CHARACTERIZATION OF CONDITIONED EFFECTS

IN THE KINDLING MODEL OF EPILEPSY AND NEUROPLASTICITY

by

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ABSTRACT

Mild periodic electrical stimulation to any one of many brain sites leads to the development and progressive intensification of elicited convulsions. This "kindling phenomenon" has been widely studied as a model of epilepsy and neuroplasticity. We recently discovered that subjects in conventional kindling experiments learn the relationship between the stimulation environment and the subsequent stimulation and convulsion and that this conditioning has significant effects on both their convulsions and interictal (between convulsions) behaviour. Specifically, the convulsions of rats kindled from the basolateral amygdala (BA) were more severe in an environment in which they had always been stimulated than in an environment in which they had never been stimulated, and they displayed more interictal defensive behaviour in the stimulation environment. The objective of the present experiments was to establish the reliability, generality, nature, and theoretical significance of this discovery.

This thesis comprises three different lines of experiments. The first line confirmed that the effects of the stimulation environment observed during BA kindling are the result of Pavlovian conditioning. The second line explored the conditioned effects associated with the kindling of brain structures other than the BA; the results suggested that kindling site determines the conditioned effects of the stimulation environment on convulsions and interictal behaviour. The third line demonstrated that conditioned effects contribute substantially to two of the defining features of the kindling phenomenon: its permanence (If a rat is left unstimulated for several months, fully generalized convulsions are quickly elicited once kindling recommences.) and its "transfer" between brain sites (If a rat is kindled from one brain site, it subsequently requires fewer stimulations to kindle from a second brain site.).

The present results not only characterize the conditioned effects of kindling, they also indicate that such effects are a general and reliable component of kindling. Clearly, the key to discovering the mechanisms underlying kindling lies, to a large degree, in the interactions of the subjects with the cues that predict each stimulation and not solely in the unconditioned consequences of the brain stimulations and convulsions.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS

AD   -afterdischarge

AN   -anterior neocortex

ANOVA   -analysis of variance

BA   -basolateral amygdala

CCR   -conditioned compensatory response

CR   -conditioned response

CS   -conditioned stimulus

DH   -dorsal hippocampus

LA   -lateral amygdala

M   -arithmetic mean

PRh   -perirhinal cortex

rms   -root mean square

SEM   -standard error of the mean

UR   -unconditioned response

US   -unconditioned stimulus

VH   -ventral hippocampus

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GENERAL INTRODUCTION

The application of periodic mild electrical stimulation via an implanted electrode to any one of many brain sites leads to the development and gradual intensification of elicited convulsions (Goddard, McIntyre, & Leech, 1969). This phenomenon, known as "kindling," has been a major focus of neuroscientific research, and several thousand studies of kindling have been published. Kindling has been the focus of intensive investigation for two main reasons: Kindling has been found to have several interesting properties that are of practical and theoretical significance, and kindling models important clinical and neuroplastic phenomena.

We recently discovered that subjects in conventional kindling experiments learn the relationship between the stimulation environment and the subsequent stimulation and convulsion and that this conditioning has large and reliable effects on both their convulsions and interictal (between convulsions) behaviour. Specifically, the convulsions of rats kindled by basolateral amygdala stimulations were more severe in an environment in which they had always been stimulated than in an environment in which they had never been stimulated, and they displayed more interictal defensive behaviour in the stimulation environment (Barnes, Pinel, Francis, & Wig, 2001). The objective of the present experiments was to establish the reliability, generality, nature, and theoretical significance of this discovery. Accordingly, this Introduction comprises the following five sections: The first describes the major properties of kindling; the second explains why kindling is believed to model important phenomena; the third argues that the standard kindling protocol has the potential to produce inadvertent conditioned effects; the fourth summarizes previous research on conditioning and kindling; and finally, the fifth describes our initial characterization of the conditioned effects of kindling and provides a general rationale for the present experiments.

<div style="text-align: center;">Major Properties of Kindling</div>

In 1967, Goddard reported his discovery[1] of the kindling effect. Then, in 1969, Goddard, McIntyre and Leech published an exhaustive study in which they documented virtually all of its major characteristics. The thoroughness of this foundation study contributed substantially to the productivity of the first generation of kindling experiments (see Wada, 1976b).

In their prototypical experiment, Goddard, McIntyre, and Leech (1969) stimulated the amygdala of rats once per day. At first, each rat displayed no obvious behavioural response, but after several stimulations, it began to display mild convulsive responses restricted to the face. Then, with each successive stimulation, the convulsive response became more generalized until each rat displayed a fully generalized convulsion (i.e., a convulsion that involves all parts of the body and is characterized by loss of equilibrium). The effect was extremely reliable: Virtually every rat with an electrode in the amygdala kindled, and their convulsions progressed through the same distinct topographic stages.

Racine (1972) subsequently developed his widely used five-point scale for measuring the increase in generalization that typically occurs during the course of kindling rat limbic sites: class 1, rhythmic mouth and face movements; class 2, facial movements and head nodding; class 3, facial movements, head nodding, and forelimb clonus; class 4, facial movements, head

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[1] Although the discovery of kindling is generally attributed to Goddard (1967), Goddard credited the discovery to Delgado and Sevallino (1961): They had reported the phenomenon six years earlier in hippocampal-stimulated cats. However, Goddard, McIntyre, and Leech (1969) were the first to characterize and demonstrate the generality and reliability of the kindling phenomenon.

nodding, and forelimb clonus with rearing; and class 5, facial movements, head nodding, forelimb clonus, and rearing with loss of equilibrium (i.e., falling). Although most kindling experiments are terminated once rats display class 5 convulsions, Pinel and Rovner (1978) found that if many more stimulations are administered, the kindled convulsions go through further development. Consequently, they added three classes to Racine's five-class scale: class 6, a class 5 pattern with multiple rearing and falling episodes; class 7, a convulsion that includes a running fit; and class 8, a convulsion that includes periods of tonus. Other systems have been devised for classifying limbic convulsions in rodents (see Michael et al., 1998), but the Racine and, where appropriate, the Pinel and Rovner extension are most widely used.

Kindling is a remarkably general phenomenon, in three different respects. First, kindling has been demonstrated in a wide variety of species. Goddard et al. (1969) demonstrated kindling in rats, cats, and rhesus monkeys; and it was subsequently demonstrated in a wide variety of other vertebrates: for example, frogs (Morrell & Tsuru, 1976), mice (Leech & McIntyre, 1976), gerbils (Cain & Corcoran, 1980), rabbits (Tsuru, Kuniyoshi, & Idenoue, 1979), dogs (Wauquier, Ashton, & Melis, 1979), and baboons (Wada, Osawa, & Mizoguchi, 1975). However, there is marked interspecies variability in the number of stimulations required to kindle fully generalized convulsions (Wada, 1976a). Although no systematic studies of human kindling exist, there are several published (e.g., Morrell, 1985; Sramka, Deslak, & Nadvornik, 1977) and unpublished (see pg. 80 of McIntyre, Poulter, & Gilby, 2002) reports of kindling-like effects in humans. Second, kindling is general in the sense that it results from the stimulation of many, but not all, brain sites. For example, Goddard et al. (1969) found that all limbic and some cortical sites can be kindled, although the sites that do kindle vary in the number of stimulations required to kindle fully generalized convulsions (Goddard et al., 1969). Because Goddard et al. established that amygdalar kindling progresses relatively quickly, requiring only about 10 stimulations to achieve

full generalization (Burnham, 1975; Goddard et al., 1969), it became the "default" structure for kindling. And third, kindling is general in the sense that kindling-like phenomena can be produced with the periodic administration of most, if not all, convulsive agents (e.g., electroconvulsive shock, intracranially or systemically administered convulsant drugs, or convulsant vapours; Pinel & Van Oot, 1976), and even some initially nonconvulsant agents (e.g., intracranially administered lidocaine; Post et al., 1984).

The most theoretically significant property of kindling has proven to be its relative permanence. Goddard et al. (1969) found that if a kindled rat is left unstimulated for an extended period of time (e.g., several months), there are substantial savings in the number of stimulations required to elicit a fully generalized convulsion once stimulations are resumed. Often a fully generalized convulsion is elicited by the second or third stimulation following the stimulation-free period (Dennison, Teskey, & Cain, 1995; Goddard et al., 1969). Thus, whatever changes in the brain underlie kindling, they are persistent, if not permanent. For this reason, kindling has attracted researchers interested in certain types of learning and memory and the retention of other types of neuroplasticity.

Another theoretically significant property of kindling is that it requires distributed, as opposed to massed, stimulations. Stimulation intervals of 24 hr or longer required the fewest number of stimulations to produce fully generalized convulsions, whereas it was often difficult to kindle at all with intervals of less than about 30 min, but see Racine, Burnham, and Gartner (1973) and Lothman and Williamson (1994). Accordingly, most kindling experiments involve the administration of one or two stimulations per day.

Yet another significant feature of kindling is that it can transfer between brain sites. Goddard et al. (1969) found that rats that had previously been kindled through an electrode implanted in the amygdala (the primary kindling site) kindled faster when stimulated through a

second electrode implanted in the contralateral amygdala or in the ipsilateral septum than did rats that had not been previously kindled. Subsequent research demonstrated that these effects can by observed in a variety of other brain sites that are either contralateral (Racine, 1972) or ipsilateral (Burnham, 1975) to the primary kindling site. Transfer effects are also observed following destruction of the primary kindling site (McIntyre & Goddard, 1973; Racine, 1972) or following forebrain commissurotomy in structures contralateral to the primary kindling site (McIntyre, 1975). This indicates that the neuroplastic changes that underlie kindling are not restricted to the primary site of stimulation. Furthermore, transfer effects also occur between different forms of kindling; for example, between electrical and any one of the many different forms of chemical kindling (Cain, 1981; Mori, Wada, & Kumashiro, 1989; Pinel, Van Oot, & Mucha, 1975; Wasterlain, Morin, & Jonec, 1982).

Goddard, McIntyre, and Leech (1969) believed that it was the repeated stimulations that produced kindling. This proved to be only partially correct. Although stimulations are necessary for electrical kindling, subsequent work by Racine (1972) demonstrated that the stimulations alone are not sufficient. Racine showed that kindling requires the elicitation of afterdischarges (ADs) by the repeated brain stimulations: Administrating subthreshold stimulations will not produce kindling. Racine (1972) demonstrated that subthreshold stimulations reduce the AD threshold until the stimulations begin to elicit ADs, and then kindling begins[2]. Racine also reported that the major electrographic correlate of the progressive intensification of elicited

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[2] For example, because different amygdalar nuclei have different AD thresholds each nuclei requires a different number of subthreshold-stimulations to kindle. But the number of ADs required to kindle all of the amygdalar nuclei is the same (Sitcoske O'shea, Rosen, Post, & Weiss, 2000).

convulsions during the course of kindling is the degree to which the afterdischarge spreads away from the point of stimulation in the brain (Racine, 1972).

<div style="text-align:center">Kindling as a Model</div>

Kindling has been widely studied because it models three important types of phenomena. It models aspects of human epilepsy, certain human psychopathologies, and various forms of neuroplasticity such as certain types of learning and memory.

<div style="text-align:center">*Kindling as a Model of Epilepsy*</div>

Goddard et al. (1969) were the first to point out the similarities between kindling and the progressive development of human epilepsy after head injury: More than 50% of individuals who experience a penetrating brain injury will, after a "silent period" of weeks to months, subsequently begin to display seizures (Hernandez, 1997). Many investigators have subsequently tried to understand the mechanisms of kindling to shed light on human epileptogenesis (e.g., Elmer, Kokaia, Kokaia, McIntyre, & Lindvall, 1998). Others have studied the antiepileptic potential of drugs by assessing their ability to block kindling (e.g., Postma, Krupp, Li, Post, & Weiss, 2000) or their ability to block the elicitation of convulsions once animals have been kindled (McNamara, 1989).

Amygdala-kindled animals are considered to be models of temporal lobe epilepsy (McNamara, 1984; McNamara et al., 1985). There are three reasons for this. First, the topography of the amygdala-kindled convulsions are similar to those observed in human

temporal lobe epilepsy (Sato, Racine, & McIntyre, 1990). Second, the effects of drugs on amygdala-kindled convulsions are predictive of their effects on partial seizures (Racine & Burnham, 1984) and tonic-clonic convulsions in humans (Loscher, 2002). And third, long-term amygdala-kindled rats display patterns of neuronal damage and axon sprouting (e.g., Cavazos, Das, & Sutula, 1994) similar to those observed in the brains of human temporal lobe epileptics (Swanson, 1995).

In the context of kindling as a model of epilepsy, a key finding is that kindling ultimately results in a syndrome characterized by spontaneously recurring convulsions. Most kindling experiments are curtailed once fully generalized convulsions are reliably elicited--after about 15 stimulations in amygdala-kindled rats. However, it has been demonstrated that if kindling is not curtailed at this point and is continued (for about 250 stimulations in the rat), convulsions begin to recur spontaneously (Pinel & Rovner, 1978; Wada et al., 1975; Wada, Sato, & Corcoran, 1974).

Kindling as a Model of Psychopathologies

Kindling has also been used to model other clinical disorders. It is regarded as a model of pathological neuroplastic changes that are presumed by some to underlie such disorders as bipolar disorder, depression, and schizophrenia. Similar to kindling, repeated bouts of mania, depression, or psychosis seem to increase the risk and severity of subsequent bouts (Kraus, 2000; Weiss & Post, 1998). Most importantly, insights gained from the kindling model (Post & Weiss, 1989) have led to the successful employment of anticonvulsants for the treatment of bipolar disorder (Ketter, Manji, & Post, 2003; Post et al., 1998), depression (Post, Altshuler, Ketter, Denicoff, & Weiss, 1991), and most recently posttraumatic stress disorder (Taylor, 2003).

Kindling as a Model of Certain Types of Learning and Memory

Kindling has also been widely studied as a model of neuroplasticity--particularly of certain types of learning and memory--for several reasons. First, many of the characteristics of kindling are similar to certain types of learning and memory: Kindling progresses more efficiently with distributed practice (Dempster, 1996); its effects are relatively permanent (Dennison et al., 1995); and the changes that maintain the kindled state are stored diffusely in the brain (Goddard et al., 1969). Second, it has been shown that kindling is associated with specific neuroplastic changes in the brain, such as alterations in neuronal structure and function (Mody, 1999), and increases in neurogenesis (e.g., Nakagawa et al., 2000). Third, kindling has often been compared to, and implicated in, long-term potentiation, the most widely studied neurophysiological model of learning and memory (Cain, 1989).

Kindling as a Model of Interictal Behavioural Disorders

Although most kindling research has focused on the convulsions themselves or on the neuroplastic changes that accompany their development, kindling has also been used to model the interictal behavioural changes that accompany seizures in some human epileptics. Two kinds of kindling-produced changes in interictal behaviour have been investigated: first, the interictal impairments in learning and memory (Hannesson & Corcoran, 2000), and second, the abnormal interictal behaviours, which are presumed to model the interictal psychopathology that is problematic in many cases of temporal lobe epilepsy. Because interictal behavioural abnormalities associated with kindling are often studied to shed light on the interictal psychopathology of temporal lobe epileptics, the effects of amygdala kindling on interictal

behaviour have been most widely investigated (Kalynchuk, 2000). The main finding has been that amygdala kindling increases interictal defensive behaviour in cats (Adamec, 1975) and rats (Kalynchuk, Pinel, & Treit, 1999; Pinel, Treit, & Rovner, 1977).

Potential for Kindling to Produce Conditioned Effects

In many ways, conventional kindling experiments are ideal for the generation of conditioned effects. In the typical kindling experiment, each subject is repeatedly stimulated through an implanted electrode. Each time, the subject is removed from its cage; the stimulation lead is attached; the subject is placed in the stimulation environment; and the current is delivered. Accordingly, there is ample opportunity for kindled animals to learn the predictive relation between antecedent events and the subsequent stimulation and convulsion. The fact that the potential for such conditioned effects has received so little attention is paradoxical given that kindling is of such widespread interest as a model of certain types of learning and memory. Moreover, 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.

The potential for the kindling procedure to produce inadvertent conditioned effects has been addressed in only a handful of studies. There have been only three positive reports. Two of these studies purportedly demonstrated the elicitation of seizure-like electrographic activity in response to a conditioned stimulus when an amygdalar stimulation and convulsion served as the unconditioned stimulus (Janowsky, Laxer, & Rushmer, 1980; Yoshii & Yamaguchi, 1963). However, both of these studies had procedural flaws (see Mostofsky & Myslobodsky, 1982); the

effect was observed in only a few of the subjects, and others have failed to replicate it (Freeman & Mikulka, 1986; Myslobodsky, Mintz, Lerner, & Mostofsky, 1983; Wyler & Heavner, 1979).

More recently, Corcoran, Lanius, and Duren (1992) used a place preference paradigm to demonstrate that rats can discriminate between an environment in which kindled stimulations were administered and one in which no stimulations were delivered. Theirs was the first study to demonstrate that interictal behaviours can be conditioned by amygdala kindling.

First Systematic Demonstration of the Conditioned Effects of Amygdala Kindling on Convulsions and Interictal Defensive Behaviour

Because the kindling paradigm seemed ideal for the generation of conditioned effects, I was sceptical of previous failures to document them. In 2001, we (Barnes, Pinel, Francis, & Wig) reported that a standard kindling protocol produces robust conditioned effects on both the convulsions and interictal behaviour of rats. Rats received 53 stimulations to the basolateral amygdala in one conditional stimulus (CS) 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 much less severe than in the CS+. In short, our data indicated that the learned association of the stimulation environment with the stimulations and convulsions was a significant contributor to the interictal behaviour and

convulsions of kindled rats (Barnes et al., 2001). These data were the starting point for the present thesis[3].

Rationale, Purposes, and General Methodological Approach

If conditioned effects are a fundamental part of the kindling phenomenon, a complete understanding of kindling and of the many phenomena for which kindling is a model (e.g., neuroplasticity, epilepsy, affective disorders) is not likely to emerge without considering them. This thesis was based on this premise.

The general purposes of this thesis were to establish the reliability, generality, nature, and theoretical significance of our previously observed effects of the stimulation environment on the convulsions and interictal behaviour of basolateral amygdala (BA) kindled rats. To achieve these general purposes, this thesis addressed the following three questions. First, are the previously observed effects of the stimulation environment on the ictal (during convulsion) and interictal behaviour of BA-kindled rats the result of Pavlovian[4] conditioning? Second, is such conditioning associated with the kindling of brain structures other than the BA? Third, do

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[3] These data were the focus of my Master's thesis.

[4] A qualification of the use of the term "Pavlovian" is necessary. In the present experiments, the CS was the stimulation environment, but Pavlovian conditioning has traditionally employed discrete CSs (e.g., lights or tones). Based on the traditional distinction between cue and context conditioning (Bouton, 1993), which is a contestable one (see (Murphy, Baker, & Fouquet, 2001), the protocol employed in the present thesis could also be called "context conditioning."

conditioned effects contribute to the major features of the kindling phenomenon? Each of these questions corresponds to one of the three lines of experiments contained in this thesis.

We assumed that the failure of previous investigators to demonstrate the conditioned effects of kindling was attributable to the insensitivity of their methods. Therefore, the Barnes et al. (2001) study incorporated several new methodological approaches. In view of the success of this study, these same methodological approaches were used, when appropriate, in the present experiments.

The following were the four main methodological innovations. First, most previous efforts to demonstrate conditioned effects in kindling experiments were attempts to elicit convulsions with a CS (e.g., Janowsky et al., 1980); instead, I assessed the ability of a CS to modulate the convulsions elicited by stimulation. Second, rather than focusing exclusively on convulsions, I also assessed the ability of CSs to influence the interictal behaviour of the kindled rats. Third, previous efforts were comparisons between conditioned and unconditioned subjects; in contrast, most of my experiments employed a more sensitive, within-subjects design--in most experiments, each rat's responses to a CS+ (the stimulus that always predicted a stimulation and convulsion) and a CS- (the stimulus that never predicted a stimulation and convulsion) were compared. Fourth, most previous efforts had focused on the results of a single test at the end of the experiment; instead, I often recorded and compared the behaviour of subjects in the presence of the CS+ and CS- throughout the experiment.

Another important aspect of my methodological approach was that, as much as my objectives permitted, I attempted to adhere to widely used kindling protocols. I did this because my purpose was not merely to demonstrate that kindling can generate conditioned effects; I wanted to demonstrate that such conditioned effects are inherent features of most kindling experiments.

GENERAL METHODS

This section describes the methods common to most of the experiments in this thesis. Variations in this general methodology are described within the Methods sections of the individual experiments. All experimental procedures were approved by the University of British Columbia Animal Care Committee in accordance with the guidelines of the Canadian Council on Animal Care.

Subjects

The subjects in all of the experiments were experimentally naïve, male Long-Evans rats (Charles River Laboratories, St. Constant, Quebec, Canada) that were between 10 and 12 weeks old at the beginning of each experiment. They were housed in groups in steel hanging cages before each experiment and, individually thereafter. All 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. All experimental procedures were administered during the light phase of the light-dark cycle.

Surgery

Prior to surgery, each rat was handled daily for 1.5 min for at least 5 consecutive days. Each time, the rat was removed from its home cage, held, and lightly stroked. Following this

period of presurgery handling, a single bipolar stimulation electrode (Plastic Products Company, MS-303-2) was implanted in the site of interest under combined ketamine (100 mg/kg, i.p.) and xylazine (20 mg/kg, i.p.) anesthesia. Standard stereotaxic protocols were followed. In most of my experiments, an electrode was implanted in the left basolateral amygdala (BA) of each rat. Left BA electrode tips were aimed 2.8 mm posterior, 5.0 mm left, and 9.0 mm ventral to the skull surface at bregma with the incisor bar set at −3.3 mm--the coordinates were derived from Paxinos and Watson (1986). Following a postsurgery recovery period of at least 7 days, the rats were habituated to the stimulation lead and handled, as they had been during presurgical handling, for a minimum of 5 consecutive days.

Behavioural Procedures

Following postsurgery handling, the rats underwent a regimen of training in which they received both stimulations and sham stimulations (the stimulation procedure was followed assiduously except that no current was delivered)--all training and testing procedures were conducted in the colony room. In all of the experiments, each stimulation was administered in one test chamber (the CS+), and each sham stimulation was administered in a second similar, but distinctive, chamber (the CS-). During testing, interictal behaviour and convulsions were recorded with a video camera mounted above the test chamber.

During stimulation trials, the rats were allowed to move freely around the CS+ chamber for 30 s (the preadministration interval) while the experimenter stood immobile. After this preadministration interval, the experimenter pressed the button on the stimulator, which

delivered a stimulation (1-s, 60-Hz sine wave, 400 μA rms) or no stimulation, depending on whether it was a stimulation trial or a sham-stimulation trial.

Sham-stimulation trials were identical to the stimulation trials except that the rats were tested in the CS- and no current was passed through the implanted electrode: The stimulation lead was attached to each subject, the stimulator button was pressed, but no stimulation was delivered because the stimulation lead was not connected to the stimulator. Accordingly, any differences that developed in the behaviour of a subject in response to the CS+ and CS- could be attributed only to the differences between the CS+ and CS-. The assignment of each of the two test environments as the CS+ or as the CS- was counterbalanced among the subjects within each experiment; because no systematic differences ever developed between these conditions, the data were always combined for analysis. For the sake of clarity, the detailed counterbalancing measures employed in each individual experiment will not be described. It should be understood, however, that whenever rats were divided into new groups or subgroups, those groups were counterbalanced with respect to any prior differences in treatment amongst the subjects. For example, in most of the experiments, the CS+ for half of the subjects was one chamber and the CS+ for the other half was another chamber. If the rats were subsequently divided into two new groups, half the subjects who had one of the chambers as their CS+ would be randomly chosen to be included in one of the new groups and the other half would be chosen to be included in the second new group; and the same would be true for the rats whose CS+ was the other chamber. As an example of the counterbalancing measures employed in the present experiments, Appendix A provides a detailed description and illustration of the counterbalancing measures employed in Experiment 1 of this thesis.

Measuring Kindled Convulsions

The severity of each convulsive response was rated according to Pinel and Rovner's (1978) extension of Racine's (1972) limbic convulsion scale: class 1, rhythmic mouth and face movements; 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; class 6, a class 5 pattern with multiple rearing and falling episodes; class 7, a convulsion that includes a running fit; and class 8, a convulsion that includes tonus. In addition, both the latency to the onset of the convulsion and the duration of the convulsion 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.

Measuring Interictal Behaviour

The main purpose of the preadministration interval (i.e., the interval between the placement of the rat in the test chamber and the subsequent administration of a stimulation or sham stimulation) was to permit a comparison of each subject's behaviour prior to the stimulations in the CS+ with its behaviour prior to the sham stimulations in the CS-. The particular behaviours that were quantified from videotaped recordings of the preadministration intervals depended somewhat on the site of stimulation, but they always included the following two: (1) general activity--the number of boundary lines of a 3x3 square grid placed in front of the video monitor that were crossed by the tip of the rat's nose; and (2) freezing--the percent of the

2-s preadministration-interval epochs during which the rat was freezing (i.e., made no movements other than those associated with breathing).

