. After several stimulations the BA rats displayed significantly less ambulatory activity, more freezing and less rearing in the stimulation environment (CS+) than in the sham stimulation environment (CS-); but they displayed few wet-dog shakes in either environment. The AN rats displayed significantly less ambulatory activity, less rearing and more wet-dog-shakes in the CS+ than in the CS-; but they displayed little freezing in either environment.

Conditioning of interictal behaviours

The effects of the stimulation (CS+) and sham stimulation (CS-) environments on the ambulatory activity, freezing and rearing recorded during the pre-administration tests are illustrated in panels A, B and C of Fig. 2, respectively. Overall, the BA rats displayed less ambulatory activity, and more freezing in the CS+ than in the CS-; but they did not display significantly less rearing in the CS+ than in the CS-. The AN rats also displayed less ambulatory activity in the CS+ than in the CS-; but they reared more in the CS+ than in the CS-, and they did not freeze in either environment. Notice also in Fig. 2 that the BA rats displayed substantially less activity, more freezing, and less rearing than the AN rats irrespective of the particular environment. The effects of the stimulation and sham stimulation contexts on the number of wet-dog-shakes during the last 16 days of the kindling phase are illustrated in Fig. 2D. The BA rats displayed few wet-dog-shakes in either environment, but the AN rats displayed more wet-dog-shakes in the CS+ than in the CS-.

Activity

Figure 2A illustrates the mean number of line crossings by the BA and AN rats in the CS+ and CS- during the preadministration tests, which occurred prior to every fourth stimulation. The BA rats were significantly less active in the CS+ than in the CS- during block 3 (days 25–33), $F_{1,93} = 4.84$, $P = 0.030$, but not during block 1 (days 1–9), $F_{1,93} = 0.25$, $P = 0.62$, block 2 (days 13–21), $F_{1,93} = 3.02$, $P = 0.086$, and block 4 (days 37–45), $F_{1,93} = 3.537$, $P = 0.063$. In contrast, the AN rats were significantly less active in the CS+ than in the CS- during block 1 (days 1–9), $F_{1,93} = 4.70$, $P = 0.032$, block 2 (days 13–21), $F_{1,93} = 7.93$, $P = 0.0059$, and block 3 (days 25–33), $F_{1,93} = 9.16$, $P = 0.0032$, but not during block 4 (days 37–45), $F_{1,93} = 0.94$, $P = 0.34$.

Freezing

Figure 2B illustrates the mean duration of freezing of the BA and AN rats in the CS+ and CS- during the pre-administration tests. The BA rats displayed significantly more freezing in the CS+ than in the CS- during block 2 (days 13–21), $F_{1,93} = 7.97$, $P = 0.0057$, block 3 (days 25–33), $F_{1,93} = 11.53$, $P = 0.0010$, and block 4 (days 37–45), $F_{1,93} = 11.83$, $P = 0.00087$, but not during block 1 (days 1–9), $F_{1,93} = 0.0011$, $P = 0.97$. In contrast, the AN rats displayed virtually no freezing in either environment for the duration of the kindling phase, all $P > 0.25$.

Rearing

Figure 2C illustrates the mean number of times the BA and AN rats reared in the CS+ and CS- environments during the preadministration tests. Although Fig. 2C suggests that the BA rats did rear consistently less in the CS+ than in the CS-, these differences were not statistically significant, all $P > 0.10$. In contrast, the AN rats reared significantly less in the CS+ than in the CS- during block 2 (days 13–21), $F_{1,93} = 12.24$, $P = 0.00072$, but not during block 1 (days 1–9), $F_{1,93} = 3.55$, $P = 0.063$, block 3 (days 25–33), $F_{1,93} = 0.054$, $P = 0.82$, or block 4 (days 37–45), $F_{1,93} = 2.67$, $P = 0.10$.