Histology

At the conclusion of each experiment, all subjects were killed with CO_2 according to the Canada Council on Animal Care guidelines. Then, 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 structure in which the electrode or electrodes had been implanted. 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 slices using the Paxinos and Watson stereotaxic atlas (1986).

Statistical Procedures

The statistical significance of the results of each of the experiments in this thesis were analyzed using three types of parametric techniques. First, activity and freezing time-series data were analyzed using planned orthogonal contrasts--which use the within-cell error term from an omnibus ANOVA (Keppel & Zedeck, 1989, p. 362; Winer, Brown, & Michels, 1991, p. 342-343 and p. 526). The activity and freezing time-series data were blocked to reduce the increase in the probability of Type-I errors that occurs with multiple comparisons between means (Tukey, 1977). Because multiple ANOVAs were employed for the analysis of the activity and freezing

time-series data, the p-value required for a rejection of the null hypothesis was calculated using the Bonferroni correction: $p < .025$. Second, the convulsion-severity time-series data (i.e., convulsion class and duration) were analyzed using between-within ANOVAs. Because multiple ANOVAs were employed for the analysis of the convulsion-severity time-series data, the p-value required for a rejection of the null hypothesis was calculated using the Bonferroni correction: $p < .025$. Third, nontime-series data were analyzed using independent-samples t-tests, dependent-samples t-tests, or between-within ANOVAs. When multiple t-tests or multiple ANOVAs were employed for the analysis of nontime-series data, the p-value required for a rejection of the null hypothesis was calculated using the Bonferroni correction.

LINE 1: SUPPORT FOR A PAVLOVIAN MECHANISM

In the Barnes et al. (2001) experiment, which served as the major stimulus for the experiments in this thesis, rats received periodic stimulations to the basolateral amygdala (BA) in one conditional environment (CS+) and an equal number of sham stimulations in a second environment (CS-). As kindling progressed, the rats became more defensive in the CS+ environment than in the CS- environment; and, when they were finally stimulated in the CS-, their convulsions were less severe than in the CS+. We concluded that these results were a product of Pavlovian conditioning: Because the stimulations and sham stimulations were predicted only by the respective environments, it seems that the emergence of differences in ictal (during convulsion) and interictal (between convulsions) behaviour in the two environments reflected the conditioned association of the environments and their consequences.

These results are, however, open to alternative interpretations. For example, Rescorla (1967) has argued that discriminative conditioning is not a sufficient control for Pavlovian conditioning; because it cannot establish whether inhibitory or excitatory conditioning has occurred. If the effects observed in the Barnes et al. (2001) experiment were the sole result of inhibitory effects conditioned to the CS-, this would complicate our assertion that conditioned effects are part of many kindling experiments because most kindling experiments do not employ a sham stimulation chamber. Another potential interpretation of the results of the Barnes et al. (2001) experiment is that the observed effects were a combination of nonassociative effects; for example, sensitization to the stimulation environment and habituation to the sham stimulation environment.

Accordingly, the general purpose of this first line of experiments was to provide additional support for the conclusion that the effects of the stimulation environment on BA-kindled convulsions and interictal behaviour are a product of Pavlovian conditioning.

Experiment 1: Discrimination Reversal Confirms a Pavlovian Mechanism for the Influence of the Stimulation Environment on Convulsions and Interictal Behaviour

The specific purpose of Experiment 1 was to demonstrate that the ictal and interictal behavioural effects that come to be associated with the stimulation environment during BA-kindling can be diminished. The Barnes et al. (2001) experiment used a discrimination procedure to demonstrate those effects: Rats were stimulated in one environment (CS+) and sham stimulated in the other environment (CS-). Experiment 1 used a discrimination procedure to replicate those effects and then a discrimination-reversal procedure to diminish them. I administered 45 stimulations in one environment (CS+) and 45 sham stimulations in another environment (CS-); then, I assessed the effects of interchanging the original CS+ and CS-environments.

Pavlov's view was that the discrimination-reversal procedure involves two forms of extinction: the simultaneous extinction of inhibitory (learned responses to the CS-) and excitatory (learned responses to the CS+) conditioning (Pavlov, 1928, p. 323-324). Others have argued that it involves elements of both extinction and conditioning of an excitatory response (see Mackintosh, 1974). In either view, the reversal of a conditional discrimination would be consistent with the idea that the effects of the stimulation environment are the result of Pavlovian conditioning.

Methods

Apparatus

The test environments were two stimulation chambers positioned at opposite ends of the colony room. Both chambers were constructed of transparent Plexiglas and were 75 cm long, 75 cm wide, and 50 cm high. The floor of each chamber was covered with 2.5 cm of bedding material. To render the chambers more distinctive, one of two unique sets of plastic objects and cutout paper shapes were placed around each chamber.

Kindling Phase: Kindling and Conditioning Procedure

A single bipolar electrode was implanted in the left basolateral amygdala (BA) of each of 36 rats. Following postsurgery handling, all 36 rats were stimulated in one of the two test chambers (the CS+) and sham stimulated in the other (the CS-). For a detailed description and illustration of the counterbalancing measures employed in the present experiment, please refer to Appendix A.

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 three restrictions: (1) there were 45 stimulations and 45 sham stimulations; (2) no more than three stimulations or sham stimulations ever occurred consecutively; (3) and every fourth day (e.g., day 1, day 5, day 9, etc.) was a preadministration-test day, which always comprised one stimulation and one sham-stimulation trial in counterbalanced sequence. The preadministration interval was always 30 s.

Kindling Phase: Switch Tests

Immediately following their final stimulation of the kindling phase, the rats were divided into two equal groups of 18 rats each. The between- and within-subjects switch tests were both conducted the following day, the switch-test day. The experimenter who scored the convulsions observed during the switch tests was blind to which chamber had previously served as the original CS+ for each rat.

Between-subjects switch test. The between-subjects switch test was conducted during the morning of the switch-test day. The rats in one of the groups received a test stimulation in their CS- environment, whereas the rats in the other group received a test stimulation in their CS+ environment.

Within-subjects switch test. The within-subjects switch test, which was conducted during the afternoon of the switch-test day, involved only one of the two groups of rats: The rats that had received a test stimulation in their CS+ during the between-subjects switch test. These rats received a test stimulation in their CS-. This permitted a within-subjects comparison of the severity of the convulsion elicited by each rat's final stimulation in their CS+ with the severity of the convulsion elicited by their subsequent stimulation in their CS-.

The other group of rats--the rats that received a test stimulation in their CS- during the between-subjects switch test--were also stimulated during the afternoon of the switch-test day, in the CS+ environment. The purpose of this stimulation was to equate the rats in terms of the total number of stimulations they had received during the kindling-phase switch-test day.

Reversal Phase: Kindling

The day after the kindling-phase switch tests, the rats were redivided into two groups of 18 rats each (see Appendix A). The rats in one group, the no-interchange group, were tested as

before: During the reversal phase, they received 45 stimulations in their original CS+ and 45 sham stimulations in their original CS-. The rats in the other group, the interchange group, had their original (i.e., kindling phase) CS+ and CS- interchanged: During the reversal phase, they received 45 stimulations in their original CS- and 45 sham stimulations in their original CS+. All other reversal-phase procedures were identical to those of the kindling phase.

Reversal Phase: Switch Tests

Immediately following their final stimulation of the reversal phase, each of the two groups of rats (i.e., the no-interchange and interchange groups) was subdivided into two equal subgroups--to create four subgroups of 9 rats each. The between- and within-subjects switch tests were both conducted the following day, the reversal-phase switch-test day. The experimenter who scored the convulsions observed during the reversal-phase switch tests was blind to which chamber had previously served as the original CS+ for each rat.

Between-subjects switch test. The between-subjects switch test was conducted during the morning of the switch-test day. The rats in one of the no-interchange subgroups received a test stimulation in their original CS-, whereas the rats in the other no-interchange subgroup received a test stimulation in their original CS+; and the rats in one of the interchange subgroups received a test stimulation in their original CS+ (i.e., the environment where they received sham stimulations during the reversal phase), whereas the rats in the other interchange subgroup received a test stimulation in their original CS-.

Within-subjects switch test. The within-subjects switch test was conducted during the afternoon of the switch-test day, and it involved only one of the two groups of no-interchange rats and one of the groups of interchange rats: The no-interchange rats that had received a test stimulation in their original CS+ during the between-subjects switch test and the interchange rats

that had received a test stimulation in their original CS- during the between-subjects switch test. For the within-subjects switch test, the group of no-interchange rats received a test stimulation in their original CS-, and the group of interchange rats received a test stimulation in their original CS+. This permitted a within-subjects comparison of the severity of the convulsion elicited by each rat's final stimulation in their original CS+ with the severity of the convulsion elicited by their final stimulation in their original CS-.

Blocking of Time-Series Data

The kindling-phase time-series data (i.e., the activity and freezing data) from all of the rats were blocked into four blocks, each block consisted of three consecutive preadministration-test days. The reversal-phase time-series data were also blocked into four blocks, but were analyzed separately for the no-interchange rats and the interchange rats.

Planned Statistical Analyses

Five different kinds of analyses were conducted to assess the statistical significance of the between-group and within-group differences. First, the activity and freezing time-series data from the kindling phase and the reversal phase were analyzed using planned orthogonal contrasts between the CS+ and CS- for each separate block of the kindling phase (i.e., blocks 1 to 4) and the reversal phase (i.e., blocks 1 to 4). Second, the four measures of convulsion severity from the kindling phase between-subjects switch test were analyzed using independent-samples *t* tests. The latter *t* tests were one-tailed because the kindling phase of the present experiment constituted a replication of two previous experiments (Barnes & Pinel, 2001; Barnes, Pinel, Wig, Stuettgen, & Holzel, 2003). Third, to confirm the results of these latter analyses, the statistical significance of the differences in the severity of the convulsions elicited 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. Fourth, the four measures of convulsion severity from the reversal-phase between-subjects switch test were analyzed using a 2-way ANOVA, with CS and group as between-subjects factors. Simple-main-effects analyses were used to investigate significant interactions. Fifth, the four measures of convulsion severity from the reversal-phase within-subjects switch test were analyzed using a 2-way between-within ANOVA, with group and CS as the between- and within-subjects factors, respectively. Because multiple *t*-tests and multiple ANOVAs were employed for the analysis of the convulsion severity data from the kindling phase and the reversal phase, respectively, the *p*-value required for a rejection of the null hypothesis was calculated using the Bonferroni correction: *p*<.0125.

Histology

Upon completion of the present experiment, the rats were used as subjects in a neurogenesis experiment (not a component of the present thesis). While slicing the brains for the purposes of the neurogenesis experiment, an experimenter who was blind to the purposes of the present experiment verified that each rat's electrode tip lay within the BA.

Results

During the kindling phase, the stimulation and sham-stimulation environments began to exert differential effects on interictal behaviour, and the switch tests administered at the end of the kindling phase indicated that these environments also exerted differential effects on the convulsions. Specifically, the rats displayed more defensive behaviour in the CS+ environment

than in the CS- environment, and when the rats were finally stimulated for the first time in the CS-, their convulsions were less severe than they had been in the CS+.

During the reversal phase, the no-interchange rats continued to display more defensive behavior in their original CS+ than in their original CS-; whereas the interchange rats began to display more defensive behaviour in their original CS- than in their original CS+. At the end of the reversal phase, there was little remaining evidence of conditioned effects on the convulsions of the no-interchange rats, making it difficult to unambiguously evaluate the effect of the interchange on the convulsions.

Kindling

The first stimulations of the kindling phase 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 a loss of equilibrium. In other words, the development of the convulsions was virtually always characterized by a progression through the classic limbic convulsion classes (i.e., 1 to 6). Moreover, the first few convulsions of the 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 of the rats consistently displayed convulsions culminating in a loss of equilibrium (i.e., of a class 5 or higher) and lasting more than 40 s. The rats required a mean of 13.4 stimulations before they displayed three convulsions of class 5 or greater, a commonly used criterion of kindling. Only one of the rats displayed a convulsion greater than class 6 during the kindling phase; that rat displayed class 7 convulsions (i.e., a convulsion with running fits) in response to each of the last three stimulations.

During the reversal phase, all of the rats continued to display convulsions of a class 5 or greater. Five of the rats displayed convulsions that were greater than class 6: One of the no-interchange rats displayed a class 7 convulsion in response to most of the reversal-phase stimulations (This was the same rat that had displayed three class 7 convulsions during the kinding phase.); one of the interchange rats and one of the no-interchange rats displayed a few class 7 convulsions towards the end of the experiment; and one of the interchange rats and one of the no-interchange rats displayed a few class 8 convulsions, but no class 7 convulsions, towards the end of the experiment.

Conditioning of Interictal Behaviours

The effects of the stimulation (CS+) and sham stimulation (CS-) environments on the ambulatory activity and freezing recorded during the preadministration tests of the kindling phase and the reversal phase are illustrated in Figure 1. During the kindling phase, the rats began to display less ambulatory activity and more freezing in the CS+ environment than in the CS- environment. During the reversal phase, the no-interchange rats continued to display less activity and more freezing in their original CS+ than in their original CS-; whereas, the interchange rats began to display less ambulatory activity and more freezing in their original CS- than in their original CS+.

Activity. The left side of panels A and B of Figure 1 illustrate the mean number of line crossings displayed by the no-interchange and the interchange rats, respectively, in the CS+ and CS- during the kindling-phase preadministration tests, which occurred prior to every fourth stimulation. During the kindling phase, the rats were significantly less active in the CS+ than in the CS- during block 3 (days 25-33), $F(1,85)=10.13$, $p=.0020$, and block 4 (days 37-45),

Figure 1. Experiment 1: Conditioning of Interictal Behaviours. The mean ambulatory activity

(A) and freezing (C) displayed by the no-interchange rats in their original CS+ and CS-

environments during each of the four blocks of test days of the kindling phase and of the reversal

phase. The mean ambulatory activity (B) and freezing (D) displayed by the interchange rats in

their original CS+ and CS- during each of the four blocks of test days of the kindling phase and

of the reversal phase. Error bars represent the standard error of the mean (*SEM*).

No-interchange

Interchange

A

● Original CS+
○ Original CS-

B

C

D

Mean Activity Score

Mean Duration of Freezing (%)

Kindling Phase
Block

Reversal Phase
Block

Kindling Phase
Block

Reversal Phase
Block
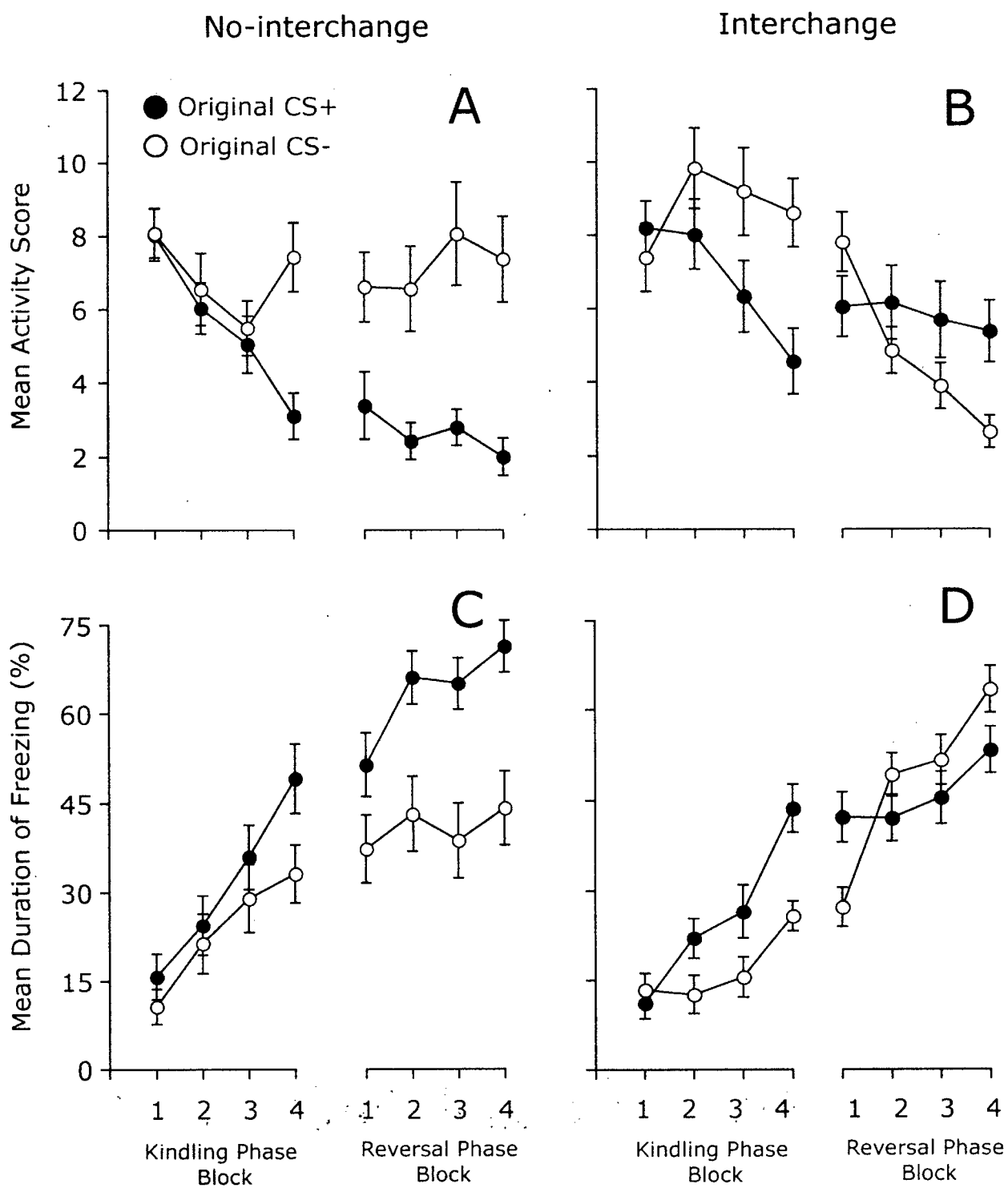
$F(1,85)=64.67$, $p<.00000001$, but not during block 1 (days 1-9) and block 2 (days 13-21), both ps$>.027$.

The right side of panels A and B of Figure 1 illustrate the mean number of line crossings by the no-interchange rats and the interchange rats, respectively, in their original CS+ and CS- during the reversal-phase preadministration tests. The no-interchange rats were significantly less active in their original CS+ than in their original CS- during block 1 (days 1-9), $F(1,102)=20.15$, p=.000019, block 2 (days 13-21), $F(1,102)=33.11$, $p=.00000009$, block 3 (days 25-33), $F(1,102)=53.88$, p<.00000001, and block 4 (days 37-45), F(1,102)=55.97, $p<.00000001$. In contrast, the interchange rats were significantly less active in their original CS+ than in their original CS- only during block 1, $F(1,102)=7.86$, $p=.0071$. They were significantly less active in their original CS- than in their original CS+ during block 3, $F(1,102)=6.26$, $p=.0014$, and block 4, $F(1,102)=14.38$, $p=.00025$, but not during block 2, $F(1,102)=3.26$, $p=.074$.

Freezing. The left side of panels C and D of Figure 1 illustrate the mean duration of freezing displayed by the no-interchange and the interchange rats, respectively, in the CS+ and CS- during the kindling-phase preadministration tests. During the kindling phase, the rats displayed significantly more freezing in the CS+ than in the CS- during block 2 (days 13-21), $F(1,105)=7.037$, $p=.0092$, block 3 (days 25-33), $F(1,105)=14.54$, $p=.00023$, and block 4 (days 37-45), $F(1,105)=51.66$, $p<.00000001$, but not during block 1 (days 1-9), $F(1,105)=.36$, $p=.55$.

The right side of panels C and D of Figure 1 illustrate the mean duration of freezing displayed by the no-interchange rats and the interchange rats, respectively, in their original CS+ and CS- during the reversal-phase preadministration tests. The no-interchange rats displayed significantly more freezing in their original CS+ than in their original CS- during block 1 (days 1-9), $F(1,102)=13.32$, $p=.00042$, block 2 (days 13-21), $F(1,102)=35.11$, $p=.00000004$, block 3 (days 25-33), $F(1,102)=46.39$, $p<.00000001$, and block 4 (days 37-45), $F(1,102)=49.57$,

p<.00000001. In contrast, the interchange rats displayed significantly more freezing in their original CS+ than in their original CS- only during block 1, $F(1,102)=14.94$, $p=.00020$. They displayed significantly more freezing in their original CS- than in their original CS+ during block 4, $F(1,102)=6.74$, $p=.011$, but not during blocks 2 and 3, both ps>.065.

Conditioning of Convulsions

Kindling-phase between-subjects switch test. The left side of panels A, B, C, and D of Figure 2 illustrate the means of the four measures of the severity of the convulsions that were elicited on the switch-test day of the kindling phase, when half of the rats received a test stimulation in the CS- environment while the other half received a test stimulation in the CS+ environment. The convulsions of the rats that were tested in the CS- were weaker than those of the rats that were tested in the CS+: The convulsions were significantly shorter, $t(34)=4.65$, $p=.000012$; they were of a significantly lower class, $t(34)=3.38$, $p=.00046$; and they involved significantly fewer falls, $t(34)=3.06$, $p=.0011$; but their latencies were not quite significantly longer, $t(34)=1.47$, $p=.038$.

Kindling-phase within-subjects switch test. The left side of panels A, B, C, and D of Figure 3 illustrate the means of the four measures of the severity of the convulsions that were elicited in the rats by their final kindling-phase stimulation in the CS+ and by their final kindling-phase stimulation in the CS-. These within-groups comparisons confirmed the results of the between-groups comparisons (see Figure 2). When the rats were stimulated in the CS-, their convulsions were weaker than when they were stimulated in the CS+: Their convulsions were significantly shorter, $t(35)=7.61$, p<.00000001; they were of a significantly lower class, $t(35)=4.78$, $p=.0000080$; they had significantly longer latencies, $t(35)=3.43$, $p=.00039$; and they involved significantly fewer falls, $t(35)=5.69$, $p=.00000050$.

Figure 2. Experiment 1: Kindling-Phase and Reversal-Phase Between-Subjects Switch Tests.

The mean duration (A), class (B), latency (C), and number of falls (D) of the convulsions elicited

during the between-subjects switch test which occurred at the end of the kindling phase (left

side): Half of the rats received a test stimulation in their CS- environment and the other half

received a test stimulation in their CS+ environment. Also illustrated are the mean duration (A),

class (B), latency (C), and number of falls (D) of the convulsions elicited during the between-

subjects switch test which occurred at the end of the reversal phase (right side): Half of the no-

interchange rats and half of the interchange rats received a test stimulation in their original CS-,

and the remaining rats received a test stimulation in their original CS+. Error bars represent the

SEM.