Wet-dog-shakes

Figure 2D illustrates the mean number of wet-dog-shakes that the BA subjects had in the CS+ and CS-, during the last 16 days of the kindling phase (i.e. from the time the wet-dog-shakes began to be systematically recorded on day 30 to the end of the kindling phase on day 45). The BA rats displayed few wet-dog-shakes during this period in either the CS+ and CS-, all $P > 0.70$. However, the AN rats displayed significantly more wet-dog-shakes in the CS+ than in the CS- during block 2 (days 34–37), $F_{1,93} = 6.39$, $P = 0.013$, and block 4

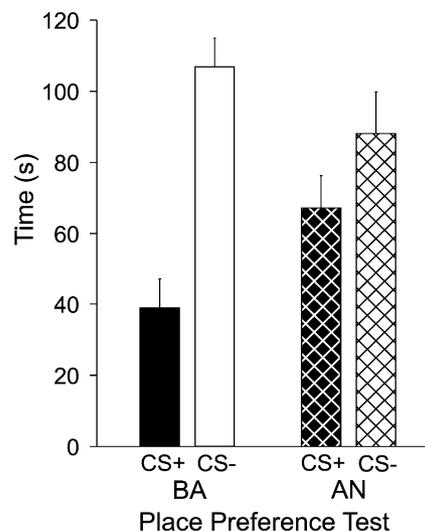


FIG. 3. Conditioned place preference test. The mean amount of time spent by the BA and the AN rats in the CS+ and CS- during the 5-min place-preference test on day 46. The BA rats spent significantly less time in the CS+ than in the CS-. The AN rats spent an equivalent amount of time in both CSs.

(days 42–45), $F_{1,93} = 5.82$, $P = 0.018$, but not during block 1 (days 30–33), $F_{1,93} = 1.32$, $P = 0.25$, or block 3 (days 38–41), $F_{1,93} = 5.82$, $P = 0.018$.

Conditioned place-preference

Figure 3 shows the total amount of time that the BA and AN rats spent in the CS+ and CS- during the conditioned place-preference test. The BA rats spent significantly less time in the CS+ than in the CS- ($M = 106.87$ versus 39.00), $t_{14} = 5.42$, $P = 0.000045$. In fact, 14 of the 15 BA rats spent less time in the CS+; four did not enter the CS+ at all, and 12 of the 15 chose to enter the CS- first, $\chi^2_1 = 8.07$, $P = 0.0045$. In contrast, the amount of time that AN rats spent in the CS+ did not differ significantly from the amount of time they spent in the CS- (mean; $M = 88.06$ versus 67.17), $t_{17} = 1.12$, $P = 0.28$. Because two of the 18 AN rats had developed convulsions that were similar in topography and duration to those of the BA rats a posthoc analysis was conducted on their place preference data. Like the BA rats, these two AN rats spent significantly less time in the CS+ than in the CS- ($M = 97.00$ versus 43.00), $t_1 = 27.00$, $P = 0.024$.

Conditioning of convulsions

Figure 4A–D illustrates the means of the four measures of the severity of the convulsions that were elicited on day 55, when half of the BA and AN rats were stimulated for the first time in the CS- while the other half were stimulated as usual in the CS+. The convulsions of the BA rats stimulated for the first time in the CS- were associated with significantly shorter durations ($M = 27.88$ versus 46.71), $t_{13} = 2.25$, $P = 0.025$; lower classes ($M = 3.38$ versus 5.14), $t_{13} = 2.30$, $P = 0.019$; and longer latencies ($M = 10.12$ versus 1.29), $t_{13} = 2.25$, $P = 0.02$; but not fewer falls ($M = 0.50$ versus 1.71), $t_{13} = 1.68$, $P = 0.059$. Indeed, one BA rat failed to respond with any convulsion when stimulated in the CS-, despite previously displaying 18 consecutive generalized convulsions (i.e. of a class 5 or higher) in the CS+. In contrast to the BA rats, the AN rats displayed significantly longer, rather than shorter convulsions in the CS- ($M = 13.11$ versus 1.00), $t_{16} = 2.69$, $P = 0.016$; but the other measures of convulsion severity did not differ significantly in the two environments: class ($M = 3.11$ versus 1.89), $t_{16} = 1.60$, $P = 0.13$; latencies ($M = 5.33$ versus 0), $t_{16} = 1.58$, $P = 0.13$; or number of falls ($M = 0.11$ versus 0), $t_{16} = 1.00$, $P = 0.33$.