33

CS+   CS-

CS+ CS-   CS+ CS-
No-interchange   Interchange

Kindling-Phase
Test

Reversal-Phase Test

Figure 3. Experiment 1: Kindling-Phase and Reversal-Phase Within-Subjects Switch Tests. The

mean duration (A), class (B), latency (C), and number of falls (D) of the convulsions elicited

during the within-subjects switch test which occurred at the end of the kindling phase (left side):

All of the rats received a test stimulation in their CS- environment and a test stimulation in their

CS+ environment. Also illustrated are the mean duration (A), class (B), latency (C), and number

of falls (D) of the convulsions elicited during the within-subjects switch test which occurred at

the end of the reversal phase (right side): All of the no-interchange rats and all of the interchange

rats received a test stimulation in their original CS- and a test stimulation in their original CS+.
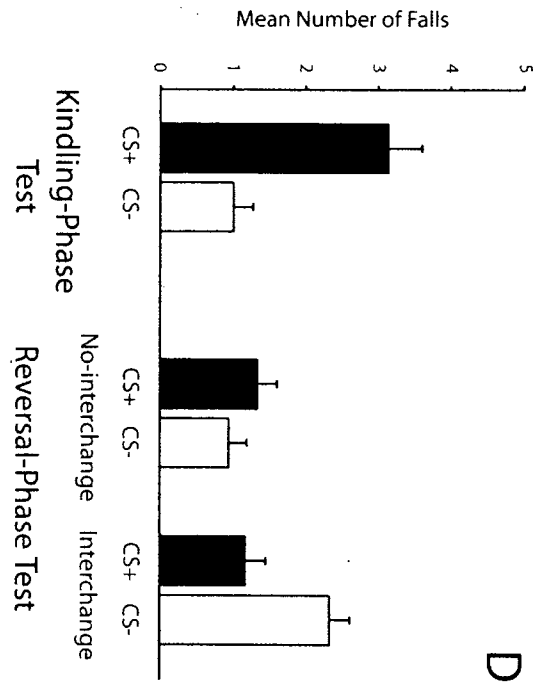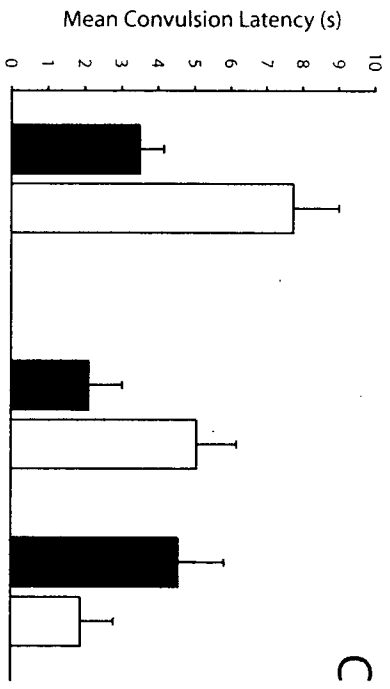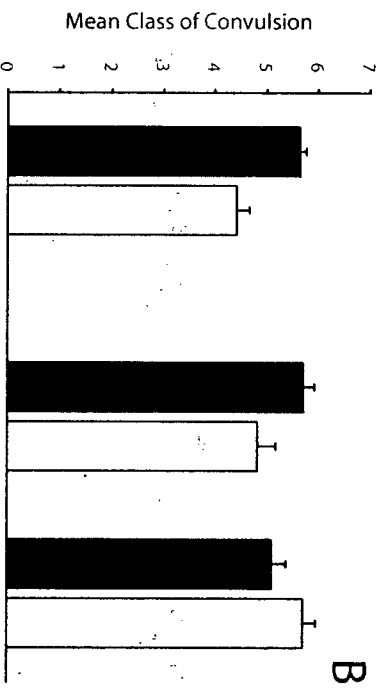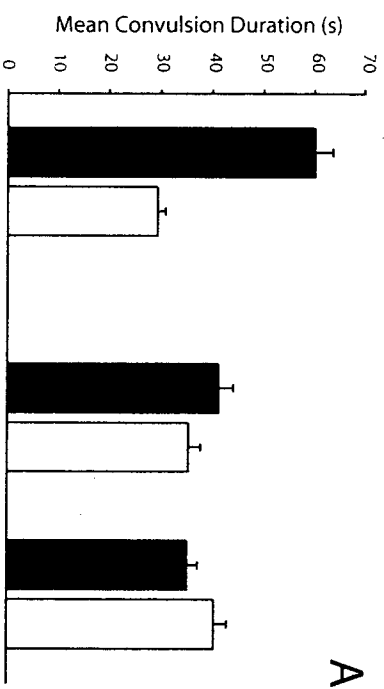
Error bars represent the *SEM.*

Reversal-phase between-subjects switch test. The right side of panels A, B, C, and D of Figure 2 illustrate the means of the four measures of the severity of the convulsions that were elicited in the no-interchange and interchange rats on the last day of the reversal phase. The effect of the between-subjects switch test on the class of convulsions displayed by the no-interchange rats was different from its effect on the class of convulsions displayed by the interchange rats, $F(1,32)=5.95, p=.02$. As predicted, the convulsions of the no-interchange rats stimulated in their original CS- were significantly lower in class than those of the no-interchange rats stimulated in their original CS+, $F(1,32)=7.86, p=.0085$. In contrast, there was no significant difference in the class of the convulsions displayed by the interchange rats stimulated in their original CS+ and the class of the convulsions displayed by the interchange rats stimulated in their original CS-, $F(1,32)=.42, p=.52$. There were no significant interactions in the other three measures of convulsion severity, all $ps>.56$.
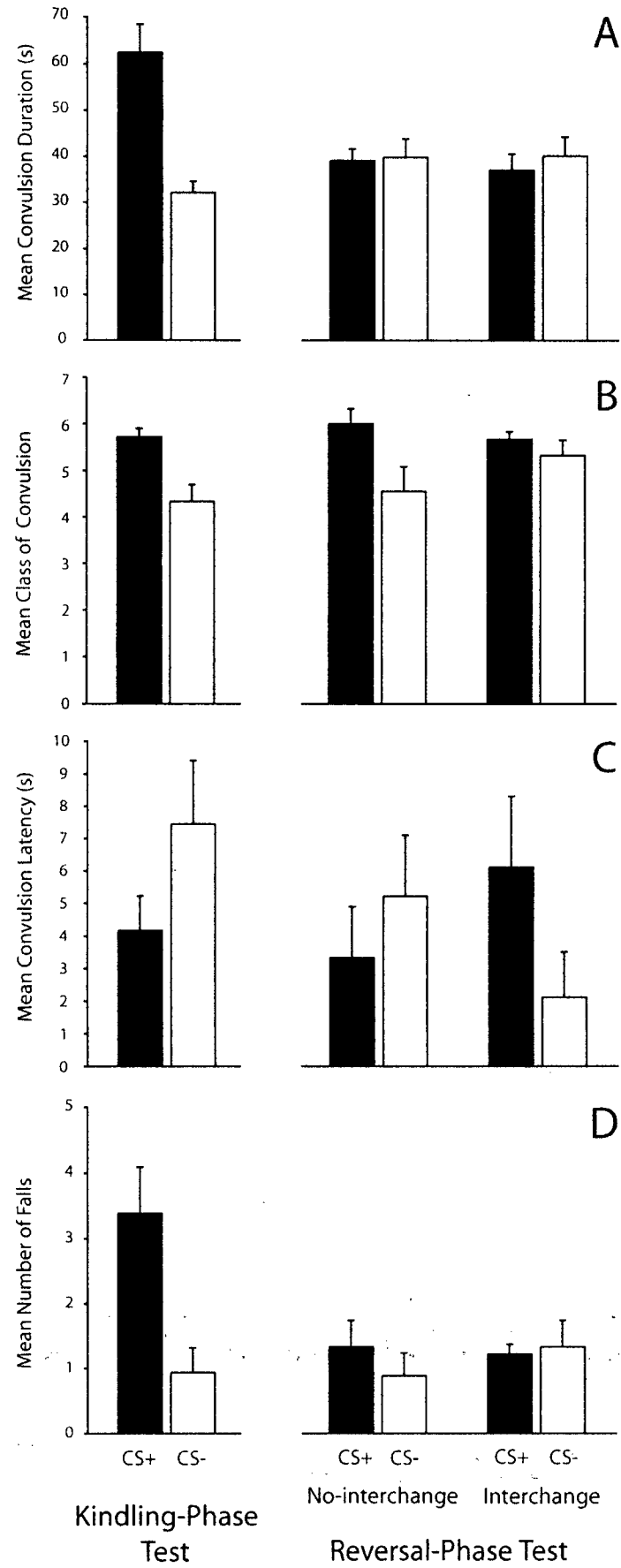
Reversal-phase within-subjects switch test. The right side of panels A, B, C, and D of Figure 3 illustrate the means of the four measures of the severity of the convulsions that were elicited in the no-interchange and interchange rats by their last stimulation of the reversal phase in their original CS+ and by their test stimulation in their original CS-. The effect of the within-subjects switch test on the class of convulsions displayed by the no-interchange rats was different from its effect on the class of convulsions displayed by the interchange rats, $F(1,34)=13.23$, $p=.001$. As predicted, the convulsions of the no-interchange rats in their original CS- were significantly lower in class, $F(1,34)=9.28, p=.0044$. In contrast, the convulsions of the interchange rats in their original CS- were significantly greater in class, $F(1,34)=9.64, p=.0038$. The effect of the within-subjects switch test on the number of falls displayed during the convulsions of the no-interchange rats was also different from its effect on the number of falls displayed during the convulsions of the interchange rats, $F(1,34)=11.09, p=.002$. The

convulsions of the interchange rats in their original CS- involved significantly more falls, $F(1,34)=9.64$, $p=.0038$, whereas the number of falls of the no-interchange rats in their two CSs were not significantly different, $F(1,34)=3.42$, $p=.073$. There were also significant interactions in the duration, $F(1,34)=6.31$, $p=.017$, and latency of the convulsions, $F(1,34)=10.26$, $p=.003$; but there were no statistically significant within-group differences in these two measures, all $ps>.023$.

Discussion of Experiment 1

The purpose of Experiment 1 was to demonstrate that the ictal and interictal behavioural effects that come to be associated with the stimulation environment during BA-kindling can be diminished. There were four major findings. First, as the kindling phase progressed, the rats began to display significantly less activity and significantly more freezing in the stimulation environment (the CS+) than in the sham-stimulation environment (the CS-). Second, at the end of the kindling phase, when the rats were stimulated for the first time in the CS-, they displayed significantly milder convulsions than they had in the CS+. Third, during the reversal phase, the no-interchange rats continued to display significantly more defensive behaviour in their original CS+ than in their original CS-, whereas the interchange rats began to display significantly less ambulatory activity and more freezing in their original CS-. Fourth, at the end of the reversal phase, when the no-interchange rats were stimulated in their original CS-, their convulsions were significantly milder than in their original CS+, but in terms of only one of the four measures of convulsion severity (i.e., class). In contrast, when the interchange rats received a test stimulation in their original CS+, their convulsions were significantly milder than in their original CS-, but in terms of only two of the four measures of convulsion severity (i.e., class and number of falls).

The present results confirm the reliability of the effects of the stimulation environment on convulsions and interictal behaviour: The results of the kindling phase were nearly identical to those of the Barnes et al. (2001) experiment. More importantly, they clearly support the view that the effects of the stimulation environment on interictal behaviour are the result of Pavlovian conditioning: At the end of the reversal phase, the pattern of defensive behaviour displayed by the interchange rats was the reverse of what they had displayed during the kindling phase and the reverse of that displayed by the no-interchange rats. That is, at the end of the reversal phase, the interchange rats displayed significantly more freezing and less activity in their original CS- than in their original CS+. Although the present results also provide support for the view that Pavlovian conditioning is responsible for the effects of the stimulation environment on convulsions, the effects were less consistent--only five of the eight predicted interactions were statistically significant.

Why was the effect of the interchange on the convulsions so inconsistent? The main problem seems to have been that by the end of the reversal phase, the effect of the stimulation environment on the convulsions of the no-interchange rats was so small that it was difficult to demonstrate a significant effect of the interchange. Why was the effect of the stimulation environment on the convulsions of the no-interchange rats at the end of the reversal phase smaller than it had been at the end of the kindling phase? One possibility is that the one stimulation they had received in the CS- environment at the end of the kindling phase was sufficient to substantially diminish that effect.

In addition to the inconsistency of the reversal-phase convulsion data, there is a problem related to the logic of the discrimination-reversal procedure as a measure of extinction, and therefore as a means of determining the Pavlovian nature of an observed effect. Even though the class of the convulsions of the no-interchange rats was affected by the switch test, the absence of

that same effect in the interchange rats complicates a Pavlovian interpretation. Because a discrimination-reversal may involve elements of both extinction and conditioning (e.g., Mackintosh, 1974), the reverse of the effect seen in the no-interchange rats must be observed in the interchange rats to conclude that extinction had occurred. For example, in the present experiment, a reversal was observed in the effects of the stimulation environment on interictal behaviour: During the reversal phase, the interchange rats displayed more freezing and less activity in their original CS- than in their original CS+, and the no-interchange rats displayed more freezing and less activity in their original CS+ than in their original CS-. A comparable reversal was not observed on the convulsions of the interchange rats. Although there was evidence for a reversal of the effects of the stimulation environment on the convulsions of the interchange rats in the within-subjects switch test, it was observed in only two of the four measures of convulsion severity (i.e., class and number of falls), and a comparable effect was not observed in the between-subjects switch test.

Experiment 2: Latent Inhibition Confirms a Pavlovian Mechanism for the Influence of the

Stimulation Environment on Kindled Convulsions

The general purpose of Experiment 2 was to provide additional support for the view that the stimulation environment influences kindling-related behaviour through Pavlovian conditioning. Unlike Experiment 1, it focused exclusively on kindled convulsions.

The specific purpose of Experiment 2 was to demonstrate that the convulsive effects that come to be associated with the stimulation environment can be affected by latent inhibition. If convulsions are more severe in an environment where the rats had always been stimulated than

in an environment where they had never been stimulated, as they were in Experiment 1, then pre-exposure to the stimulation environment might exert inhibitory effects during subsequent kindling. Does pre-exposure to the stimulation environment attenuate the effects conditioned to the stimulation environment during subsequent kindling? In other words, is kindling attenuated if rats are kindled in an environment that they have previously learned is not associated with brain stimulations and convulsions?

Methods

Apparatus

The test environments were two stimulation chambers positioned at opposite ends of the colony room. The only difference between these chambers and the two used in Experiment 1 was their size. The chambers used in the present experiment were slightly smaller: 50 cm long, 75 cm wide, and 50 cm high.

Pre-exposure Phase

As in Experiment 1, a single bipolar electrode was implanted in the left basolateral amygdala (BA) of each rat (n=20). Following postsurgery handling, all 20 rats received 60 sham stimulations: 58 in one of the two test chambers (CS1) and 2, in the other (CS2). There were two sham-stimulation sessions each day, and as in Experiment 1, the interval between the two sessions on a given day was between 2 and 6 h, and the preadministration interval was 30 s. The two sham stimulations in the CS2 were administered on the fifth day before the end of the pre-exposure phase (i.e., on day 26). The purpose of these two sham stimulations was to familiarize the rats with the CS2 because there had been suggestions that lack of familiarity with the

stimulation environment has effects on kindling rate (see Wintink, Young, Davis, Gregus, & Kalynchuk, 2003).

Kindling Phase

The day after their final sham stimulation of the pre-exposure phase, the rats were divided into two groups of 10 rats each. The rats in one group, the no-pre-exposure group, received 30 stimulations in the CS2 and 30 sham stimulations in the CS1, in a quasirandom order over 30 days. Whereas, the rats in the other group, the pre-exposure group, received all of the 30 stimulations and 30 sham stimulations in the CS1, also in a quasirandom order over 30 days. The reason the sham stimulations were still administered in the CS1 during the kindling phase was to ensure that any effects of the CS1 pre-exposure did not deteriorate during the early stages of kindling (i.e., the first five stimulations) when there are usually no convulsions displayed, so that those effects could be observed once convulsions emerged. Accordingly, the two groups of rats were treated in the same way during the kindling phase except that the pre-exposure rats were stimulated and sham stimulated in the CS1, and the no-pre-exposure rats were stimulated in the CS2 and sham stimulated in the CS1. The order of the stimulation and sham-stimulation trials was random, with the one restriction that no more than three stimulations or sham stimulations ever occurred consecutively.

Measuring the Kindled Convulsions and the Kindling Rate

In Experiment 2, only two of the four measures of convulsion severity that were used in Experiment 1 were recorded: duration and class. Latency and number of falls, the other two measures, were not recorded in this experiment because it focused on the early stages of kindling, when there are few convulsions. Experiment 2 also used measures that were not used

in Experiment 1: two different commonly used measures of kindling rate. The first was the number of stimulations to the first class 5 or greater convulsion, and the second was the number of stimulations to three class 5 or greater convulsions.

Planned Statistical Analyses

Two different kinds of planned analyses were conducted to assess the statistical significance of the between-group differences. First, the kindling-rate data were analyzed using an independent-samples *t* test. Second, the convulsion-severity time-series data from the kindling phase were analyzed using 2-way between-within ANOVAs with group (no-pre-exposure vs. pre-exposure) as the between-subjects factor and stimulation number (1 to 30) as the within-subjects factor. Significant interactions were followed up with simple-main-effects analyses at each level of the within-subjects factor (i.e., stimulation number).

Results

As predicted, pre-exposure to the stimulation environment attenuated kindled convulsions. However, the nature of the attenuation was unexpected. Pre-exposure to the stimulation environment did not produce a significant overall reduction in the class or duration of the convulsions, nor did it significantly reduce kindling rate, but the pre-exposure rats periodically failed to display a convulsive response to the stimulation or they displayed a substantially milder convulsive response than their response to the previous stimulation, even after they had been kindled.

Histology

Figure 4 illustrates the locations of the electrode tips in the left basolateral amygdala of 16 of the 20 rats that completed the experiment. Of these 16 rats, 14 had an electrode tip in the BA, and 2 had an electrode tip in the lateral amygdala. The electrode placements of the other 4 rats that completed the experiment could not be determined because the sections taken from their brains did not capture the full length of the electrode track. Because no systematic differences were observed among the convulsions of the 14 rats with an electrode tip in the BA, of the 2 with an electrode tip in the lateral amygdala, and of the 4 whose placements could not be verified; the data of all 20 rats were combined and subjected to analysis.

Kindling

The 30 stimulations administered during the kindling phase of the experiment were effective in kindling all of the subjects. All except for one of the rats displayed convulsions of class 5 or greater; and the one exception, a pre-exposure rat, did display several class 4 convulsions[5].

Table 1 provides the mean number of stimulations that the no-pre-exposure and pre-exposure rats required before they met the two criteria of kindling. None of the between-group differences in kindling rate was statistically significant.

About halfway through the kindling phase, it was observed that there was substantial

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[5] For the calculation of the two kindling criteria (see Table 1) this rat was assigned scores of 31 and 33 (the total number of stimulations administered during the kindling phase plus 1 and plus 3, respectively) as the number of stimulations required before it displayed one class 5 or greater convulsion or three class 5 or greater convulsions, respectively.

Figure 4. Experiment 2: Histology. The location of the electrode tips in the left basolateral amygdala of 16 of the 20 rats that completed Experiment 2. The electrode placements of the other 4 rats that completed the experiment could not be determined because the sections taken from their brains did not capture the full length of the electrode track. Each black dot represents the location of an electrode tip in one of the subjects. Distances are measured from bregma.

-2.30 mm

-2.56 mm

-2.80 mm

-3.14 mm

-3.30 mm

-3.60 mm
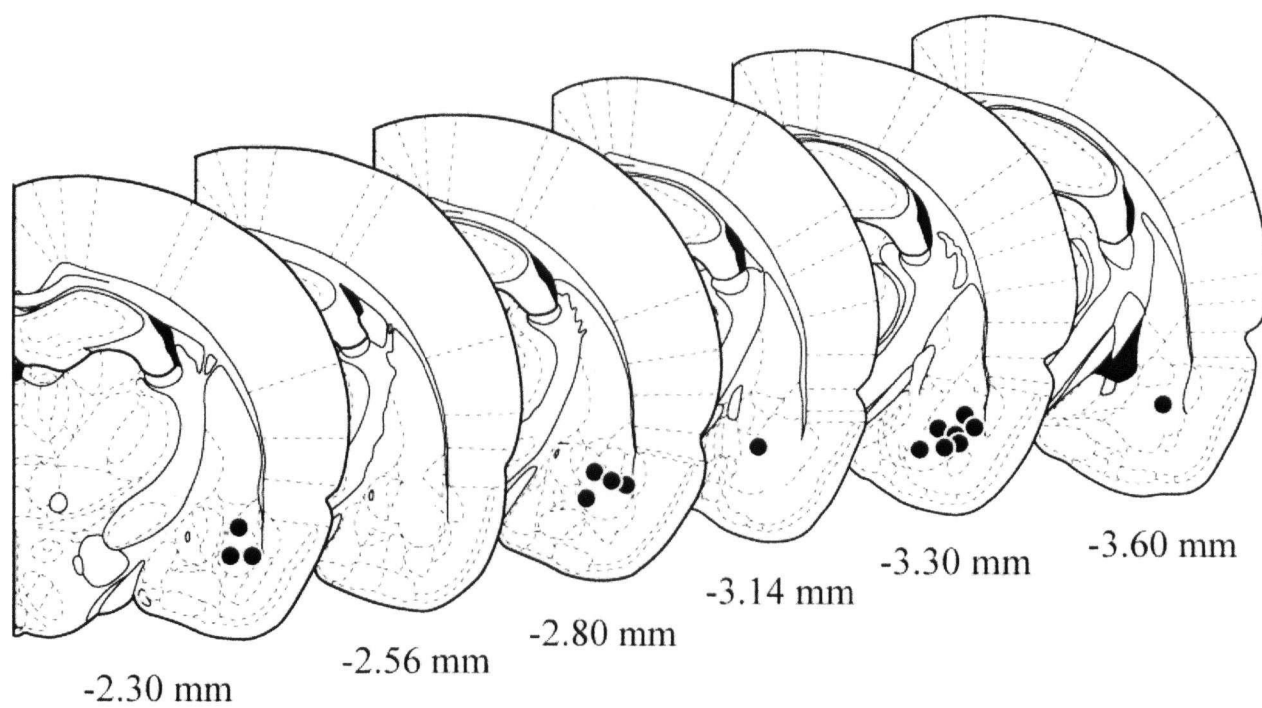
Table 1.

Number of stimulations to achieve two different kindling criteria.

Number of Stimulations to	No-pre-exposure Rats		Pre-exposure Rats	
	Mean	*SEM*	*Mean*	*SEM*
first class 5 or greater convulsion	11.10	.75	14.30	2.19
three class 5 or greater convulsions	16.60	1.46	20.50	2.52

day-to-day variation in the convulsions of some of the rats: For example, if a class 3 or greater convulsion was elicited by a stimulation, some of the rats would often display a much milder convulsive response, or even not respond at all, to the next stimulation. The proportion of these "drop days"[6] was quantified by counting the number of instances when a rat's convulsive response was three or more classes below that of its convulsive response to the previous stimulation, and then dividing that total by the total number of class 3 or greater convulsions displayed by the rat. This measure was more appropriate since the rat's kindling rate would skew a simple "total drop days" measure. An analysis of these data revealed that the pre-exposure rats displayed a greater proportion of drop days than did the no-pre-exposure rats. During the kindling phase, the mean proportion of drop days displayed by the no-pre-exposure rats was .053, whereas the mean proportion of drop days displayed by the pre-exposure rats was

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[6] Seidel and Corcoran (1986) used the term "days off" to describe a comparable phenomenon commonly observed in anterior-neocortex kindled rats: They periodically fail to display a convulsive response to stimulation. The term "drop days" was a more appropriate term for the present data because, in addition to displaying days off, they periodically displayed large declines in the strength of their convulsive response.

.17, $t(18)=2.61$, $p=.016$. Figure 5 illustrates this phenomenon: It illustrates the class of the convulsions displayed by each of three of the no-pre-exposure rats and by each of three comparable pre-exposure rats. These particular rats were selected because they corresponded to the first, second, and third quartiles of the proportion of drop days displayed by their groups.

Kindling: Time Series Data

The effects of pre-exposure to the stimulation chamber on the convulsive responses elicited by the 30 stimulations are illustrated in Figure 6. Overall, the convulsions displayed by the no-pre-exposure rats did not differ significantly from those displayed by the pre-exposure rats; however, there were significant between-group differences in the mean duration (Figure 6A) and class (Figure 6B) of the convulsive responses to some of the stimulations.

Convulsion duration. Overall, the convulsions displayed by the no-pre-exposure rats were not significantly different in duration from those displayed by the pre-exposure rats (see Figure 6A), $F(1,18)=.812$, $p=.38$. However, there were statistically significant differences in the duration of the convulsions displayed by the no-pre-exposure and pre-exposure rats in response to four of the stimulations, resulting in a significant interaction effect, $F(29,522)=2.51$, $p=.000032$. The convulsions of the pre-exposure rats were significantly shorter than those of the no-pre-exposure rats in response to the 15th, $F(1,522)=18.50$, $p=.000020$, 25th, $F(1,522)=6.89$, $p=.0089$, and 29th stimulations, $F(1,522)=6.14$, $p=.014$, but not in response to the others, all $ps>.11$. The convulsions of the no-pre-exposure rats were significantly shorter in response to only the 22nd stimulation, $F(1,522)=6.16$, $p=.013$.