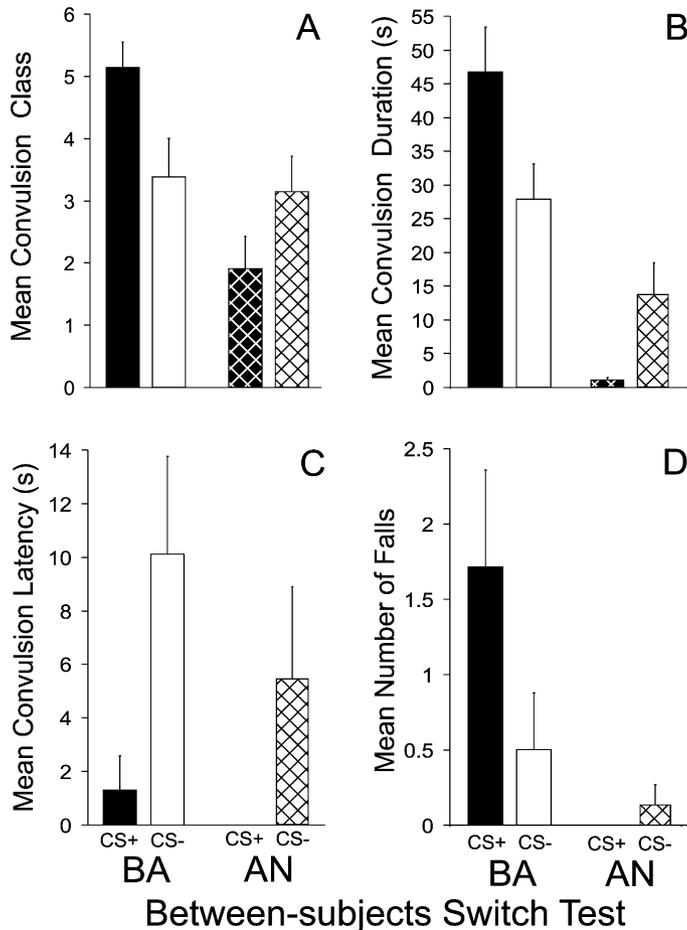


FIG. 4. Conditioning of convulsions. Between-subjects comparisons. The (A) mean class (B) duration (C) latency and (D) number of falls associated with convulsions elicited on day 55, when half of each of the BA and AN rats was stimulated for the first time in the CS- while the other half of each group was stimulated as usual in the CS+. The convulsions of the BA rats stimulated in the CS- were significantly less severe than the convulsions of the BA rats stimulated in the CS+. In contrast, the convulsions of the AN rats stimulated in the CS- were significantly more severe than the convulsions of the AN rats stimulated in the CS+.

Figure 5A–D illustrates the means of the four measures of the severity of the convulsions elicited in the BA and AN rats in the CS- in relation to the convulsions elicited by their final stimulation in the CS+. These within-groups comparisons confirmed the findings of the between-groups comparisons (see Fig. 4). The convulsions of the BA rats in the CS- were characterized by shorter durations ($M = 28.27$ versus 45.20), $t_{14} = 2.74$, $P = 0.0080$; lower classes ($M = 4.00$ versus 5.27), $t_{14} = 3.67$, $P = 0.0012$; longer latencies ($M = 8.73$ versus 60), $t_{14} = 3.83$, $P = 0.00092$; and significantly fewer falls ($M = 0.53$ versus 1.67), $t_{14} = 3.90$, $P = 0.00080$, indicating weaker convulsions in the CS-. In contrast, the convulsions of the AN rats in the CS- were characterized by longer durations ($M = 9.67$ versus 3.22), $t_{17} = 2.58$, $P = 0.019$; and higher classes ($M = 3.33$ versus 2.33), $t_{17} = 2.47$, $P = 0.024$, both indicating more severe convulsions in the CS-. However, there were no significant differences in latency ($M = 2.67$ versus 0), $t_{17} = 1.51$, $P = 0.15$; or in the number of falls ($M = 0.11$ versus 11), $t_{17} = 0.00$, $P = 1.00$. The two anomalous AN rats, like the BA rats, displayed a decrease, rather than an increase, in convulsion severity when stimulated in the CS- relative to their last stimulation in the CS+. The first rat had a class 5 convulsion of 15 s in duration after its last stimulation in the CS+, but then only a class 4 convulsion of 2 s

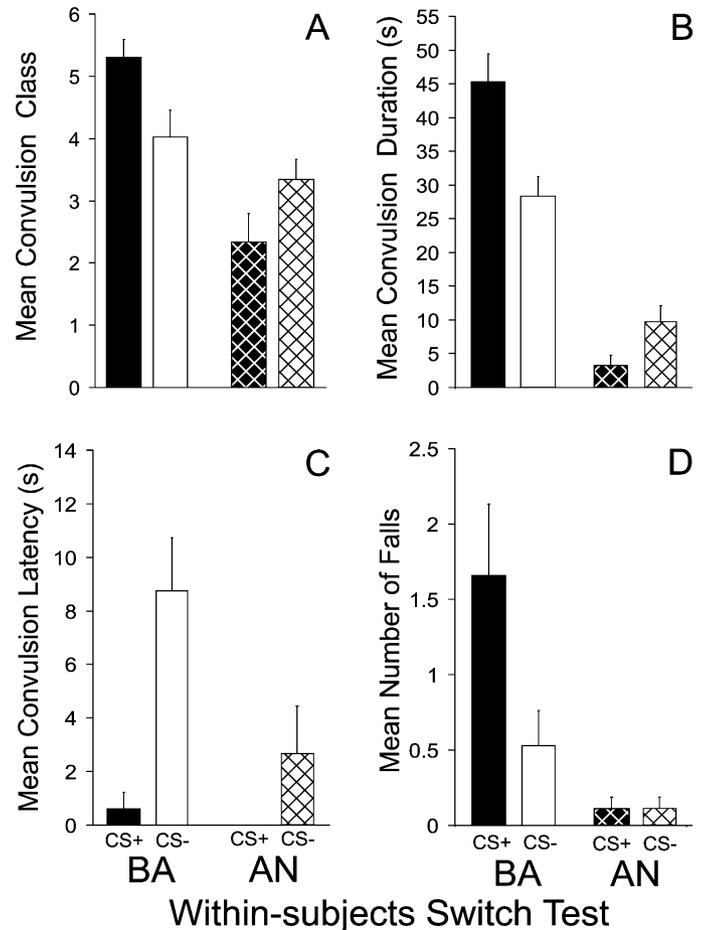


FIG. 5. Conditioning of convulsions. Within-subjects comparisons. The (A) mean class (B) duration (C) latency and (D) number of falls associated with convulsions elicited on day 55, when all of the BA and AN rats were stimulated for the first time in the CS-. The convulsions of the BA rats were significantly less severe in the CS- than in the CS+. In contrast, the convulsions of the AN rats were significantly more severe in the CS- than in the CS+.

in duration when stimulated in the CS-. The second rat had a class 6 convulsion of 25 s in duration after its last stimulation in the CS+, but then only a class 4 convulsion of 19 s in duration when stimulated in the CS-.

Correlational analyses

Two types of analyses were performed to confirm that a significant relationship existed between the number of wet-dog-shakes and convulsion severity: between-subjects correlations analyses and within-subjects correlations.

Between-subjects correlations

For the BA rats, there was no significant correlation between wet-dog-shakes and convulsion class, $r = 0.065$, $t_{60} = 0.49$, $P = 0.62$, nor between wet-dog-shakes and convulsion duration, $r = 0.19$, $t_{60} = 1.47$, $P = 0.15$. In contrast, those AN rats that displayed more wet-dog-shakes also had convulsions of a lower class, $r = -0.37$, $t_{72} = 3.33$, $P = 0.0014$, and of a shorter duration, $r = -0.27$, $t_{72} = 2.34$, $P = 0.022$.

Within-subjects correlations

Because the BA rats displayed a median of 0.0 wet-dog-shakes over the last 16 days of the kindling phase, the period over which they were systematically recorded, these analyses could not be performed. In

contrast, the AN rats displayed a median of 0.50 wet-dog-shakes over the last 16 days of the kindling phase. There were significant correlations between the number of wet-dog-shakes displayed by individual AN rats in a particular block and the class, $t_{17} = 2.85$, $P = 0.011$, and duration, $t_{17} = 2.73$, $P = 0.014$, of convulsions that they displayed in the same blocks.