Convulsion class. Overall, the convulsions displayed by the no-pre-exposure rats were not significantly different in class from those displayed by the pre-exposure rats (see Figure 6B), $F(1,18)=4.32$, $p=.052$. However, there were statistically significant differences in the class of the

Figure 5. Experiment 2: Kindling-Phase Convulsions of Selected Rats. The class of the convulsions displayed by each of three of the no-pre-exposure rats and three of the pre-exposure rats in response to each of the 30 stimulations administered during the kindling phase. The proportion of drop days displayed by these particular rats corresponded to either the first, second, or third quartile of the proportion of drop days displayed by their respective groups.
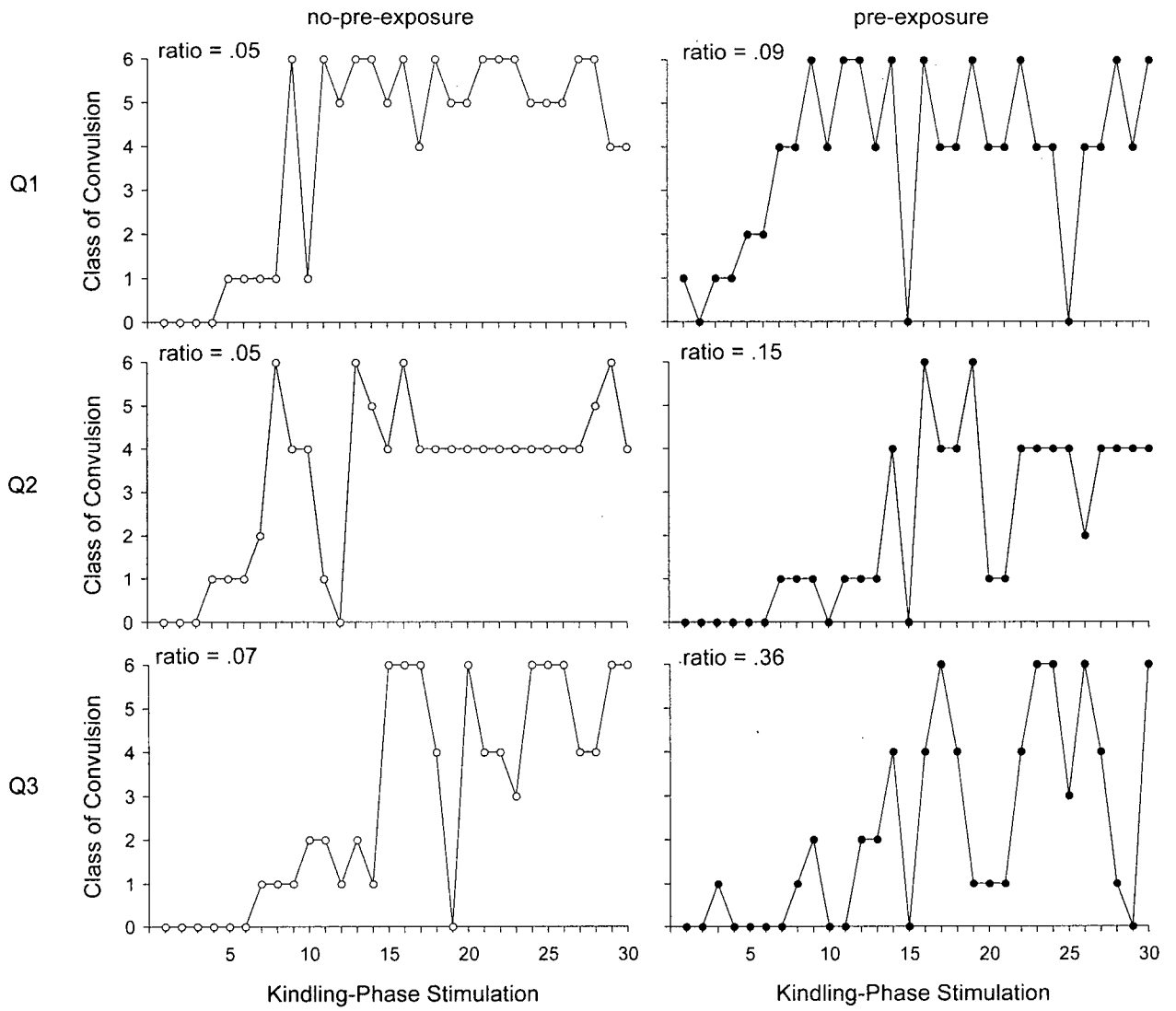
Figure 6. Experiment 2: Kindling-Phase Convulsions. The mean duration (A) and mean class

(B) of the convulsions displayed by the no-pre-exposure and pre-exposure rats in response to

each of the 30 stimulations administered during the kindling phase. Error bars represent the

SEM.
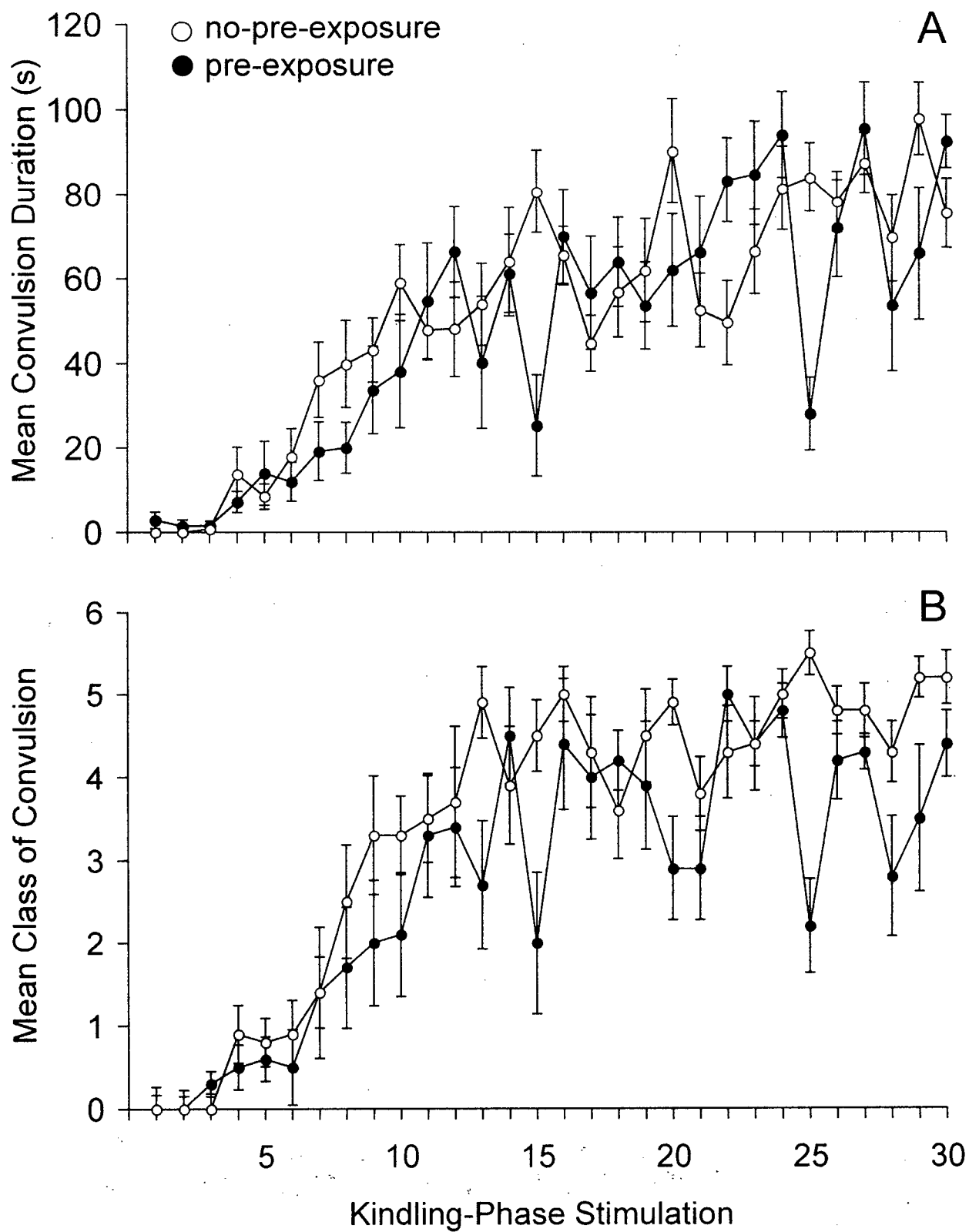
convulsions displayed in response to five of the stimulations resulting in a significant interaction, $F(29,522)=1.86, p=.0046$. The convulsions of the pre-exposure rats were significantly lower in class than those of the no-pre-exposure rats in response to the 13th, $F(1,522)=10.05, p=.0016$, 15th, $F(1,522)=12.98, p=.00034$, 20th, $F(1,522)=8.31, p=.0040$, 25th, $F(1,522)=22.61, p=.0000026$, and 29th stimulations, $F(1,522)=6.00, p=.015$; but not in response to the others, all $ps>.031$.

The statistically significant differences in means largely reflect the concordance of drop days by several pre-exposure rats. For example, the largest between-group differences were observed in the convulsive responses to the 15th and 25th stimulations (see Figure 6); 4 pre-exposure rats displayed a drop day in response to the 15th stimulation and 3 displayed a drop day in response to the 25th stimulation.

Discussion of Experiment 2

Experiment 2 demonstrated that pre-exposure to the stimulation environment can influence the elicitation of kindled convulsions. In contrast to the typical pattern displayed by amygdala-kindled rats, the pre-exposure rats occasionally displayed a convulsion that was much milder than their response to the previous stimulation--sometimes they failed to display any convulsive response whatsoever. In other words, the pre-exposure rats displayed many more drop days than did the no-pre-exposure rats.

The present results support the view that the effects of the stimulation environment on kindled convulsions are a product of Pavlovian conditioning. There was an obvious inhibitory effect of pre-exposure to the stimulation environment on the subsequent elicitation of amygdala-kindled convulsions. This implies that effects conditioned by amygdalar kindling to the

stimulation environment are excitatory and that latent inhibition can attenuate the development of those effects.

The considerable day-to-day variability in the severity of the convulsions of the pre-exposure rats is reminiscent of a comparable phenomenon in anterior-neocortex kindled rats. Anterior-neocortex kindled rats sometimes fail to display a convulsive response to stimulation after having displayed a class 3 or greater convulsion in response to the previous stimulation. Seidel and Corcoran (1986) termed those instances "days off." In the present experiment, the pre-exposure rats, unlike the no-pre-exposure control rats, displayed many days off as defined by Seidel and Corcoran. Moreover, once they were well kindled, they displayed drops of three or more classes on consecutive days to a class 1, 2, or 3. Because drops of three or more classes were rare in the control rats, they were included with days off under the umbrella term "drop days" (i.e., instances when their convulsions were three or more classes lower than their previous convulsion). The present experiment constitutes the first demonstration of drop days (or days off) in amygdala-kindled rats, and it thus raises the possibility that days off in anterior-neocortex kindled rats are a product of conditioning.

In 1974, Pinel, Phillips, and Deol studied the effect of current intensity on the reliability of amygdala-kindled convulsions. They found that a high current intensity produced kindled convulsions that were particularly reliable in their severity; once kindled, rats stimulated at 500 μA rms consistently displayed convulsions of the same class and duration. The fact that a comparably high current intensity (i.e., 400 μA rms) was employed in the current experiment makes the variability observed in the pre-exposure rats all the more noteworthy. Moreover, it suggests that the effects of pre-exposure to the stimulation environment on kindled convulsions might be much greater at near-threshold stimulation intensities.

Discussion of Line 1

The general purpose of this first line of experiments was to provide support, over and above that provided by Barnes et al. (2001), for the view that the effects of the stimulation environment on BA-kindled convulsions and interictal behaviour are a product of Pavlovian conditioning. The results of the two experiments composing line 1 both support this view. The results of Experiment 1 demonstrated that a discrimination reversal could diminish the effects of the stimulation environment on interictal behaviour. It also suggested that a discrimination reversal could significantly diminish the effects of the stimulation environment on convulsions, but the effects on convulsions in this study were somewhat equivocal. In contrast, Experiment 2 demonstrated unequivocally that pre-exposure to the stimulation environment can reduce the reliability with which kindled convulsions can be elicited.

Although the finding that Pavlovian mechanisms can influence kindled convulsions is at odds with current thinking about the factors that influence kindled convulsions, it is well established that Pavlovian conditioning to predictable stimuli can influence a variety of other physiological responses. For example, functional drug tolerance (Siegel, 1976; Siegel, Hinson, Krank, & Mccully, 1982), sensitization to stimulants (Hinson & Poulos, 1981), development of premeal hunger (Woods & Ramsay, 2000), salivation (Pavlov, 1928), baroreflexes (Dworkin & Dworkin, 1995), and immune-system responses (Cohen, Moynihan, & Ader, 1994) have all been shown to be influenced by conditioned effects. And now kindling joins that list.

LINE 2: STIMULATION-SITE SPECIFICITY

The purpose of the second line of experiments in this thesis was twofold. One purpose was to establish that effects are conditioned to the stimulation environment during the kindling of sites other than the basolateral amygdala (BA). The other purpose was to determine whether such conditioned effects are different from those associated with BA kindling.

Experiment 3: Conditioned Effects of Kindling in the Anterior Neocortex and Amygdala[7]

Experiment 3 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 AN was selected as the second 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 (e.g., Burnham, 1978; Pinel, 1981; Racine, 1975; Seidel & Corcoran, 1986). My 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

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[7] The results of Experiment 3 have been published elsewhere: Barnes, Pinel, Wig, Stuettgen, & Hölzel (2003). Stimulation site determines the conditioned effects of kindling in rats: Anterior neocortex vs. amygdala. *European Journal of Neuroscience, 17,* 1671-1679.

stimulation in the same subject, I assumed that an analysis of the conditioned effects of AN kindling might provide insight into the source of variability in AN-kindled convulsions--the results of Experiment 2 had suggested that conditioning may play a role in this variability.

Methods
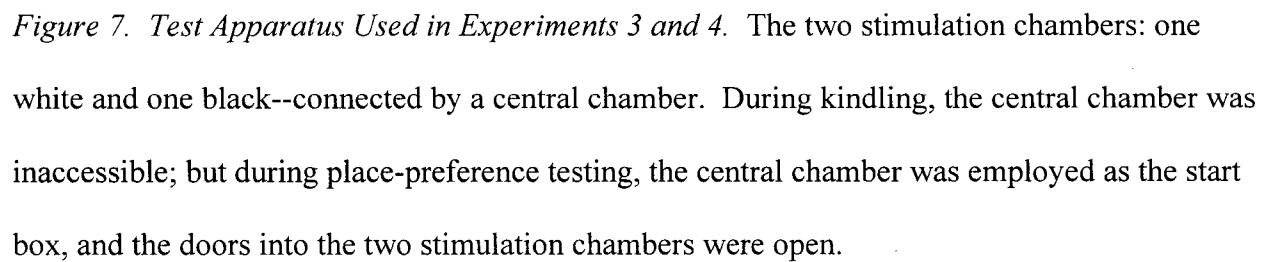
Apparatus

Figure 7 illustrates the test apparatus used in Experiment 3. It comprised two similar, but discriminable, stimulation chambers--one white and one black--connected by a central chamber which was half white and half black. 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.

Kindling Phase

A single bipolar electrode was implanted in the left BA of each of 18 rats, and in the left AN of each of another 18 rats. The electrode tip 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. Following postsurgery handling, all 36 rats were stimulated in one of the two test chambers (the CS+) and sham stimulated in the other (the CS-). The preadministration interval was 30 s.

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

Figure 7. Test Apparatus Used in Experiments 3 and 4. The two stimulation chambers: one

white and one black--connected by a central chamber. During kindling, the central chamber was

inaccessible; but during place-preference testing, the central chamber was employed as the start

box, and the doors into the two stimulation chambers were open.
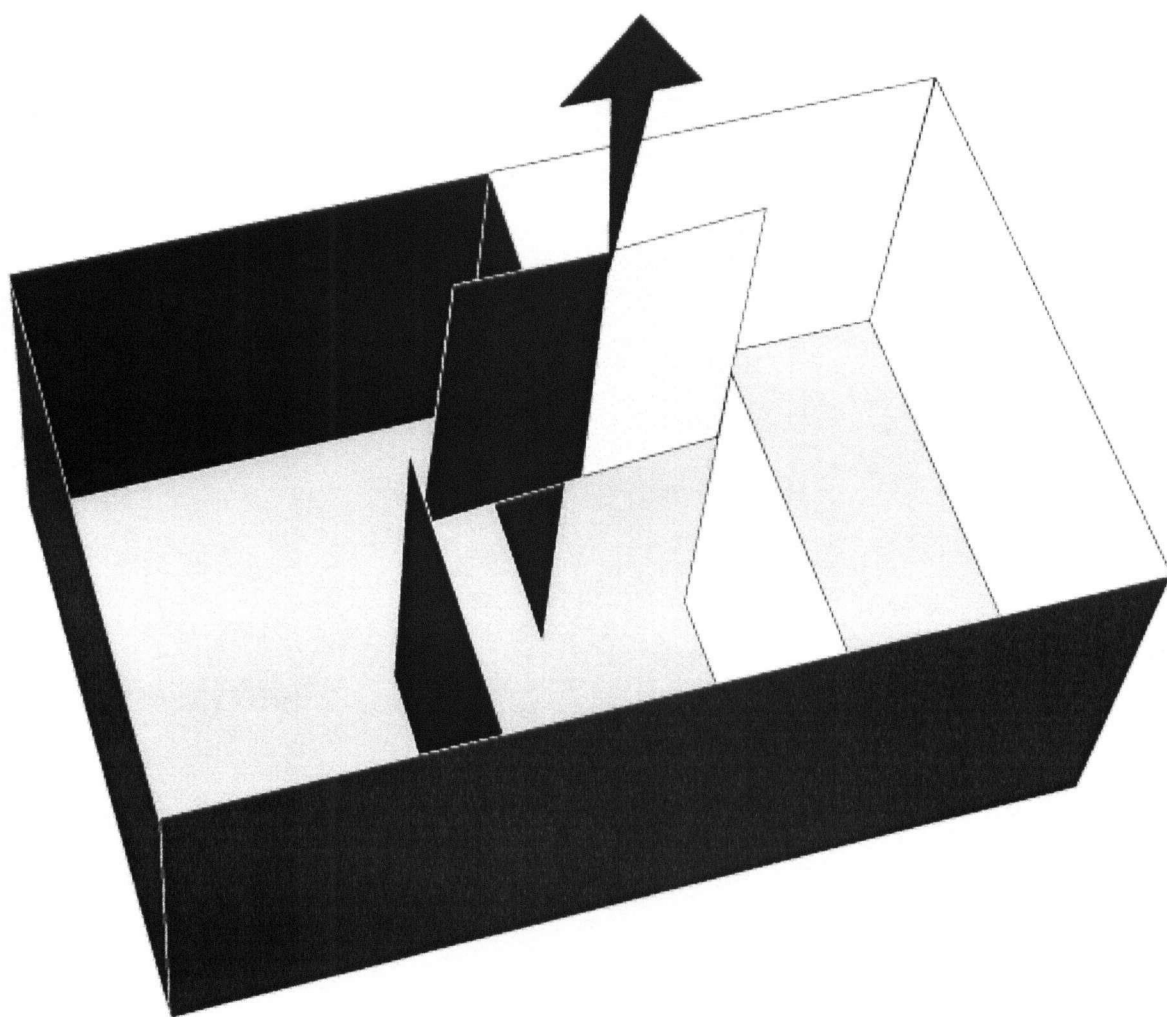
between the two sessions on a given day was between 2 and 6 h. The order of stimulation and sham-stimulation trials was quasirandom and was determined according to the following two restrictions: (1) there were 45 stimulations and 45 sham stimulations; (2) no more than three stimulations or sham stimulations ever occurred consecutively; (3) and every fourth day (e.g., day 1, day 5, day 9, etc.) was a preadministration-test day, which always comprised one stimulation trial and one sham-stimulation trial in counterbalanced sequence.

On day 29, I noticed that several rats displayed wet dog shakes in the preadministration interval. 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 & Pycock, 1977). After day 29, the experimenter regularly recorded their incidence during each preadministration interval in the CS+ and CS-. In contrast, the activity and freezing data were recorded only on preadministration 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 conditioned 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

Immediately following their final stimulation of the kindling phase, each of the two groups of rats (i.e., the AN and BA rats) was subdivided into two equal subgroups--to create four subgroups of 9 rats each. The between- and within-subjects switch tests were both conducted the following day, the switch-test day. The experimenter who scored the convulsions observed during the switch tests was blind to which chamber had previously served as the CS+ for each rat.

Between-subjects switch test. The between-subjects switch test was conducted during the morning of the switch-test day. The rats in one of the BA groups and one of the AN groups received a test stimulation in their CS- environment, whereas the rats in the other two groups received a test stimulation in their CS+ environment.

Within-subjects switch test. The within-subjects switch test was conducted during the afternoon of the switch-test day, and it involved only one of the BA groups and one of the AN groups, the rats that had received a test stimulation in their CS+ during the between-subjects switch test. These rats received a test stimulation in their CS-. This permitted a within-subjects comparison of the severity of the convulsion elicited by each rat's final stimulation in their CS+ with the severity of the convulsion elicited by their final kindling-phase stimulation in their CS-.

Blocking of Time-Series Data

The activity and freezing time-series data were blocked into four blocks of three consecutive preadministration-test days each. 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).

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, freezing, and wet-dog-shake time-series data were analyzed separately for each group using planned orthogonal contrasts between the CS+ and CS- for each separate block (i.e., blocks 1 to 4). Second, the place-preference data were analyzed using a between-within ANOVA, with group (AN or BA) 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 convulsion severity from the between-subjects switch test were analyzed using 2-way ANOVAs, with test-stimulation location (CS+ or CS-) and group (AN or BA) 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 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 between-within ANOVAs, with group as the between-subjects factor and CS as the within-subjects factor. Because multiple ANOVAs were employed 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: $p < .0125$.

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*'s 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 mean 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 4 blocks was calculated. Then, this median value was used to divide the convulsion 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, 3 were eliminated from the main analysis--1 for having a defective electrode that precluded the development of convulsions and 2 because the tips of their electrodes lay outside the amygdala. Panel A of Figure 8 illustrates the locations 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 2 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 4 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.

Panel B of Figure 8 illustrates the locations 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 2 rats had their

Figure 8. Experiment 3: Histology. The location of the electrode tips in the left basolateral amygdala (BA) of the 15 BA rats that completed Experiment 3 (A); and in the left anterior neocortex (AN) of the 18 AN rats that completed Experiment 3 (B). Each black dot represents the location of an electrode tip in one of the subjects.

A

Bregma -2.30 mm

-2.56 mm

-2.80 mm

-3.14 mm

-3.30 mm

B

Bregma 0.70 mm

0.48 mm

0.20 mm

-0.26 mm

-0.30 mm
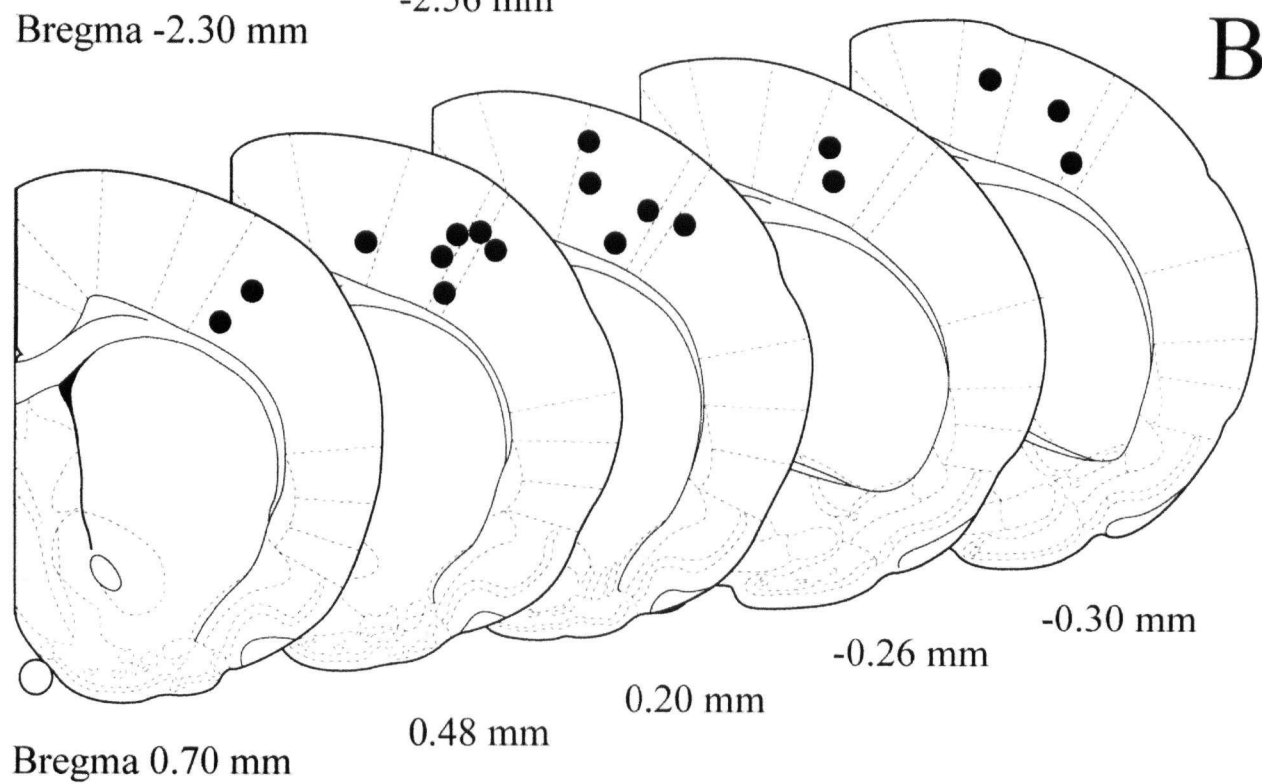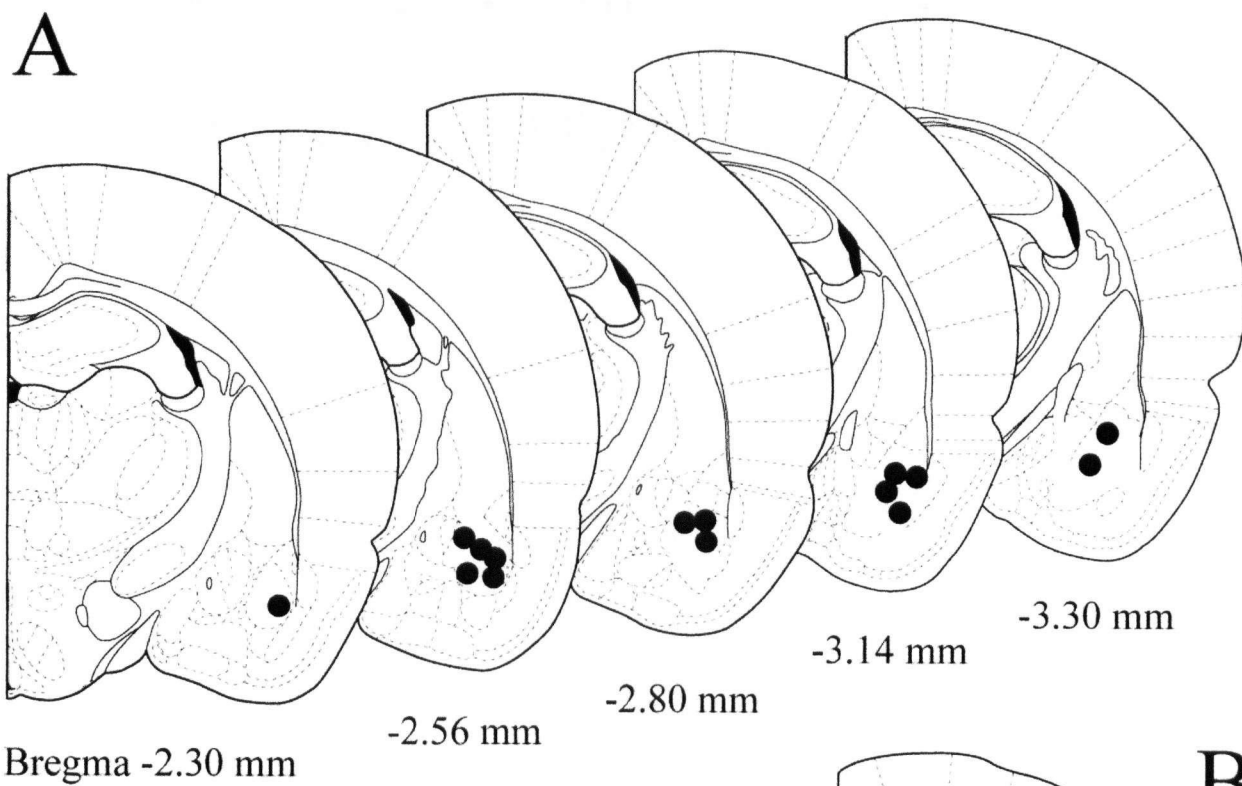
electrode tips in the motor cortex. Because no systematic differences were observed between the behaviour of the 16 AN rats with electrode tips in the somatosensory cortex and the behaviour of the 2 AN rats with electrode tips in the motor cortex, the data of all 18 AN rats were subjected to analysis.

Kindling

As previously reported (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., 1 to 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 (see 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 (i.e., usually less than 10 s) in comparison to the convulsions of the BA rats (see Della Paschoa, Kruk, Hamstra, Voskuyl, & Danhof, 1997). In addition to their brevity, all AN convulsions began coincidently with the stimulation (i.e., there was no apparent latency) and always involved

an initial brief clonic response (i.e., of 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 (see Burnham, 1978; Racine, 1975). If a convulsion were elicited by a

stimulation, the AN rats tended not to respond to the next stimulation; that is, they often

displayed what Seidel & Corcoran (1986) termed "days off." Towards the end of the kindling

phase, 2 of the 15 AN rats began to display convulsions that were similar in topography to those

of the BA rats (see Burnham, 1978).

Conditioning of Interictal Behaviours

The effects of the stimulation (CS+) and sham stimulation (CS-) environments on the

ambulatory activity and freezing recorded during the preadministration tests are illustrated in

panels A, B, C, and D of Figure 9. Overall, the BA rats displayed less ambulatory activity, and

more freezing in the CS+ than in the CS-. The AN rats also displayed less ambulatory activity in

the CS+ than in the CS-, but they did not freeze in either environment. Notice also in Figure 9

that the BA rats displayed substantially less activity and more freezing than the AN rats

Figure 9. Experiment 3: Conditioning of Interictal Behaviours. The mean ambulatory activity (A), freezing (C), and wet dog shakes (E) displayed by the basolateral-amygdala (BA) 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 (B), freezing (D), and wet dog shakes (F) displayed by the anterior-neocortex (AN) 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 *SEM*.

BA-kindled

AN-kindled
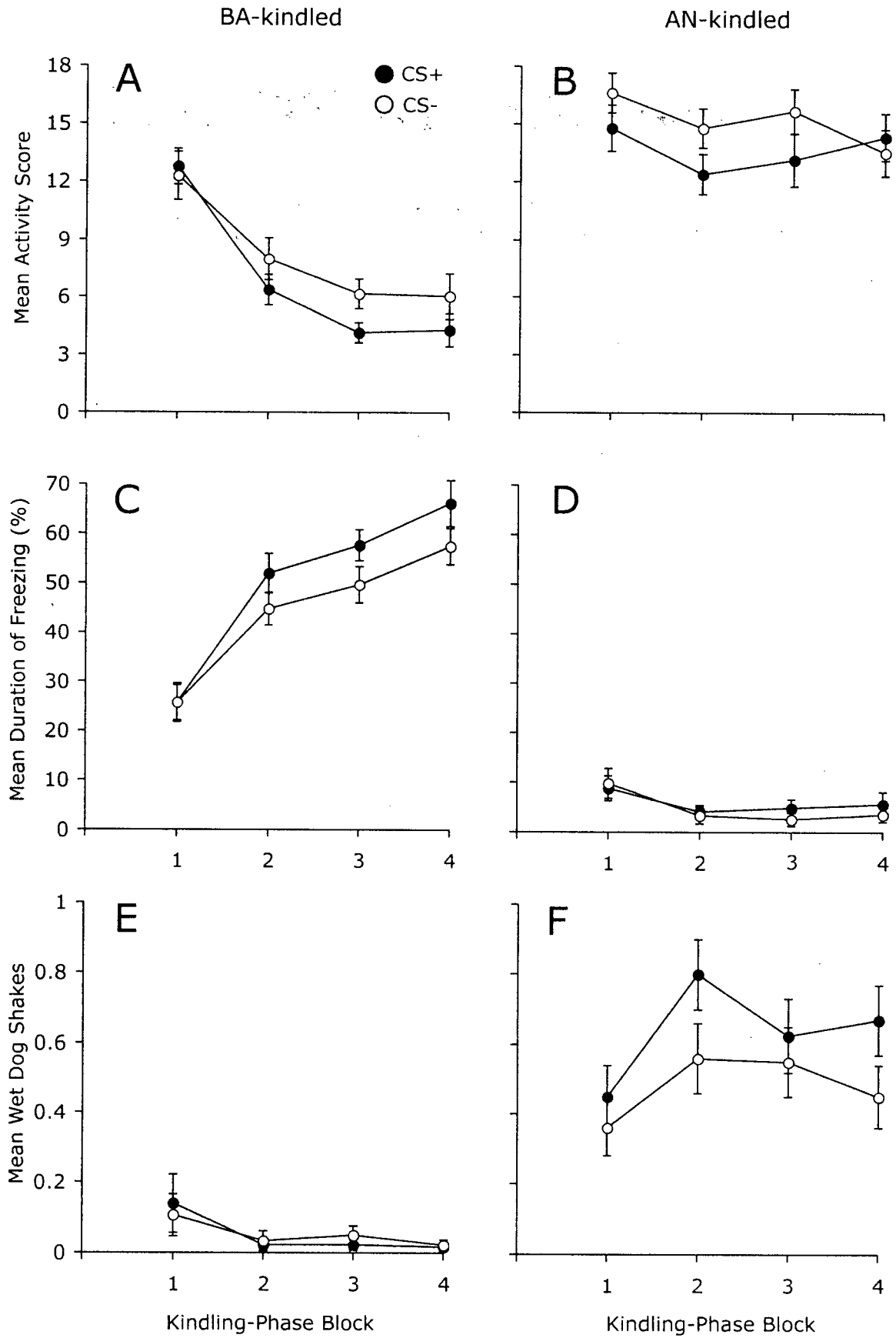
irrespective of the particular environment. The effects of the stimulation and sham stimulation environments on the number of wet dog shakes during the last 16 days of the kindling phase are illustrated in panels E and F of Figure 9. The BA rats displayed few wet dog shakes in either environment, but the AN rats displayed more wet dog shakes in the CS+ than in the CS-.

Activity. Figures 9A and 9B illustrate the mean number of line crossings displayed by the BA and AN rats, respectively, 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), $F(1,42)=6.03$, $p=.018$, but not during block 1 (days 1-9), block 2 (days 13-21), and block 4 (days 37-45), all $ps>.038$. In contrast, the AN rats were significantly less active in the CS+ than in the CS- during block 2, $F(1,51)=6.73$, $p=.012$, and block 3, $F(1,51)=7.78$, $p=.0074$, but not during block 1, $F(1,51)=3.96$, $p=.052$, and block 4, $F(1,51)=.80$, $p=.38$.

Freezing. Figures 9C and 9D illustrate the mean percentage of freezing of the BA and AN rats, respectively, in the CS+ and CS- during the preadministration tests. The BA rats displayed significantly more freezing in the CS+ than in the CS- during block 2, $F(1,42)=6.09$, $p=.018$, block 3, $F(1,42)=8.45$, $p=.0058$, and block 4, $F(1,42)=9.91$, $p=.0030$, but not during block 1, $F(1,42)=.00071$, $p=.98$. In contrast, the AN rats displayed virtually no freezing in either environment for the duration of the kindling phase, all $ps>.27$.

Wet dog shakes. Figures 9E and 9F illustrate the mean number of wet dog shakes displayed by the BA and AN rats 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 $ps>.74$; however, the AN rats displayed significantly more wet dog shakes in the CS+ than in the CS- during block 2 (days 34-37), $F(1,51)=4.45$, $p=.040$,

and block 4 (days 42-45), $F(1,51)=4.04$, $p=.050$, but not during block 1 (days 30-33),

$F(1,51)=.90$, $p=.35$, or block 3 (days 38-41), $F(1,51)=.59$, $p=.45$.

Conditioned Place-Preference

Figure 10 shows the total amount of time that the BA and AN rats spent in the CS+ and

CS- during the conditioned place-preference test. Overall, the BA and AN rats spent

significantly more time in the CS+ than in the CS-, $F(1,31)=14.40$, $p=.00064$. However, there

were statistically significant differences in the amount of time that the BA rats spent in the CS+

and CS- resulting in an interaction effect that was marginally nonsignificant, $F(1,31)=4.03$,

$p=.053$. The BA rats spent significantly less time in the CS+ than in the CS-, $F(1,31)=15.43$,

$p=.00045$. In fact, 14 of the 15 BA rats spent less time in the CS+; 4 did not enter the CS+ at all;

and 12 of the 15 chose to enter the CS- first, $\chi^2(1)=8.07$, $p=.0045$. In contrast, the amount of

time that the AN rats spent in the CS+ did not differ significantly from the amount of time they

spent in the CS-, $F(1,31)=1.75$, $p=.20$. Because 2 of the 18 AN rats had developed convulsions

that were similar in topography and duration to those of the BA rats a post-hoc analysis was

conducted on their place preference data. Like the BA rats, these 2 AN rats spent significantly

less time in the CS+ than in the CS- ($M=97.00$ vs. 43.00), $t(1)=27.00$, $p=.024$.

Conditioning of Convulsions

Between-subjects switch test. Panels A, B, C, and D of Figure 11 illustrate 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 received a test stimulation in the CS- environment while the other half

received a test stimulation in the CS+ environment. The effect of the between-subjects switch

Figure 10: *Experiment 3: Conditioned Place-Preference Test.* The mean amount of time spent by the BA-kindled and the AN-kindled rats in the CS+ and CS- during the 5-min place-preference test on day 46. Error bars represent the *SEM*.
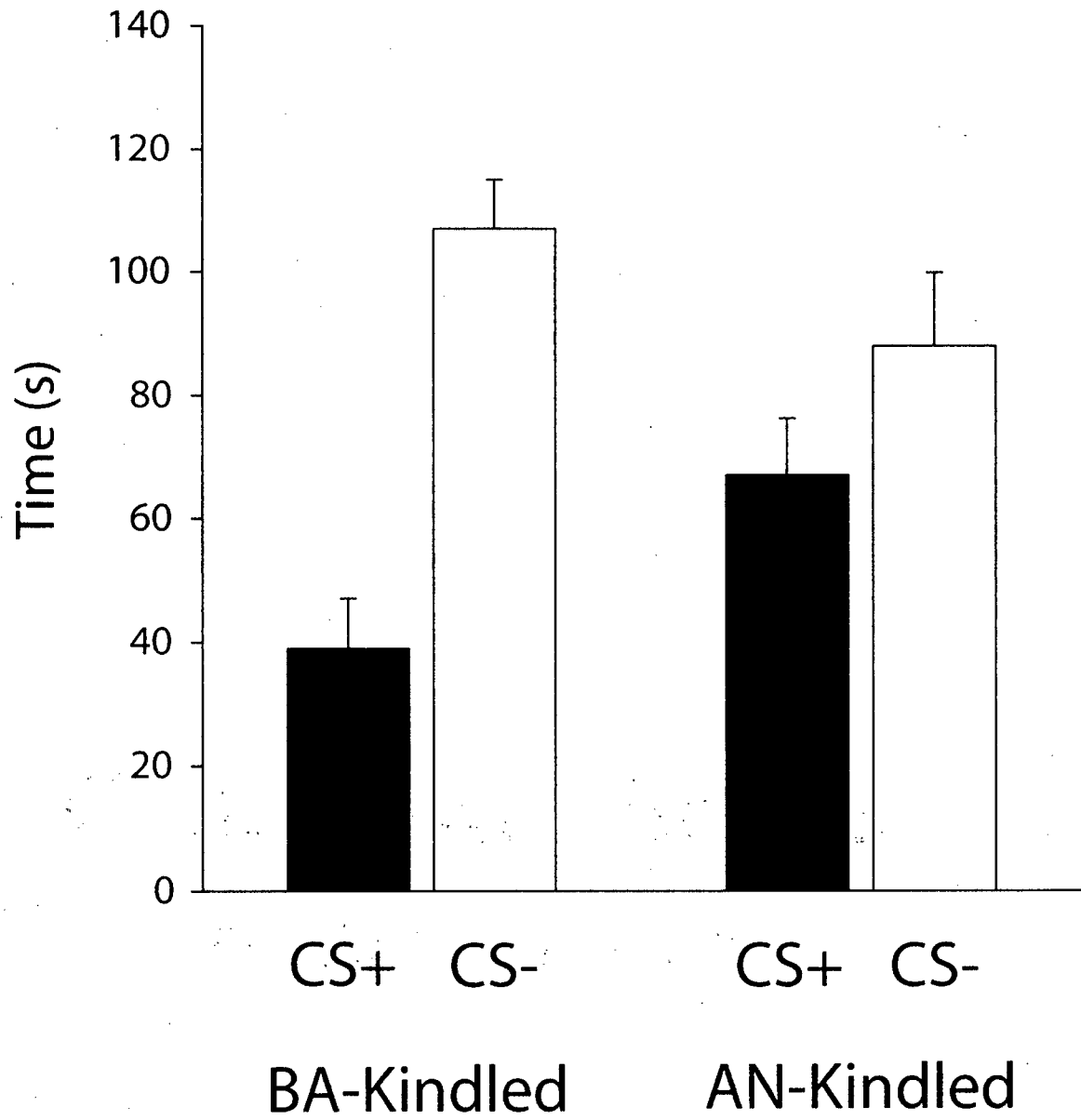
Figure 11. Experiment 3: Between-Subjects Switch Test. The mean duration (A), class (B), latency (C), and number of falls (D) of the convulsions elicited during the between-subjects switch test which occurred at the end of the kindling phase: Half of the BA-kindled rats and half of the AN-kindled rats received a test stimulation in their CS- environment and the other rats received a test stimulation in their CS+ environment. Error bars represent the *SEM*.

Mean Number of Falls

Mean Convulsion Latency (s)

Mean Class of Convulsion

Mean Convulsion Duration (s)

BA-kindled

AN-kindled

CS+   CS-   CS+ CS-

A

B

C

D
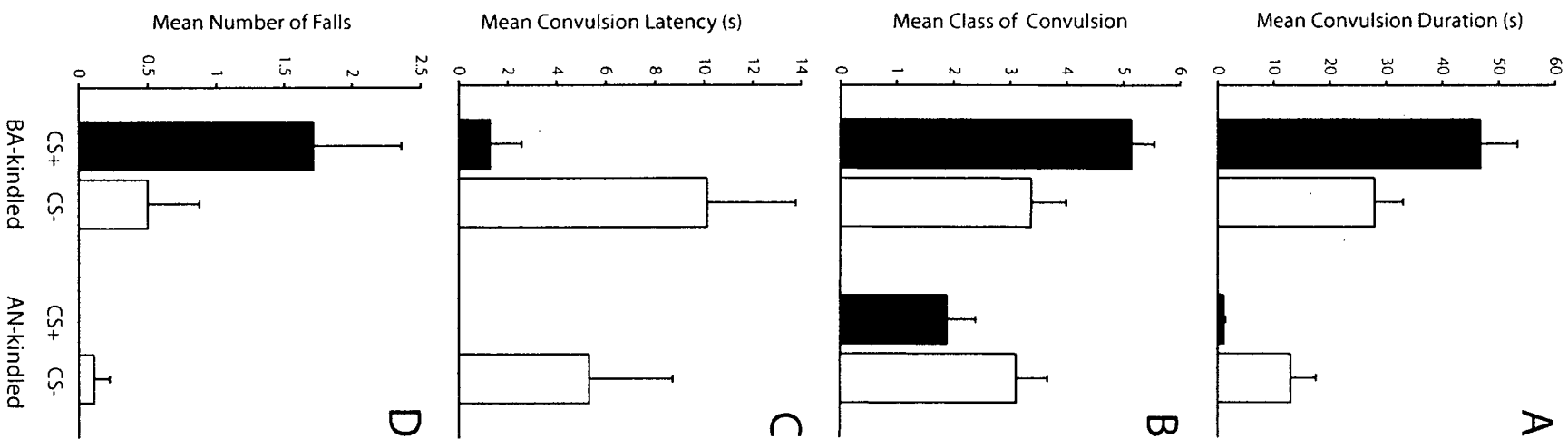
test on the severity of the convulsions displayed by the BA rats was different from its effect on the severity of the convulsions displayed by the AN rats, in terms of duration, $F(1,29)=11.68$, $p=.002$, and class, $F(1,29)=7.50$, $p=.010$, but not in terms of number of falls, $F(1,29)=3.95$, $p=.056$, and latency, $F(1,29)=.44$, $p=.51$--though the between-subjects switch test did have a significant overall effect on latency, $F(1,29)=7.26$, $p=.012$.

The convulsions of the BA rats that were tested in the CS- were significantly weaker than those of the BA rats that were tested in the CS+: Their convulsions were significantly shorter, $F(1,29)=7.91$, $p=.0087$; but not quite significantly lower in class, $F(1,29)=4.80$, $p=.037$. Indeed, one BA rat failed to respond with any convulsion when tested 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 convulsions of the AN rats that were tested in the CS- were significantly more severe than those of the AN rats that were tested in the CS+: Their convulsions were significantly longer, $U=11.00$, $p=.008$[8]; but they were not of a significantly lower class, $F(1,29)=2.77$, $p=.11$.

Within-subjects switch test. Panels A, B, C, and D of Figure 12 illustrate the means of the four measures of the severity of the convulsions that were elicited in the BA and AN rats by their final stimulation in the CS+ and by their final stimulation in the CS-. These within-groups comparisons confirmed the results of the between-groups comparisons (see Figure 11). The effect of the within-subjects switch test on the severity of the convulsions displayed by the BA rats was different from its effect on severity of the convulsions displayed by the AN rats, in

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[8] This effect on convulsion duration was not significant when tested by ANOVA, $F(1,29)=2.77$, $p=.11$. However, because the variance was heterogeneous (see Figure 11A), a Mann-Whitney U-test was performed.

Figure 12. Experiment 3: Within-Subjects Switch Test. The mean duration (A), class (B),

latency (C), and number of falls (D) of the convulsions elicited during the within-subjects switch

test which occurred at the end of the kindling phas. All of the BA- and AN-kindled rats received

a test stimulation in their CS- environment and a test stimulation in their CS+ environment.

Error bars represent the *SEM*.
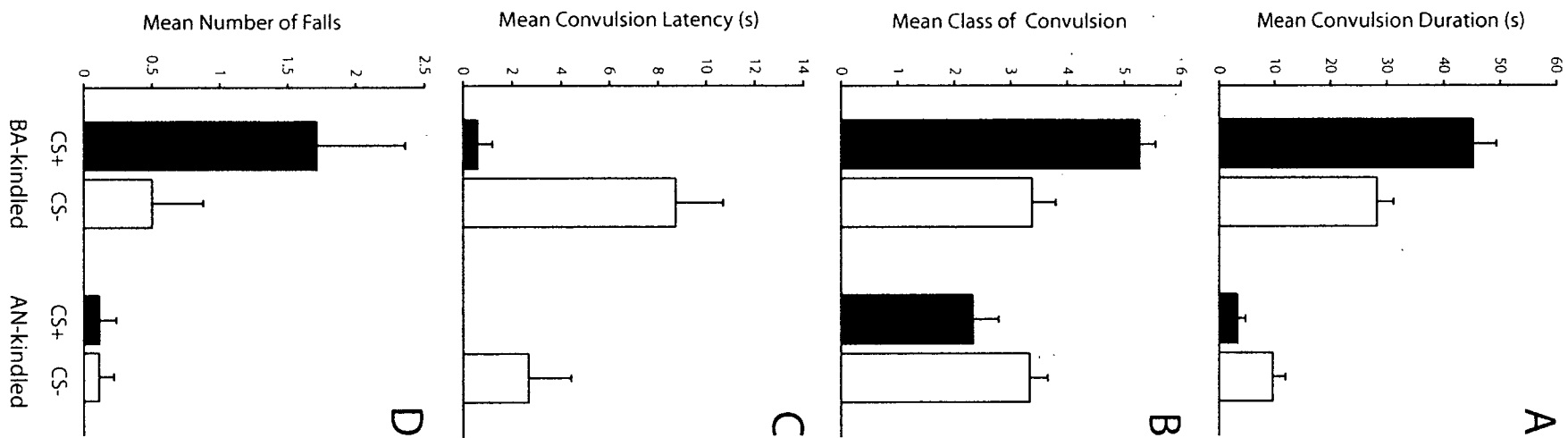
terms of duration, $F(1,31)=13.94$, $p=.00076$, class, $F(1,31)=17.39$, $p=.00023$, and number of falls, $F(1,31)=14.99$, $p=.00052$, but not in terms of latency, $F(1,29)=.44$, $p=.51$--though the within-subjects switch test did have a significant main effect on latency, $F(1,31)=15.59$, $p=.00042$.

When the BA rats were stimulated in the CS-, their convulsions were weaker than when they were stimulated in the CS+: Their convulsions were significantly shorter, $F(1,31)=13.41$, $p=.00092$; they were of a significantly lower class, $F(1,31)=9.95$, $p=.0036$; and they involved significantly fewer falls, $F(1,31)=27.44$, $p=.000011$. In contrast, when the AN rats were stimulated in the CS-, their convulsions were more severe than when they were stimulated in the CS+: Their convulsions were of a significantly lower class, $F(1,31)=7.44$, $p=.010$; but they were not significantly longer in duration, $F(1,31)=2.33$, $p=.14$, and there were no significant differences in their number of falls, $F(1,31)=0.00$, $p=1.00$. 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 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 and within-subjects correlations.

Between-subjects correlations. For the BA rats, there was no significant correlation between wet dog shakes and convulsion class, $r=.065$, $t(60)=.49$, $p=.62$, nor between wet dog shakes and convulsion duration, $r=.19$, $t(60)=1.47$, $p=.15$. In contrast, those AN rats that displayed more wet dog shakes also had convulsions of a lower class, $r=-.37$, $t(72)=3.33$, $p=.0014$, and of a shorter duration, $r=-.27$, $t(72)=2.34$, $p=.022$.

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 of 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, $t(17)=2.85$, $p=.011$, and duration, $t(17)=2.73$, $p=.014$, of convulsions that they displayed in the same blocks.