Discussion

In the present experiment, we compared the conditioned effects of basolateral amygdala (BA) kindling with those of anterior neocortex (AN) kindling. There were seven major findings. First, as kindling progressed both the BA-kindled and AN-kindled rats began to display less activity in the CS+ environment than in the CS- environment. Second, as kindling progressed the BA-kindled rats began to display more freezing in the CS+ environment than in the CS- environment, whereas the AN-kindled rats did not display freezing in either the CS+ or CS- environments. Third, as kindling progressed the AN-kindled rats began to display less rearing in the CS+ environment than in the CS- environment. Fourth, as kindling progressed the AN-kindled rats began to display more wet-dog shakes in the CS+ environment than in the CS- environment, whereas BA-kindled rats did not display wet-dog-shakes in either the CS+ or CS- environments. Fifth, the BA-kindled rats avoided the CS+ environment during a conditioned place preference test, whereas the AN-kindled rats did not. Sixth, the number of wet-dog-shakes displayed by the AN-kindled rats was negatively correlated with convulsion severity, both between AN-kindled rats, and within each individual AN-kindled rat from stimulation to stimulation. Seventh, when finally stimulated in the CS- environment, the BA-kindled rats displayed milder convulsions, whereas the AN-kindled rats displayed more severe convulsions.

The present findings confirm the previously reported conditioned effects of the stimulation environment in BA-kindled rats (Barnes *et al.*, 2001). More importantly, by comparing the conditioned effects of BA and AN kindling, we established for the first time that conditioned effects are not restricted to BA kindling and that the nature of such conditioned effects are a function of kindling site.

The present data indicate that conditioned effects are an important component of the kindling phenomenon – at least for BA and AN kindling. This finding has an important theoretical implication; the search for the neural mechanisms of kindling phenomena and their interictal manifestations cannot be entirely successful without considering the contributions of conditioned effects. For example, the increases in interictal defensive behaviour produced by amygdala kindling, which are currently being used to model epilepsy-related psychopathology (Kalynchuk, 2000), likely have both conditioned and unconditioned components that undoubtedly have different mechanisms; variables that influence the expression of interictal behaviour in kindled animals could act on either of these mechanisms.

The previously reported pattern of conditioned effects produced by BA kindling (i.e. less activity, less rearing and more freezing in the CS+ environment) has been characterized as defensive (Barnes & Pinel, 2001; Barnes *et al.*, 2001; Wig *et al.*, 2002). Two equally tenable interpretations can account for the development of these conditioned defensive behaviours: one is that they are a specific consequence of the amygdala's well established role in fear and defensive behaviour; the second is that they are a general consequence of the aversiveness of kindled convulsions irrespective of the site of kindling. The fact that the pattern of conditioned effects displayed by the AN rats was not indicative of increased defensiveness seems to support the first alternative. The AN rats displayed no freezing, and they did not avoid the CS+ environment during a conditioned place preference test –

declines in activity and rearing may have been an indirect consequence of the wet-dog-shakes.

Although the present results clearly establish that the conditioned effects of kindling are not the same for all kindling sites, scrutiny of the behaviour of the two anomalous AN rats suggests that kindling site is not the only factor that influences the conditioned effects of kindling. Convulsions kindled from the AN progress through several stages (Burnham, 1978). The first convulsions involve only brief clonus (i.e. always less than 10 s) – termed 'early clonus', but as kindling continues and after discharges become more generalized a period of tonus starts to follow this early clonus, and eventually a second period of clonus (i.e. 'late clonus') is added to the sequence. With extended kindling of the anterior neocortex, the topography of this late clonic component becomes increasingly similar to 'limbic' convulsions, such as those elicited by BA kindling (Pinel, 1981). In the present experiment, only two AN rats developed convulsions that were topographically similar to limbic convulsions. Interestingly, at the end of the experiment, these two anomalous AN rats behaved more like BA rats than the AN rats; when stimulated in the CS- environment, their convulsions were less severe than in the CS+ environment, and they also avoided the CS+ environment in the conditioned place preference test. These observations suggest that the conditioned effects on kindled convulsions and interictal behaviour may change as they generalize into new circuits and the topography of the convulsions changes. Moreover, they suggest that the conditioned interictal defensive behaviours are associated with kindled convulsions that are topographically 'limbic' in nature.

The severity of AN-kindled convulsions was negatively correlated with the expression of wet-dog-shakes in the CS+ environment, both between the AN-kindled rats and within individual AN-kindled rats from stimulation to stimulation. This negative correlation indicates that conditioned wet-dog-shakes might play a role in blocking AN-kindled convulsions. For example, the fact that more wet-dog-shakes occurred in the CS+ (Fig. 2D) may explain why the convulsions elicited in the CS+ by AN stimulation were weaker than those elicited in the CS- (Figs 4 and 5). Moreover, the correlation between wet-dog-shakes and AN-kindled convulsions suggests that the well-documented day-to-day variation in AN-kindled convulsions (Burnham, 1978; Seidel & Corcoran, 1986) may be a consequence of variations in the prevalence of wet-dog-shakes.