Discussion of Experiment 3

Experiment 3 compared the conditioned effects of basolateral amygdala (BA) kindling with those of anterior neocortex (AN) kindling. There were six 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+ than in the CS-, whereas the AN-kindled rats did not display freezing in either the CS+ or CS-. Third, as kindling progressed, the AN-kindled rats began to display more wet-dog shakes in the CS+ than in the CS-, whereas BA-kindled rats did not display wet dog shakes in either the CS+ or CS-. Fourth, the BA-kindled rats avoided the CS+ during the conditioned place-preference test, whereas the AN-kindled rats

did not. Fifth, the number of wet dog shakes displayed by the AN-kindled rats was negatively correlated with the severity of their convulsions, both between AN-kindled rats and within each individual AN-kindled rat from stimulation to stimulation. Sixth, when finally stimulated in the CS-, the BA-kindled rats displayed milder convulsions than they had in the CS+, whereas the AN-kindled rats displayed more severe convulsions in the CS-.

The present findings confirm the conditioned effects of the stimulation environment in BA-kindled rats, which were first reported by Barnes et al. (2001). More importantly, by comparing the conditioned effects of BA and AN kindling, they establish for the first time that conditioned effects are not restricted to BA kindling and that the nature of such conditioned effects are influenced by the kindling site.

Although the results of Experiment 3 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 influences the conditioned effects of kindling indirectly, through its effects on the topography of the convulsions. 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 afterdischarges 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 AN, 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 2 AN rats developed convulsions that were topographically similar to limbic convulsions. Interestingly, at the end of the experiment, these 2 anomalous AN rats behaved more like the BA-kindled rats than the AN-kindled 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 the convulsions 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+ (see Figure 9, panel F) may explain why the convulsions elicited in the CS+ by AN stimulation were weaker than those elicited in the CS- (see Figures 11 and 12). Moreover, the correlation between wet dog shakes and AN-kindled convulsions suggests that the well-documented day-to-day variation in AN-kindled convulsions (e.g., Burnham, 1978; Seidel & Corcoran, 1986) may be a consequence of variations in the prevalence of conditioned wet dog shakes.

The present results are comparable to demonstrations of the situational specificity of drug tolerance (e.g., Siegel et al., 1982) and drug sensitization (e.g., Weiss, Post, Pert, Woodward, & Murman, 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-kindled rats, these conditioned effects seemed to potentiate, rather than counteract, the

effects of the stimulations--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-kindled rats. Accordingly, the conditioned response in the BA-kindled rats seem to be similar to conditioned drug sensitization; and the conditioned response in the AN-kindled rats seem to be similar to conditioned drug tolerance.

Just as conditioned effects play a role in the development of drug tolerance or sensitization, 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 unconditioned stimulus (US). In the context of the analysis of Ramsay and 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 could begin to elicit CCRs that would offset the effects of the URs. The nature of these CCRs would 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, in the context of kindling, the maladaptive nature of a CCR does not negate its potential existence; 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.

Experiment 4: Conditioned Effects of Kindling Three Different Sites in the Hippocampal

Complex

The primary purpose of Experiment 4 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--Experiment 3 had focused on

comparing the conditioned effects of topographically distinct kindled convulsions. My 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?

I compared the behavioural 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).

Methods

Kindling Phase

A single bipolar electrode was implanted in the left PRh of 18 rats, in the left VH of

another 18 rats, and in the left DH of another 18 rats. 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. Following postsurgery handling, all 54 rats were stimulated in one of the two test chambers (the CS+) and sham stimulated in the other (the CS-). The test apparatus (see Figure 7) and behavioural testing protocol were identical to those of Experiment 3.

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 behavioural testing protocol, the three groups were significantly, but not equivalently, kindled. After comparing conditioned effects in the three groups after 53 stimulations, I attempted to bring the VH and DH rats up to a level of kindling comparable to that in the PRh rats by repeating the behavioural testing protocol for the VH and DH rats, but not the PRh rats.

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.

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

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). 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 2-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 both groups of rats (PRh, VH, or DH) by the final stimulation in the CS+ versus those elicited by the stimulation in the CS- (the within-subjects switch test) 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 employed 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 weak conditioned effects only toward the end of the experiment; and the DH rats kindled most slowly and had not demonstrated any conditioned effects by the end of the experiment. Unlike BA and AN kindling, 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 behaviour.

Histology

Figure 13 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 perirhinal cortex of the 18 PRh rats that completed the experiment. The electrodes of all 18 PRh rats lay within the boundaries of the perirhinal cortex.

Second, Figure 13 also illustrates the location of the electrode tips in the left ventral hippocampus 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 ventral hippocampus. 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 behaviour 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 electrode placements of the other 16 VH rats were accurate and because the behaviour of these 2 VH rats did not differ in any obvious fashion from that of the other 16 VH rats.

Finally, Figure 13 illustrates the location of the electrode tips in the left dorsal hippocampus of the 15 DH rats that completed the experiment. The other 3 DH rats developed severe infections around their electrode assemblies and did not complete the experiment. Of the 15 DH rats completing the experiment, the electrodes of 2 DH rats terminated in the

Figure 13. Experiment 4: Histology. The location of the electrode tips in the left perirhinal cortex (PRh) of the 18 PRh rats that completed Experiment 4 (black circles), in the left ventral hippocampus (VH) of 16 of the 18 VH rats that completed the Experiment 4 (black triangles), and in the left dorsal hippocampus (DH) of the 15 DH rats that completed Experiment 4 (black squares). Each circle, triangle, or square represents the location of an electrode tip in one of the subjects. Distances are measured from bregma.

-2.30 mm

-2.56 mm

-2.80 mm

-3.14 mm

-3.30 mm

-3.60 mm

-3.80 mm

-4.16 mm

-4.80 mm

-5.20 mm

-5.30 mm

-5.60 mm

-5.80 mm

-6.04 mm
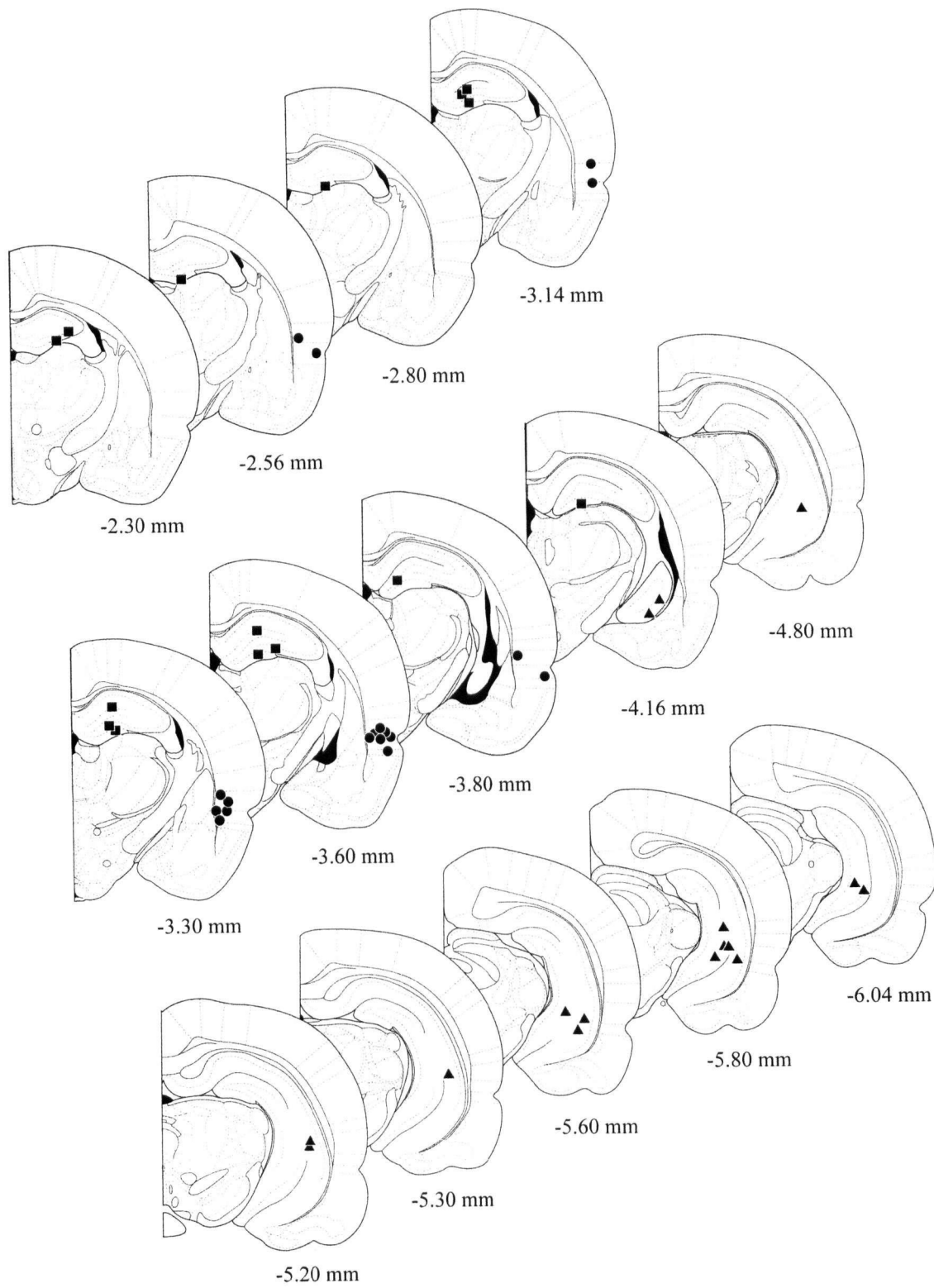
CA1-subfield of the hippocampus, the electrode of 1 DH rat terminated in the CA3-subfield of the hippocampus, and electrodes of the other 12 DH rats terminated within the boundaries of the dentate gyrus. There were no obvious differences in the behaviour of these three DH subgroups.

Kindling

Figure 14 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 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 employed 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 three rats the maximum score of 106

Figure 14. Experiment 4: Kindling-Phase Convulsions. The mean class of the convulsions

displayed by the PRh-kindled rats during each of the first four blocks of test days of the kindling

phase, and by the VH- and DH-kindled rats during each of the eight blocks of test days of the

kindling phase. Error bars represent the *SEM*.
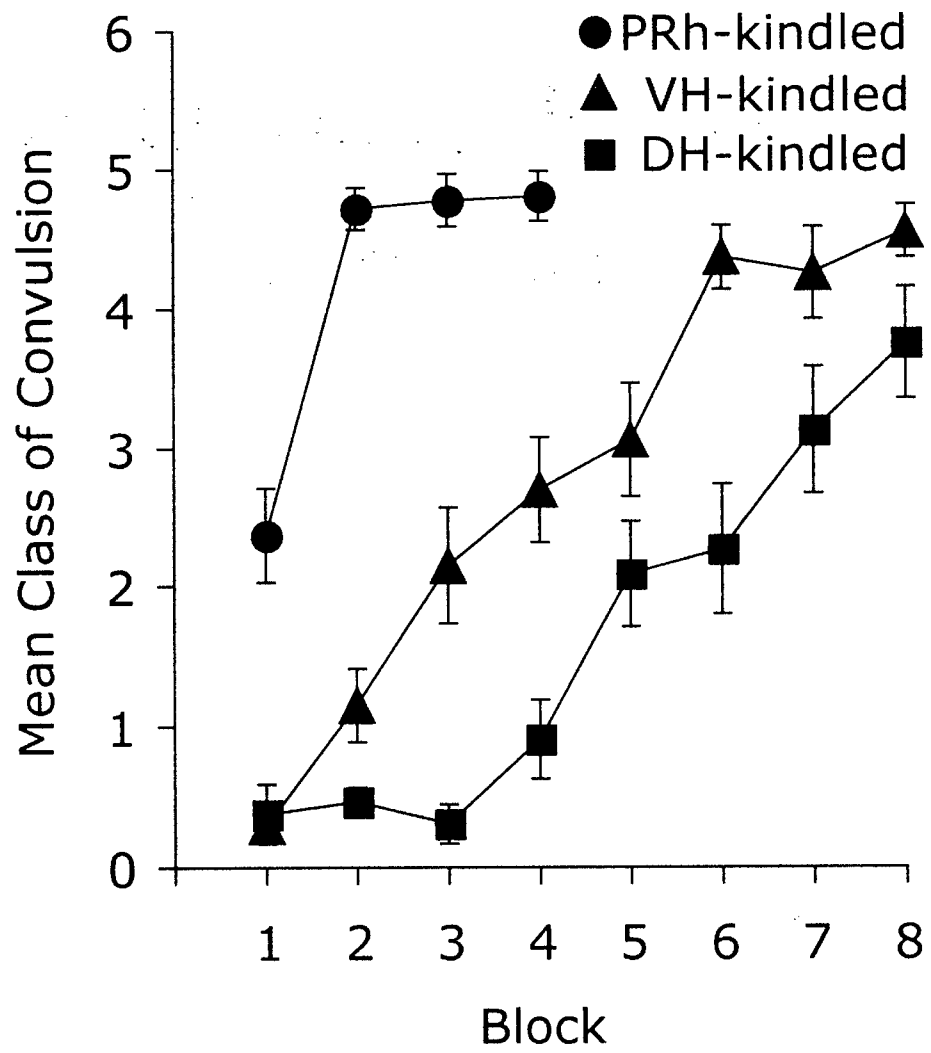
(the total number of stimulations administered to the DH and VH rats in the experiment) the DH rats required a mean of 74.1 stimulations before they displayed three convulsions with a class of 5 or greater. During the first series of 53 stimulations, the VH and DH rats displayed a mean of 6.2 and .8 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 Behaviours

The effects of the stimulation (CS+) and sham stimulation (CS-) environments on the ambulatory activity and freezing recorded during the preadministration tests are illustrated in Figure 15. Overall, the PRh rats displayed less ambulatory activity and more freezing in the CS+ than in the CS-. In contrast, the VH and DH rats did not display any 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 more freezing in the CS+ than in the CS-.

Activity. Figures 15A, 15B, and 15C 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, all $ps>.026$.

Freezing. Figures 15D, 15E, and 15F illustrate the mean percentage of freezing of the PRh, DH, and VH rats, respectively, in the CS+ and CS- during the preadministration tests. The

Figure 15. Experiment 4: Conditioning of Interictal Behaviours. The mean ambulatory activity

(A) and freezing (D) displayed by the PRh-kindled rats in their 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 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 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 *SEM.*

PRh-kindled

VH-kindled

DH-kindled

A

B

C

D

E

F

Mean Activity Score

Mean Duration of Freezing (%)

Block

Block

Block

● CS+
○ CS-

▲ CS+
△ CS-

■ CS+
□ CS-
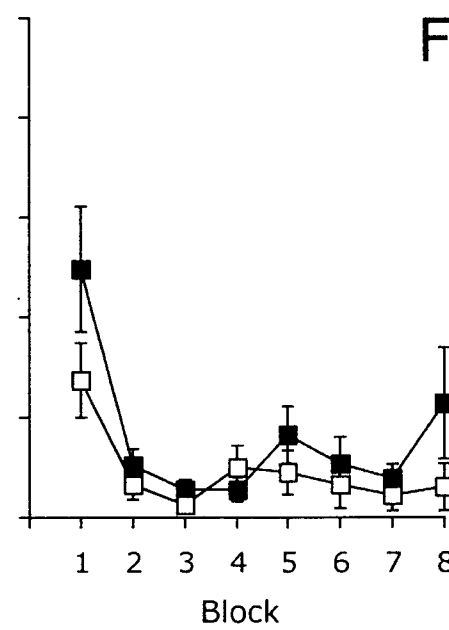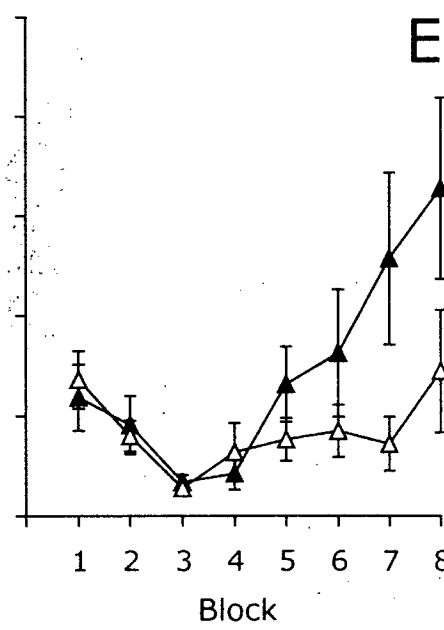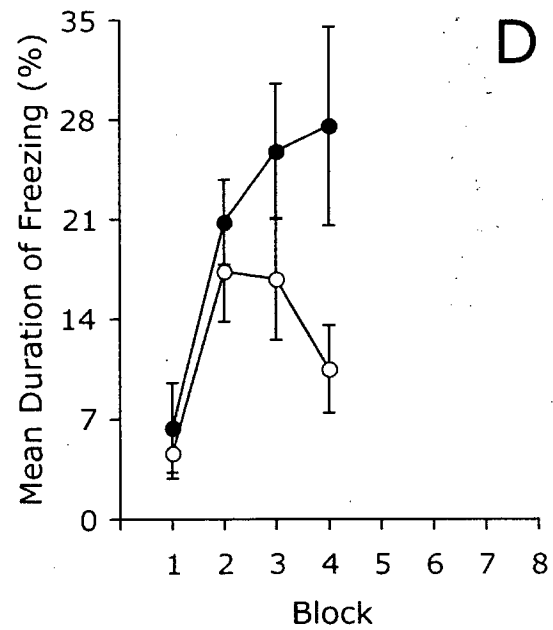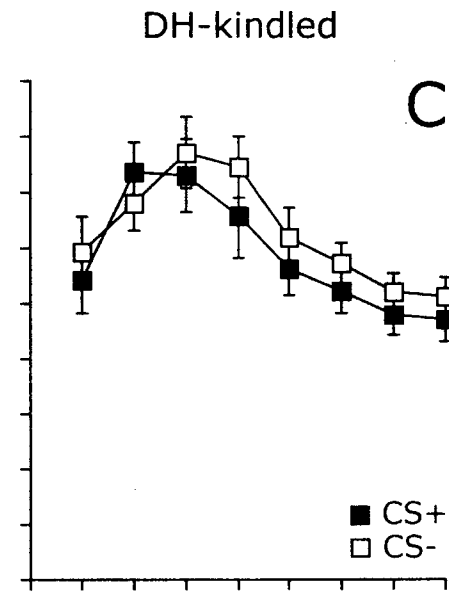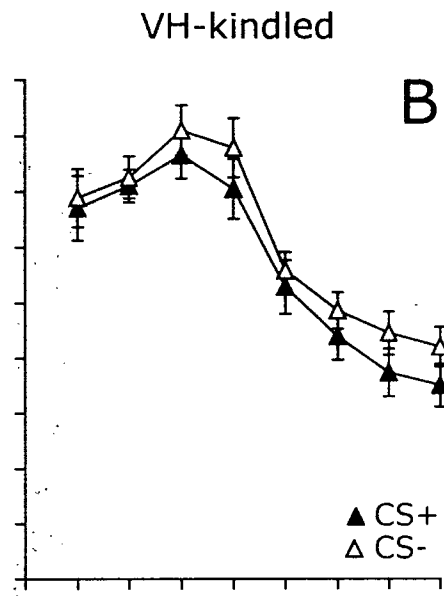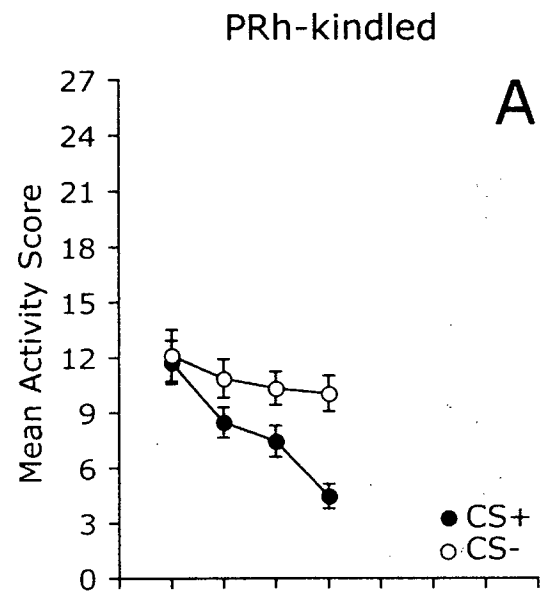
PRh rats displayed significantly more freezing in the CS+ than in the CS- during block 4, $F(1,24)=9.15, p=.0051$, but not during block 1, $F(1,24)=.10, p=.75$, block 2, $F(1,24)=.39, p=.54$, and block 3, $F(1,24)=2.63, p=.12$. Similarly, the VH rats displayed significantly more freezing in the CS+ than 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, $F(1,50)=.073, p=.79$, block 2, $F(1,50)=.027, p=.87$, block 3, $F(1,50)=.0096, p=.92$, block 4, $F(1,50)=.10, p=.75$, block 5 (days 56-64), $F(1,50)=.69, p=.41$, 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, all $ps>.089$.

Conditioned Place-Preference Test

The left half of Figure 16 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-, $F(1,48)=4.53, p=.038$. 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 16 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 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

Figure 16. Experiment 4: Conditioned Place-Preference Test. The mean amount of time spent

by the PRh-kindled rats, the VH-kindled rats, and the 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 *SEM.*
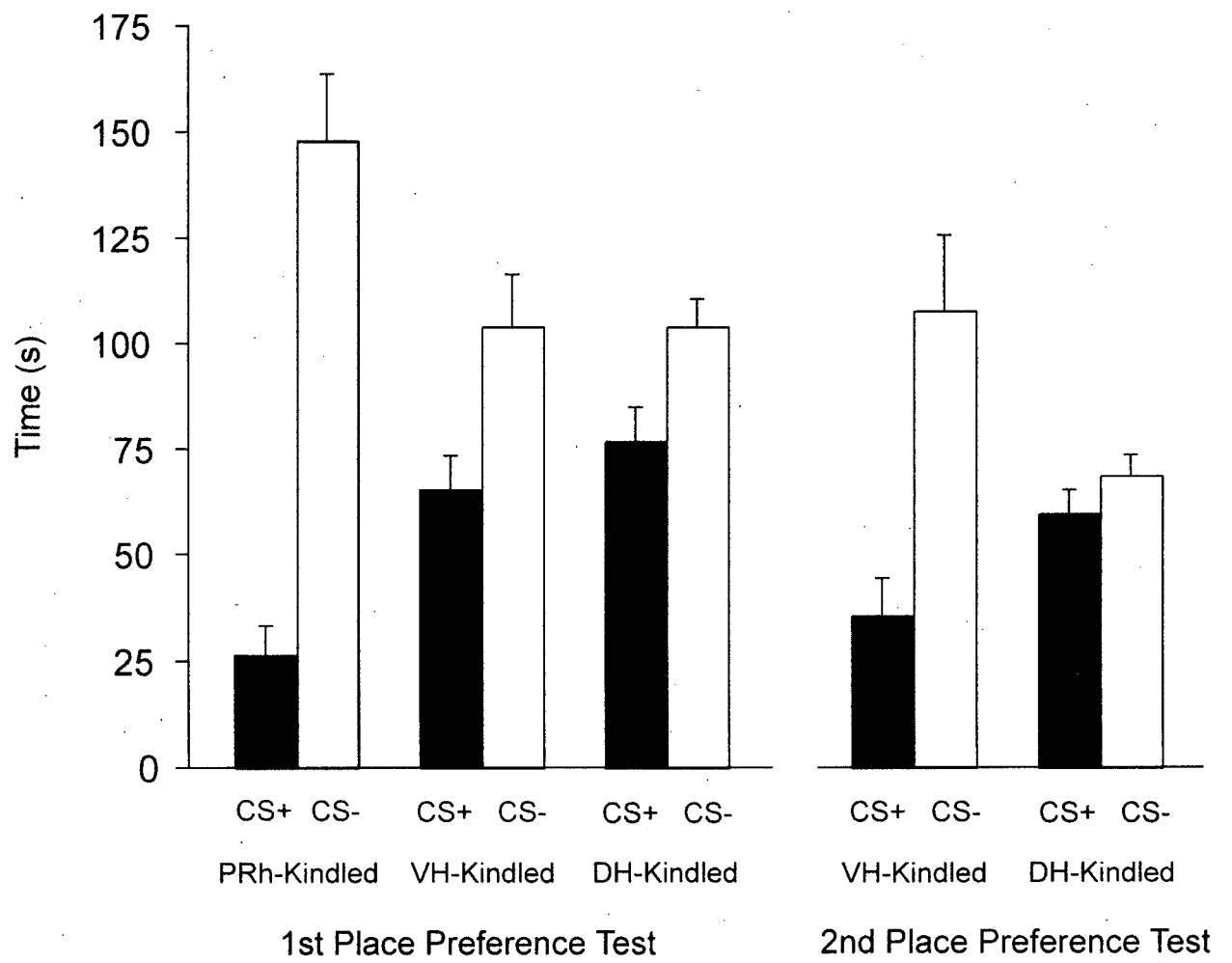
time in the CS+ than in the CS-, $F(1,31)=14.82$, $p=.00055$. In fact, 14 of the 18 VH rats spent less time in the CS+, and 4 did not enter the CS+ at all. In contrast, the amount of time that the DH rats spent in the CS+ did not differ significantly from the amount of time they spent in the CS-, $F(1,31)=.189$, $p=.67$.

Conditioning of Convulsions

After 53 stimulations, half of the PRh, VH, and DH rats were stimulated for the first time in the CS- while 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, all *ps*>.13. After 106 stimulations, half of the VH and DH rats were stimulated for the second time in the CS- while 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, all *ps*>.13.

Discussion of Experiment 4

Experiment 4 compared the effects conditioned to the stimulation environment during the kindling of each of three different sites within the hippocampal complex: the perirhinal cortex (PRh), the ventral hippocampus (VH), and the dorsal hippocampus (DH). There were three major findings. First, unlike BA and AN kindling (see Experiment 3), 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 behaviour. 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 weak conditioned effects only toward the end of the experiment; and the DH rats kindled most slowly and had not demonstrated any conditioned effects by the end of the experiment. Third, the direction of the conditioned effects observed on the interictal behaviour of the PRh and VH rats was the same: Both groups displayed more freezing in the CS+ than in the CS-, and they both avoided the CS+ in the conditioned place-preference test.

Based in part on demonstrations of BA-kindling-induced increases in defensive behaviour (e.g., Adamec, 1990; Helfer, Deransart, Marescaux, & Depaulis, 1996; Kalynchuk et al., 1999; Nieminen et al., 1992; but see Ebert & Koch, 1996; Witkin, Lee, & Walczak, 1988), the pattern of conditioned effects produced by BA 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 behaviours. The first is that they are a specific consequence of the amygdala's well-established role in fear and defensive behaviour (e.g., Davis, 1998; Fanselow & Gale, 2003; Ledoux, 2003; Maren, 2001). 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-kindled rats of Experiment 3 was not indicative of increased defensiveness seems to support the first alternative: The AN-kindled rats displayed no freezing, and they did not avoid the CS+ environment during a conditioned place preference test--declines in activity may have been an indirect consequence of the wet-dog shakes. However, the fact that the PRh- and VH-kindled rats of Experiment 4 displayed a pattern of conditioned effects that seeemed defensive in nature supports the second alternative--but with one modification: Only convulsions of a "limbic" topography, such as those observed in BA, PRh, VH, and DH kindling but not in AN kindling, can serve as an aversive

unconditioned stimulus (US) in the conditioning of interictal defensive behaviours in kindled rats.

In the experiment by Barnes et al. (2001), BA-kindled convulsions were less severe in the sham-stimulation environment (CS-) than in the stimulation environment (CS+). One possible explanation for this effect is that it is an indirect consequence of the effects of the CS+ on interictal behaviour: BA-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 behaviour. In fact, the effect of the CS+ on the interictal behaviour of the PRh-kindled rats was even more pronounced than it was in the BA-kindled rats of Experiment 3 (see panels A and C of Figure 9), but it still had no effect on their convulsions.

In their study on the conditioning of flavour aversions by amygdala kindling, Wig et al. (2001) reported a large positive correlation (i.e., $r=.90$) between kindling rate and the rate at which rats learned to discriminate between two flavours: 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 behaviour of BA-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 behaviour by kindling.

The lack of an effect of the stimulation environment on the interictal behaviour 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 seems 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 5 DH afterdischarges (Laurent-Demir & Jaffard, 1997). This might be why no conditioned effects were observed on the interictal behaviour 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.

Discussion of Line 2

The first general purpose of this second line of experiments was to establish that effects can be conditioned to the stimulation environment when sites other than the basolateral amygdala (BA) are being kindled. The experiments composing line 2 did just that: In addition to

BA-kindling, effects were conditioned to the stimulation environment during AN, PRh, and VH kindling.

The second general purpose of line 2 was to determine whether the conditioned effects of kindling other sites are different from those associated with BA kindling. Experiment 3 demonstrated that AN kindling, which produces convulsions that are topographically different from those of BA kindling, produces a pattern of conditioned effects that is distinct from that associated with BA kindling. This finding unequivocally establishes that the site of kindling influences the nature of the conditioned effects of kindling. The results of Experiment 4 also support this view: PRh and VH kindling produced conditioned effects on interictal behaviour but not on convulsions--contrary to what had been found in BA and AN kindling.

In addition to supporting the view that kindling site influences the nature of the conditioned effects of kindling on interictal behaviour, the results of Experiments 4 suggest that the topography of the convulsions is also important. PRh and VH kindling both elicit convulsions that are similar in their topography to those elicited during BA kindling, and both also produce a pattern of conditioned effects on interictal behaviour similar to that produced by BA kindling. However, unlike BA and AN kindling, PRh and VH kindling did not lead to conditioned effects on convulsions. This pattern of results suggests an interesting hypothesis: that the topography of the elicited convulsions determines the conditioned effects on interictal behaviour.

The lack of a conditioned effect on the convulsions of the PRh-, VH-, and DH-kindled rats in Experiment 4 was surprising given that these three brain regions play important roles in certain forms of learning and memory. That result also makes it difficult to explain why an effect was observed on the convulsions of BA- and AN-kindled rats in Experiment 3. One potential explanation may be that the nature of the conditioned stimulus (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 BA and AN, 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 BA- or AN-kindled convulsions. This view is supported by the results of a recent pilot study conducted in our laboratory: A discrete light CS had an effect on the interictal behaviour of BA-kindled rats but not on their convulsions.

LINE 3: CONTRIBUTIONS OF CONDITIONING TO THE DEFINING FEATURES OF KINDLING

The purpose of the third, and final, line of experiments in this thesis was to demonstrate that conditioned effects play a role in two of the major features of kindling: its permanence and its transfer between brain sites.

Experiment 5: Conditioned Effects Contribute to the Permanence of Kindling

Kindling is relatively permanent. If a kindled rat is left unstimulated for an extended period of time (e.g., several months), there are substantial savings in the number of stimulations required to rekindle it (Dennison et al., 1995; Goddard et al., 1969). Arguably, it is this feature more than any other that has attracted the interest of researchers. The purpose of Experiment 5 was to demonstrate that effects conditioned to the stimulation environment during kindling influence the permanence of kindling. Is the permanence of kindling attenuated if rats are rekindled in a different environment from the one in which they were initially kindled?

Methods

Apparatus

The test environments were two stimulation chambers positioned at opposite ends of the colony room. Both chambers were constructed from transparent Plexiglas and were 75 cm long, 75 cm wide, and 50 cm high. The floor of each chamber was covered with 2.5 cm of bedding

material. To render the chambers more distinctive, one of two unique sets of plastic objects and cutout paper shapes were placed around each chamber.

Kindling Phase

A single bipolar electrode was implanted in the left basolateral amygdala (BA) of each of 19 rats. Following postsurgery handling, all 19 rats were stimulated in one of the two test chambers (the CS+) and sham stimulated in the other (the CS-). 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: (1) there were 25 sham stimulations and 25 stimulations; (2) no more than three stimulations or sham stimulations ever occurred consecutively. The preadministration interval was 30 s. After receiving all 25 stimulations and 25 sham stimulations, all of the kindled rats remained in their home cages for a stimulation-free period of 100 days.

Retention Phase

The day after the last day of the 100-day stimulation-free period, the rats were divided into two groups. The rats in one group, the nonswitch group ($n=9$), received another 25 stimulations and another 25 sham stimulations just as they had during the kindling phase; that is, during the retention phase they were stimulated in the CS+ environment and sham stimulated in the CS- environment. The rats in the other group, the switch group ($n=10$), also received another 25 stimulations and another 25 sham stimulations, but during the retention phase, they received

both the stimulations and sham stimulations in the former CS- environment. All other retention-phase procedures were identical to those of the kindling phase.

Measuring Kindled Convulsions, Kindling Rate, and Permanence of Kindling

Experiment 5 used the same two measures of convulsion severity that were used in Experiment 2: duration and class. Experiment 5 assessed the permanence of kindling in two ways: (1) by examining the severity of the convulsions elicited by the first stimulation following the 100-day stimulation-free period, and (2) by calculating the percent savings in the number of stimulations required to re-attain the kindling criterion of three class 5 or greater convulsions. Specifically, each percent savings score was calculated by: subtracting the number of stimulations required to achieve the kindling criterion during the retention phase from the number of stimulations required to reach that criterion during the kindling phase, dividing that sum by the number of stimulations required to reach that criterion during the kindling phase, and multiplying the result by 100%.

Planned Statistical Analyses

Two different kinds of planned analyses were conducted to assess the statistical significance of the between-group differences. First, the kindling-permanence data were analyzed using independent-samples t tests. Because multiple t tests were employed for these analyses, the p-value required for a rejection of the null hypothesis was calculated using the Bonferroni correction: $p<.01$. Second, the convulsion-severity time-series data from the retention phase were analyzed using 2-way between-within ANOVAs with group (nonswitch vs. switch) as the between-subjects factor and stimulation number (1 to 25) as the within-subjects

factor. Significant interactions were followed up with simple-main-effects analyses at each level of the within-subjects factor (i.e., stimulation number).

Results

When rats were rekindled in the same environment in which they had initially been kindled, the kindling effect proved to be remarkably permanent. The first stimulation following the 100-day stimulation-free period elicited a fully generalized (i.e., class 5 or greater) convulsion, and there were substantial savings in the number of stimulations required to kindle three fully generalized convulsions. In contrast, when rats were rekindled in a different environment from the one in which they had initially been kindled, there were no significant savings in the number of stimulations required to regain the same criterion.

Histology

Of the original 19 rats, 1 rat lost its electrode assembly prior to the end of the experiment. Figure 17 illustrates the locations of the electrode tips in the left basolateral amygdala of 16 of the 18 rats that completed the experiment. Each of these 16 rats had an electrode tip in the basolateral amygdala (BA). The electrode placements of the other 2 rats that completed the experiment could not be determined because the sections taken from their brains did not capture the full length of the electrode track. Because no systematic differences were observed among the convulsions of the 16 rats with an electrode tip in the BA and of the 2 whose placements could not be verified; the data of all 18 rats were combined and subjected to analysis.

Figure 17. Experiment 5: Histology. The location of the electrode tips in the left basolateral

amygdala of 16 of the 18 rats that completed Experiment 5. The electrode placements of the

other 2 rats that completed the experiment could not be determined because the sections taken

from their brains did not capture the full length of the electrode track. Each black dot represents

the location of an electrode tip in one of the subjects.

Bregma -1.80 mm   -2.12 mm   -2.30 mm   -2.56 mm   -2.80 mm   -3.14 mm   -3.30 mm   -3.60 mm
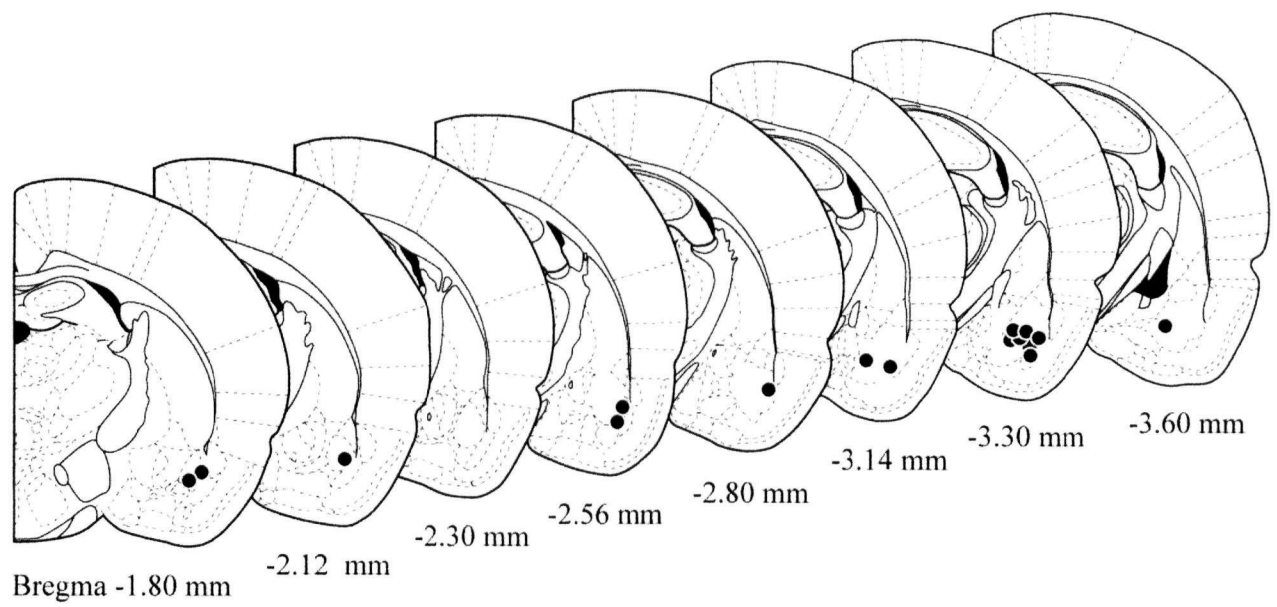
Kindling

　Kindling phase. The left side of Figure 18 illustrates the mean duration (Figure 18A) and class (Figure 18B) of the convulsions elicited by each of the 25 stimulations administered during the kindling phase of the experiment, and Table 2 provides the mean number of stimulations that the rats required before they met the criterion for kindling during the kindling phase. The 25 stimulations were effective in kindling all of the subjects: After about 20 stimulations, every rat virtually always responded with a class 5 or greater convulsion.

　Retention phase. The right side of Figure 18 illustrates the mean duration (Figure 18A) and class (Figure 18B) of the convulsions elicited by each of the 25 stimulations administered to the nonswitch and switch rats during the retention phase of the experiment, and Table 2 provides the mean number of stimulations that the rats required before they met the criterion for kindling during the retention phase. The convulsions of the nonswitch rats--the rats that were stimulated following the 100-day interval in the CS+ environment--displayed no sign of attenuation following the interval: The mean class of their convulsive response following the interval was 5.56, which was not significantly different from 5.78, $t(8)=1.00$, $p=.35$, the mean class of their convulsive response to the last stimulation of the kindling phase (see Figure 18B). Furthermore,

Table 2.

Number of stimulations to achieve three class 5 or greater convulsions.

Kindling Phase				Retention Phase			
Nonswitch rats		Switch rats		Nonswitch rats		Switch rats	
Mean	*SEM*	*Mean*	*SEM*	*Mean*	*SEM*	*Mean*	*SEM*
12.00	.55	12.67	1.04	3.33	.24	16.22	1.63

Figure 18. Experiment 5: Kindling- and Retention-Phase Convulsions. The mean duration (A)

and mean class (B) of the convulsions displayed by the nonswitch and switch rats in response to

each of the 25 stimulations administered during the kindling phase and in response to each of the

25 stimulations administered during the retention phase of Experiment 5. Error bars represent

the *SEM.*
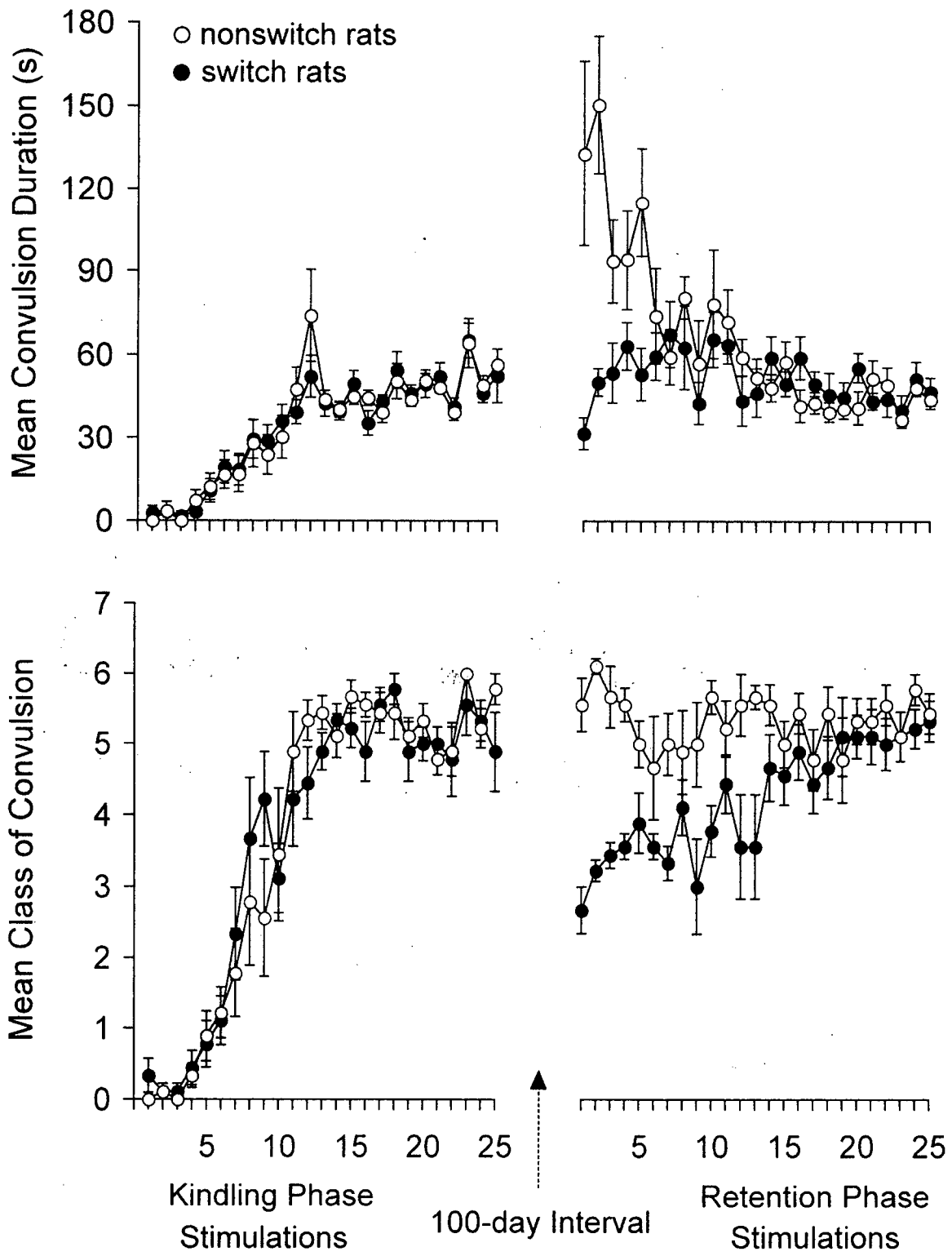
during the retention phase, they attained the criterion of three class 5 convulsions much faster than they had during the kindling phase (see Table 2); their mean savings was 72.08%, which was significantly different from no savings, $t(8)=45.36$, $p<.00000001$.

In contrast, the kindling effect was substantially attenuated following the 100-day interval in the switch rats (see Figure 18B). The first stimulation after the 100-day interval, that is, the first stimulation administered in their former CS-, elicited convulsions with a mean class of 2.67--far weaker than those observed on the same trial in the nonswitch rats, $t(16)=5.74$, $p=.000030$. Moreover, the switch rats displayed –32.79% savings--significantly less than was displayed by the nonswitch rats, $t(16)=6.41$, $p=.0000087$, and not significantly different from no savings, $t(8)=2.01$, $p=.079$.

Kindling: Retention-Phase Time-Series Data

The effects of rekindling rats in a different chamber from the one in which they were initially kindled are illustrated in Figure 18. During the retention phase, the convulsions displayed by the switch rats were significantly weaker than those displayed by the nonswitch rats in response to the first few stimulations but not thereafter.

Convulsion duration. Overall, during the retention phase, the convulsions displayed by the switch rats were significantly shorter in duration than those displayed by the nonswitch rats (see right side of Figure 18A), $F(1,16)=9.41$, $p=.0074$. There were statistically significant differences in the duration of the convulsions displayed by the nonswitch and switch rats in response to five of the stimulations, resulting in a significant interaction effect, $F(24,384)=4.48$, $p<.00000001$. The convulsions of the nonswitch rats were significantly longer than those of the switch rats in response to the 1st, $F(1,400)=47.07$, $p<.00000001$, 2nd, $F(1,400)=47.70$,

$p<.00000001$, 3rd, $F(1,400)=8.26$, $p=.0043$, 4th, $F(1,400)=5.26$, $p=.022$, and 5th

stimulations, $F(1,400)=19.15$, $p=.000015$, but not in response to the others, all $ps>.16$.

Convulsion class. Overall, during the retention phase, the convulsions displayed by the

switch rats were of a significantly lower class than those displayed by the nonswitch rats (see

right side of Figure 18B), $F(1,16)=18.02$, $p=.00062$. There were statistically significant

differences in the class of the convulsions displayed in response to ten of the stimulations

resulting in a significant interaction, $F(24,384)=2.93$, $p=.0000076$. The convulsions of the

switch rats were significantly lower in class than those of the nonswitch rats in response to the

1st, $F(1,400)=23.20$, $p=.0000020$, 2nd, $F(1,400)=26.01$, $p=.00000053$, 3rd, $F(1,400)=15.77$,

$p=.000085$, 4th, $F(1,400)=11.74$, $p=.00068$, 7th, $F(1,400)=7.86$, $p=.0053$, 9th, $F(1,400)=11.08$,

$p=.00095$, 10th, $F(1,400)=10.40$, $p=.0014$, 12th, $F(1,400)=11.66$, $p=.00071$, and 13th

stimulations, $F(1,400)=13.00$, $p=.00035$; but not in response to the others, all $ps>.036$.

Discussion of Experiment 5

The purpose of Experiment 5 was to demonstrate that effects conditioned to the

stimulation environment during kindling influence the permanence of kindling. There were two

major findings. First, the rats that were stimulated following the stimulation-free period in the

same environment in which they had been originally kindled displayed nearly perfect retention

of kindling, confirming that when there are no major alterations in the cues that predict the

stimulations--as kindling is typically studied--kindling is indeed permanent. Second, the rats that

were stimulated following the stimulation-free period in an environment that was different from

the one in which they had originally been kindled displayed only marginal retention--

demonstrating that the permanence of kindling is largely a result of effects that are conditioned to the stimulation environment during kindling.

In the present experiment, rats that were stimulated following the stimulation-free period in the same environment in which they had been initially kindled displayed nearly perfect retention of kindling. This contrasts with the results of most studies of the permanence of kindling; in most studies, there has been some decline in the class of the convulsions elicited in kindled animals following the stimulation-free period (Dennison et al., 1995; Goddard, McIntyre, & Leech, 1969; but see Homan & Goodman, 1988). One explanation for this discrepancy lies in the analysis of the content of what the rats in the present experiment learned in comparison to what rats in conventional kindling experiments learn. Rats in the present experiment learned that the stimulation environment is the best predictor of a stimulation and that all other antecedent stimuli are equally predictive of stimulations or sham stimulations. In contrast, rats in the standard kindling experiment learn that any one of the many stimuli can serve as reliable predictors of an impending stimulation: Being removed from their home cage, being handled by the experimenter, being attached to a stimulation lead, even time of day, all come to be reliable predictors of an impending stimulation and convulsion. Then, in a stimulation-free period, every time any of these predictors are present (e.g., time of day, being taken from their cage, being handled) in the absence of stimulation would function as an extinction trial, and some of their predictive value would be lost. But not in the present experiment: The rats in the present experiment were never exposed to the stimulation environment during the stimulation-free period.

In the present experiment, rats that were stimulated following the stimulation-free period in the same environment in which they had been initially kindled displayed convulsions that were twice as long as those elicited by their final stimulation prior to the rest interval (see Figure

18A). Homan and Goodman (1988) reported a comparable phenomenon: Afterdischarges elicited by stimulations administered to amygdala-kindled rats following a 45-day stimulation-free period were much longer than those elicited by the last stimulation prior to the stimulation-free period. They observed afterdischarges that lasted between 74 and 140 s, similar to the duration of the convulsions observed in the present experiment. The reasons for these increases are unclear, but they are likely related to post-seizure inhibition: Convulsions inhibit the severity of subsequently elicited convulsions, and such inhibition can take up to several days to dissipate (Mucha & Pinel, 1977). Accordingly, when the duration of the interstimulation interval is increased, there are often increases in convulsion severity, depending on the durations involved. In the present experiment and in the experiment of Homan and Goodman (1988), it appears that the interstimulation intervals were short enough that some post seizure inhibition built up and then dissipated during the stimulation-free period.

The decay of post seizure inhibition explains the increases in the duration of the convulsions observed in the rats that were rekindled in the same environment in which they had been initially kindled. But the absence of such increases in the rats that were rekindled in a different environment from the one in which they had been initially kindled clarifies the nature of the effects conditioned to the stimulation environment during kindling: Those effects appear to be excitatory in nature and, in the absence of post-seizure inhibition, they are maximally expressed.