From the present results, it is not possible to determine whether the brain stimulations, the convulsions, or an interaction between the two served as the unconditioned stimulus (US). However, two pieces of evidence suggest that the generalized convulsions in particular served as the US, at least in the conditioning of interictal defensive behaviour in the BA-kindled rats. First, in two previous experiments (Barnes *et al.*, 2001; Wig *et al.*, 2002) the emergence of a conditioned discrimination between CS+ and CS- stimuli roughly coincided with the emergence of fully generalized BA-kindled convulsions. Second, in our demonstration of the conditioning of flavor aversions by BA kindling (Wig *et al.*, 2002), we found a strong positive correlation (i.e. $r = 0.90$) between the rate of learning of a discrimination (i.e. between the CS+ and CS- flavors) and the rate of development of generalized BA kindled convulsions. Those data suggest that, for the BA-kindled rats in the present experiment, the generalized convulsions served as the US. Sorting out the nature of the US in kindling experiments will be difficult because the two most obvious ways of separating the effects of the stimulation from the effects of the resulting convulsion appear 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 (Racine, 1972b; Pinel, Skelton, & Mucha, 1976). Second,

any pharmacological procedure for keeping stimulated subjects from experiencing convulsions would confound any comparisons with those subjects experiencing both stimulations and convulsions.

The present results are similar to demonstrations of the situational specificity of drug tolerance (Burnham, 1978; Siegel *et al.*, 1982) and sensitization (Weiss *et al.*, 1989). In studies of conditioned drug tolerance and drug sensitization, subjects receive a series of drug administrations in the same environment, and that environment begins to elicit conditioned responses that offset or augment the drug effects, thus contributing to the development of tolerance or sensitization, respectively. 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 convulsions. In the BA rats, these conditioned effects seemed to potentiate, rather than counteract, the effects of the unconditioned stimulus – convulsions elicited in the usual stimulation environment were more severe than those elicited in the sham stimulation environment; whereas the reverse was true for the AN rats. Accordingly, the conditioned response in the BA rats seem to be similar to conditioned drug sensitization, and the conditioned response in the AN rats seem to be similar to conditioned drug tolerance.

Just as conditioned effects play a role in the development of drug sensitization or tolerance, the conditioned effects of kindling might play an important role in the development and maintenance of kindled convulsions; with repeated stimulation, an animal could develop a conditioned compensatory response (CCR) that would be initiated by CSs that predict the onset of the unconditional stimulus (US). In the context of the analysis of Ramsay & Woods (1997), the disruption of neural activity after the application of electrical stimulation to a particular brain site would be the US, and the elicited reactions to this disruption would be unconditioned responses (URs). The nature of these URs would be dependent on the site of stimulation. With repeated stimulation, CSs would begin to elicit CCRs that would offset the effects of the URs. The nature of these CCRs would also be dependent on the site of stimulation. We have shown in the present experiment that the stimulation environment can serve as a CS for such conditioning and that the resulting CCRs are likely a function of the kindling site.

In the present experiment, the convulsions of the BA rats were more severe in the CS+ than in the CS-. These data seem to contradict the hypothesis that CCRs play a role in the development and maintenance of amygdala kindled convulsions. However, at least in the context of kindling, the fact that a CCR appears to be maladaptive does not challenge the potential existence of that CCR, for there is no reason to believe that the intracerebral application of an exogenous stimulus (e.g. a kindling stimulation) is a situation for which an adaptive response could have evolved.

Finally, it should be emphasized that the magnitude of the conditioned effects reported in the present experiment likely underestimate those that routinely occur in kindling experiments, at an equivalent number of stimulations. For control purposes, the rats in the present experiment were required to learn a discrimination between two similar environments, whereas in conventional kindling experiments, there are innumerable environmental, behavioural and temporal cues that reliably predict each stimulation.

Abbreviations

AN, anterior neocortex; BA, basolateral amygdala; CCR, conditioned compensatory response; CR, conditioned response; CS, conditioned stimulus; *M*, mean; POC, planned orthogonal contrast; UR, unconditioned response; US, unconditioned stimulus.

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