By demonstrating that the effects of the stimulation environment on kindled convulsions are excitatory in nature, the results of the present experiment offer a solution to a long-standing mystery in the kindling literature. Mucha and Pinel (1977) demonstrated that switching from a 1.5-h to a 24-h interstimulation-interval kindling-schedule dramatically attenuates BA-kindled convulsions; the exact reverse of what should be expected based on the concept of post-seizure

inhibition. The reason for this discrepancy may lie in the analysis of what the rats in their experiment learned during the course of kindling. When their rats were being stimulated at regular 1.5-h intervals, they may have learned that time was a good predictor of a stimulation and convulsion; and when that regularity was removed, it produced the same effect that changing the environment did in the experiments of the present thesis.

Experiment 6: Conditioned Effects Contribute to the Bilateral Transfer Of Kindling

In addition to its relative permanence, another important feature of kindling is that it can transfer between brain sites. For example, rats that are first kindled through one amygdalar electrode (the primary site) kindle faster when they are subsequently kindled through another electrode implanted in the contralateral amygdala (the secondary site) than do rats that had not been previously kindled (McIntyre & Goddard, 1973). The general purpose of Experiment 6 was to demonstrate that effects conditioned to the stimulation environment during primary-site kindling influence subsequent secondary-site kindling. Is the transfer of kindling attenuated if rats undergo secondary-site kindling in a different environment from the one in which they underwent primary-site kindling?

Methods

Kindling Phase

One bipolar electrode was implanted in the left basolateral amygdala (BA) and a second bipolar electrode was implanted in the right BA of each of 36 rats. Following postsurgery

handling, all 36 rats were stimulated through one of their electrodes (the primary site) in one of the two test chambers (the CS+) and sham stimulated in the other (the CS-). The particular BA (i.e., left or right) that was stimulated during this phase was randomly selected for each rat. The test apparatus and the kindling-phase behavioural-testing protocol were nearly identical to those of Experiment 5, except for one difference: In the present experiment, after receiving all of the 25 stimulations and 25 sham stimulations, all of the kindled rats remained in their home cages for a stimulation-free period of 10, rather than 100, days. A rest between primary- and secondary-site kindling has been shown to increase the amount of transfer (McIntyre & Goddard, 1973).

Transfer Phase

The day after the last day of the 10-day stimulation-free period, the rats were divided into two groups of 18 rats each. The rats in one group, the nonswitch group (n=18), received another 25 stimulations through their second electrode (the secondary site) and another 25 sham stimulations as they had during the kindling phase; that is, during the transfer phase they were stimulated in the CS+ and sham stimulated in the CS-. The rats in the other group, the switch group (n=18), also received another 25 stimulations through their second electrode and another 25 sham stimulations, but during the transfer phase, they received both the stimulations and sham stimulations in the former CS-. All other transfer-phase procedures were identical to those of the kindling phase.

Measuring the Kindled Convulsions, the Kindling Rate, and the Transfer of Kindling

Experiment 6 used the same two measures of convulsion severity that were used in Experiment 5: duration and class. In Experiment 6, there were two measures of transfer: (1) the

number of stimulations required to re-attain the kindling criterion of three class 5 or greater

convulsions during secondary-site kindling, and (2) the percent change in the number of

stimulations required to re-attain the same criterion during secondary-site kindling. More

specifically, each percent change, or "transfer," score was calculated using the method described

by McIntyre and Goddard (1973): by subtracting the number of stimulations required to achieve

the kindling criterion (three convulsions of class 5 or greater) during secondary-site kindling

from the number of stimulations required to reach that criterion during primary-site kindling,

dividing that sum by the number of stimulations required to reach that criterion during primary-

site kindling, and multiplying the result by 100%.

Planned Statistical Analyses

Two different kinds of planned analyses were conducted to assess the statistical

significance of the between-group differences. First, the kindling-transfer data were analyzed

using independent-samples *t* tests. Because multiple *t* tests were employed for these analyses,

the *p*-value required for a rejection of the null hypothesis was calculated using the Bonferroni

correction: $p<.025$. Second, the convulsion-severity time-series data from the transfer phase

were analyzed using 2-way between-within ANOVAs with group (nonswitch vs. switch) as the

between-subjects factor and stimulation number (1 to 25) as the within-subjects factor.

Significant interactions were followed up with simple-main-effects analyses at each level of the

within-subjects factor (i.e., stimulation number).

Results

When rats were kindled through their second electrode in the same environment in which the primary-site had been kindled, there was significant transfer of kindling: Secondary-site kindling was substantially faster than primary-site kindling. In contrast, when rats were kindled through their second electrode in a different environment from the one in which the primary-site had been kindled, there was no transfer of the kindling effect; in fact, secondary-site kindling was slower than primary-site kindling.

Histology

Of the original 36 rats, 1 did not display any behavioural convulsions during the kindling phase, and 2 did not display any behavioural convulsions during the transfer phase--all three instances were the result of defective electrodes. Figure 19 illustrates the locations of the electrode tips in the right and left basolateral amygdala (BA) of the 33 rats that completed the experiment. Of these 33 rats, 28 had both electrode tips in the BA, 1 had it's primary-site electrode tip in the right central amygdala and it's secondary-site electrode tip in the left BA, 2 had their primary-site electrode tip in the right lateral amygdala (LA) and their secondary-site electrode tip in the left BA, and 2 had their primary-site electrode tip in the left BA and their secondary-site electrode tip in the right LA. Because no systematic differences were observed between the convulsions of the 28 rats with both electrode tips in the BA and the others, the data of all 33 rats were combined and subjected to analysis.

Figure 19. Experiment 6: Histology. The location of the electrode tips in the left and right

basolateral amygdala of the 33 rats that completed Experiment 6. Each black dot represents the

location of an electrode tip in one of the subjects.

Bregma -1.80 mm

-2.12 mm

-2.30 mm

-2.56 mm

-2.80 mm

-3.14 mm

-3.30 mm

-3.60 mm
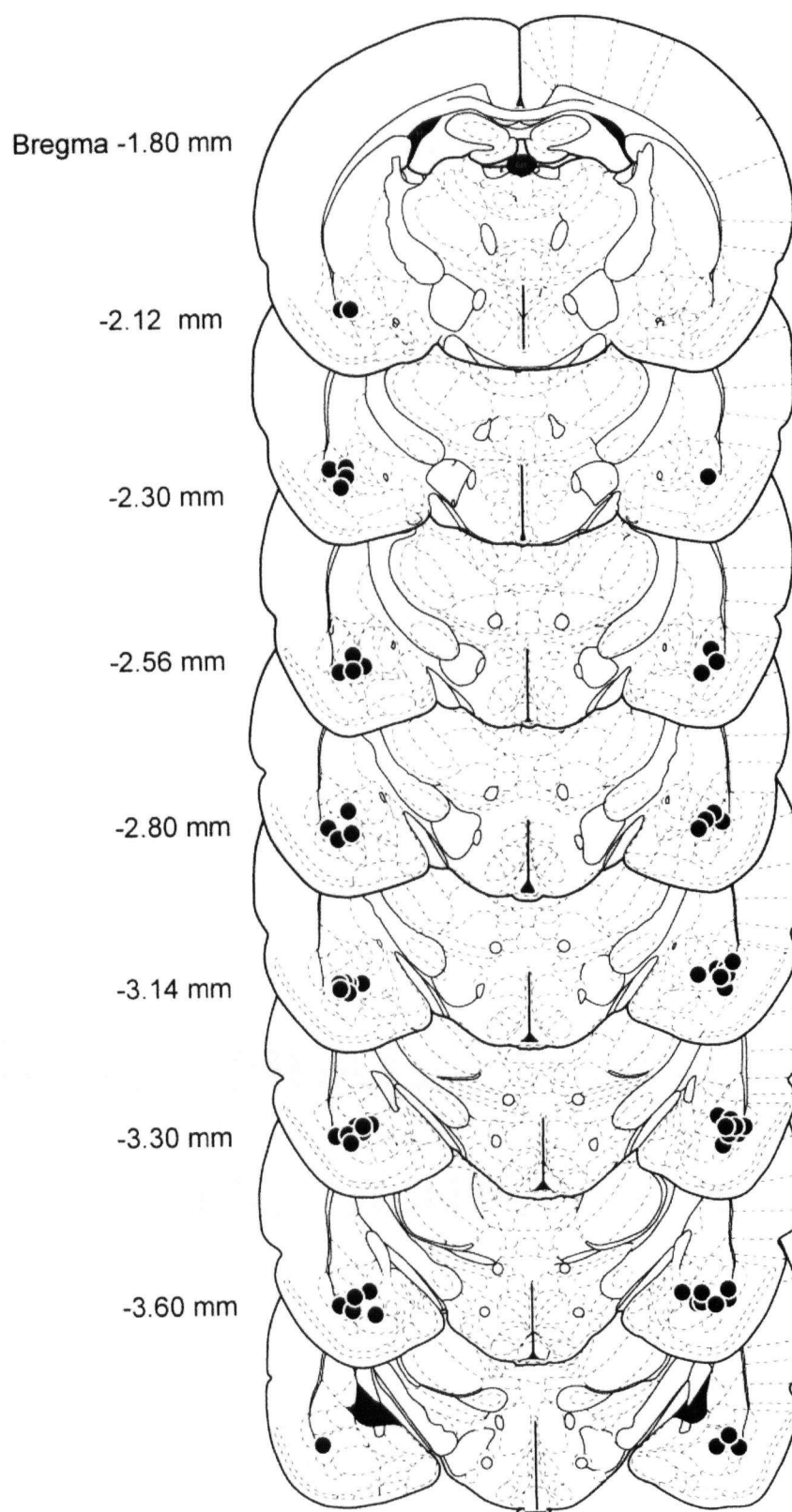
Kindling

> *Kindling phase.* The left side of Figure 20 illustrates the mean duration (Figure 20A) and class (Figure 20B) of the convulsions elicited by each of the 25 stimulations administered through the primary-site electrode of each rat during the kindling phase of the experiment. The 25 stimulations were effective in kindling all of the subjects: After about 20 stimulations, every rat virtually always responded with a class 5 or greater convulsion.

> *Transfer Phase.* The right side of Figure 20 illustrates the mean duration (Figure 20A) and class (Figure 20B) of the convulsions elicited by each of the 25 stimulations administered to the nonswitch and switch rats during the transfer phase of the experiment, and Figure 21 illustrates the mean transfer scores of the nonswitch and switch rats. During the transfer phase, the nonswitch rats--the rats who were stimulated through their secondary-site electrode in the CS+ environment--attained the criterion of three generalized convulsions much faster than they had during the kindling phase: Although they had required a mean of 14.94 stimulations to attain the criterion of three class 5 or greater convulsions during the kindling phase, they required a mean of only 11.38 stimulations to reattain the criterion during the transfer phase, $t(15)=3.35$, $p=.004$. Only one of the nonswitch rats did not reach the criterion during the transfer phase[9]. The mean transfer score of the nonswitch rats was 21.95%, which was significantly different from no transfer, $t(15)=3.61$, $p=.0026$.

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[9] For the calculation of the transfer score this rat was assigned a score of 28 (the total number of stimulations administered during the transfer phase plus 3) as the number of stimulations required before it displayed three class 5 or greater convulsions.

Figure 20. Experiment 6: Kindling- and Transfer-Phase Convulsions. The mean duration (A)

and mean class (B) of the convulsions displayed by the nonswitch and switch rats in response to

each of the 25 stimulations administered to the primary-kindling site of each rat during the

kindling phase, and to each of the 25 stimulations administered to the secondary-kindling site

during the transfer phase of Experiment 6. Error bars represent the *SEM*.
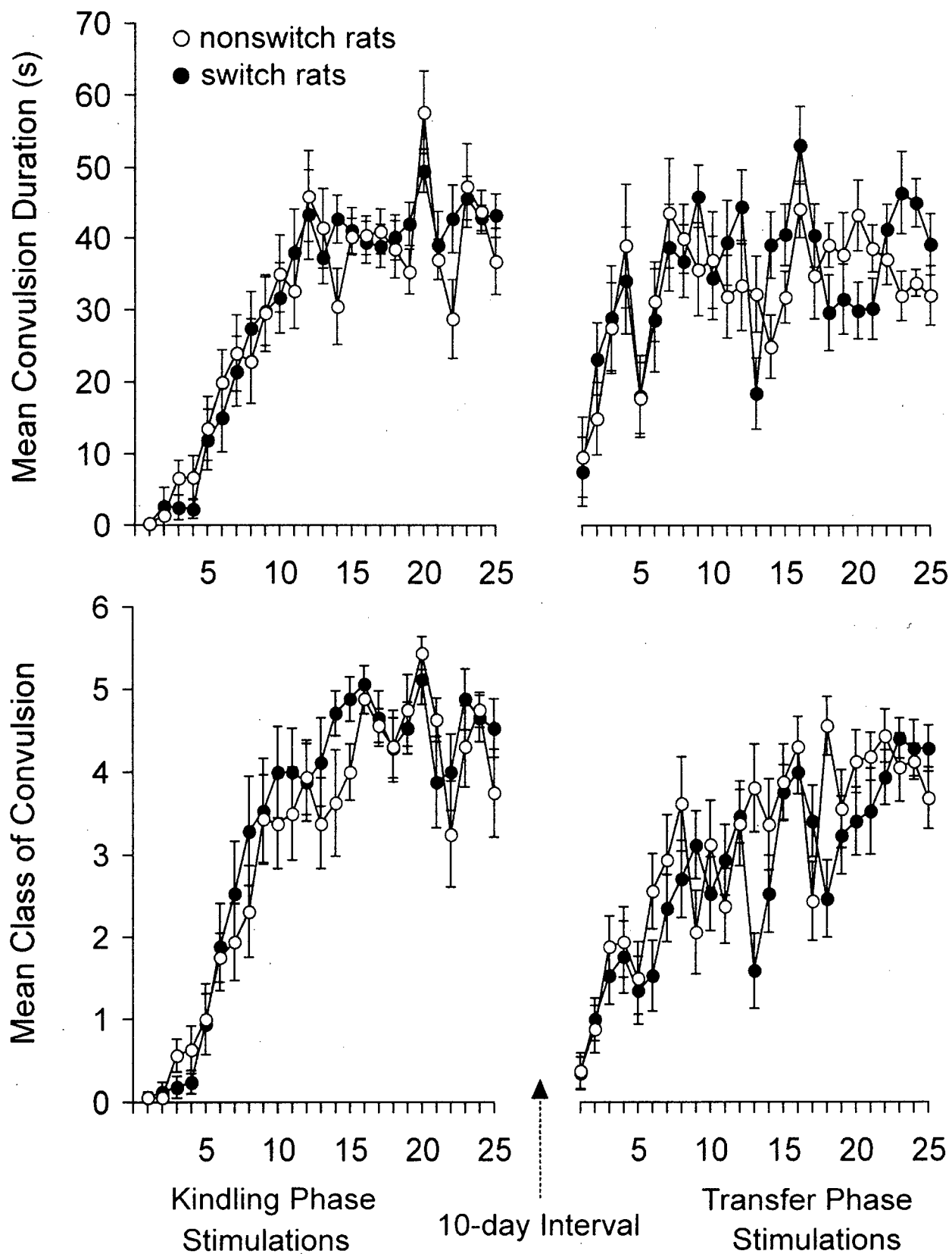
Figure 21. *Experiment 6: Transfer Scores.* The mean transfer scores of the nonswitch and switch rats; that is, the mean percent change in the number of stimulations required by the nonswitch and switch rats to re-attain the kindling criterion during secondary-site kindling. Error bars represent the *SEM.*
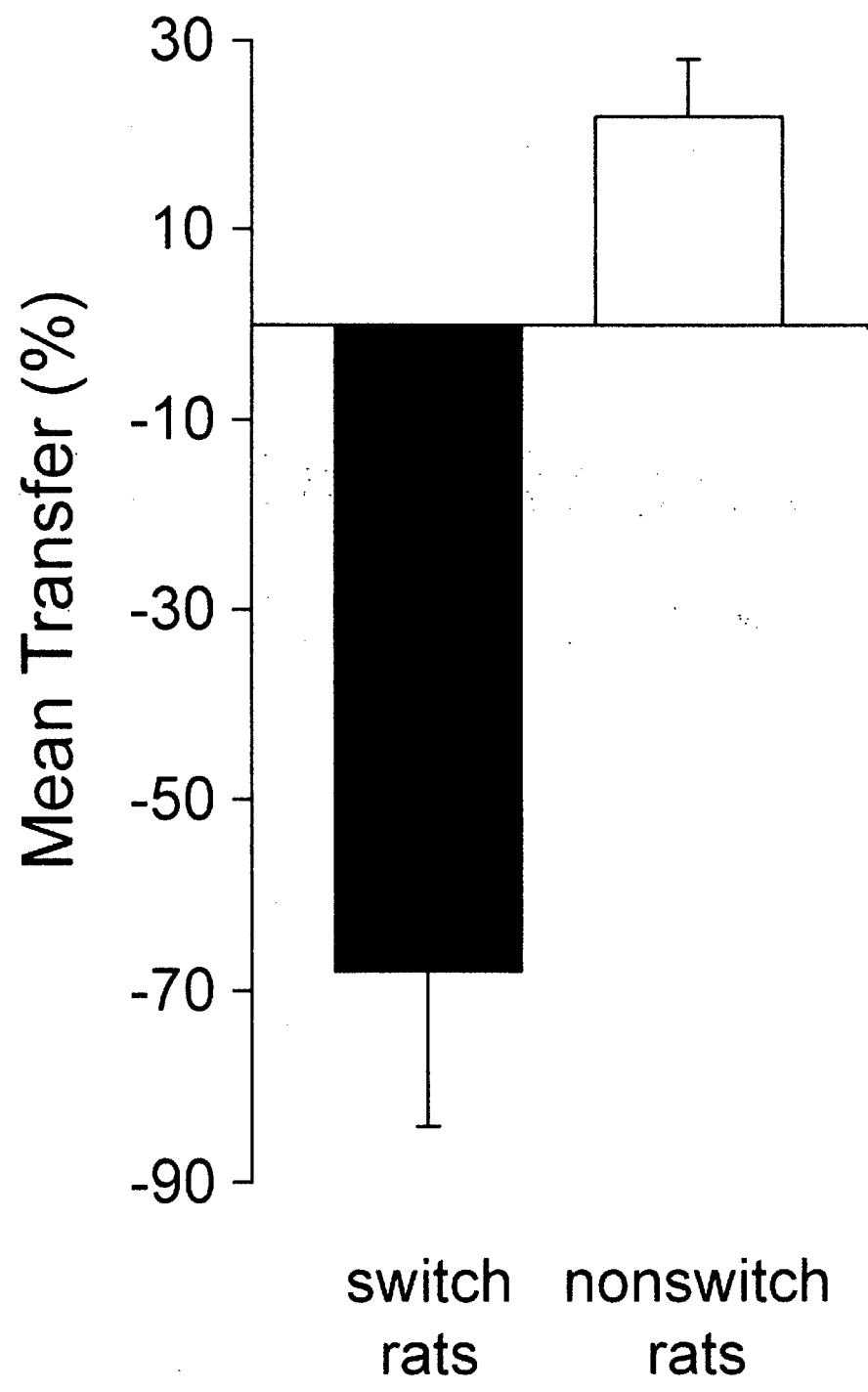
In contrast, during the transfer phase, the kindling rate of the switch rats--the rats that were stimulated through their secondary-site electrode in the CS- environment--was actually attenuated (see Figure 21). Although the switch rats had only required a mean of 13.24 stimulations to attain the criterion during the kindling phase, they required a mean of 20.65 stimulations to attain the criterion of three generalized convulsions during the transfer phase, $t(16)=4.38$, $p=.00047$. In fact, 5 of the switch rats did not reach the criterion during the transfer phase, and 2 of those 5 never displayed any class 5 convulsions during the transfer phase[10]. The mean transfer score of the switch rats was −68.01%--significantly less than was displayed by the nonswitch rats, $t(31)=5.06$, $p=.0000018$, and significantly less than no transfer, $t(16)=4.19$, $p=.00069$.

About halfway through the transfer phase, it was observed that there was substantial day-to-day variation in the convulsions of some of the rats--strikingly similar to the phenomenon observed in Experiment 2 and to that which has been reported to occur during AN-kindling (Burnham, 1978; Racine, 1975; Seidel & Corcoran, 1986). For example, if a class 3 or greater convulsion was elicited by a stimulation, some of the rats would often display a much milder convulsive response, or even not respond at all, to the next stimulation. The proportion of these drop days was quantified in the same manner as they had been in Experiment 2: by counting the number of instances when a rat's convulsive response was three or more classes below that of its convulsive response to the previous stimulation, and then dividing that total by the total number of class 3 or greater convulsions displayed by the rat. One ratio was calculated from the transfer-phase convulsion-severity data, and another was calculated from the kindling-phase convulsion-

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[10] For the calculation of the kindling criterion these rats were each assigned a score of 28. See Footnote 9.

severity data. Although an analysis of these data, using a between-within ANOVA with group (switch vs. nonswitch) as the between-subjects factor and phase (kindling vs. transfer) as the within-subjects factor, failed to reveal any significant between-group differences, it did reveal a significant between-phase difference, $F(1,31)=28.01$, $p=.0000093$: Both the nonswitch and switch rats displayed a significantly greater proportion of drop days during the transfer phase than they had during the kindling phase. The nonswitch rats displayed a mean of .20 drop days during the transfer phase, but a mean of only .095 drop days during the kindling phase; the switch rats displayed a mean of .18 drop days during the transfer phase, but a mean of only .050 drop days during the kindling phase.

Kindling: Transfer-Phase Time-Series Data

Despite the between-group difference in the transfer of kindling, there were only a few significant differences between the nonswitch and switch rats in their mean response to each individual stimulation of the transfer phase.

Convulsion duration. Overall, the convulsions displayed by the nonswitch rats during the transfer phase were not significantly different in duration from those displayed by the switch rats (see right side of Figure 20A), $F(1,31)=.23$, $p=.64$; nor was there a significant interaction effect, $F(24,744)=1.58$, $p=.04$.

Convulsion class. Overall, the convulsions displayed by the nonswitch rats during the transfer phase were not significantly different in class from those displayed by the switch rats (see right side of Figure 20B), $F(1,31)=1.34$, $p=.26$. However, there were statistically significant differences in the class of the convulsions displayed in response to two of the stimulations, resulting in a significant interaction, $F(24,744)=2.18$, $p=.001$. The convulsions of the switch rats were significantly lower in class than those of the nonswitch rats in response to the 13th,

$F(1,750)=14.04$, $p=.00019$, and 18th stimulations, $F(1,750)=12.42$, $p=.00045$; but not in response to the others, all $ps>.076$.

The two statistically significant differences in the class of the convulsions displayed by the two groups largely reflect the concordance of drop days by several switch rats. For example, the only between-group differences were observed in the convulsive responses to the 13th and 18th stimulations of the transfer phase (see Figure 20); 7 switch rats displayed a rest day in response to the 13th stimulation and 4 displayed a rest day in response to the 18th stimulation.

Discussion of Experiment 6

The purpose of Experiment 6 was to demonstrate that effects conditioned to the stimulation environment during primary-site kindling influence subsequent secondary-site kindling. There were two major findings. First, rats that were stimulated from their secondary-site in the same environment as where their primary-site had been kindled, displayed significant transfer of kindling, confirming that when there are no major alterations in the cues that predict the stimulations--as the transfer of kindling is typically studied--transfer does occur. Second, rats that were stimulated from their secondary-site in an environment different from the one in which their primary-site had been kindled displayed virtually no evidence of transfer; in fact, secondary-site kindling was actually suppressed in those rats. These results demonstrate that the transfer of kindling is largely a result of effects conditioned to the stimulation environment during primary-site kindling.

Why would kindling a secondary-site in a different environment from the primary site prevent transfer? An explanation lies in an analysis of the content of what the rats of the present experiment learned during primary-site kindling. During primary-site kindling, they learned that

the stimulation environment, and not the sham stimulation environment, was predictive of a convulsion. So if conditioning that occurs during kindling plays an integral role in kindling, as the experiments in this thesis suggest, then the rats had already learned much of what they "needed to know" at the commencement of secondary-site kindling. By this analysis, secondary-site kindling should be expected to proceed more rapidly than primary-site kindling if they occur in the same environment. Conversely, subjecting a primary-site-kindled rat to secondary-site kindling in an environment different from the one it had learned was predictive of the stimulations should prevent transfer--because that rat would have to learn about the stimulation environment all over again. However, in the present experiment, not only was transfer absent in the rats that received secondary-site kindling in a different environment from where they had received primary-site kindling, it was in fact suppressed; that is, they took longer to kindle from their secondary-site than they did from their primary-site. However, this is not surprising if one considers what those rats had to learn during secondary-site kindling. Not only did they have to learn that the sham-stimulation environment was no longer predictive of sham stimulations, they also had to learn that it was now predictive of stimulations; that is, they had to learn more than they had to during primary-site kindling.

In the present experiment, the rats that received secondary-site kindling in the same environment as primary-site kindling displayed a transfer effect comparable in magnitude to that observed in previous studies of amygdala-to-amygdala transfer (e.g., McIntyre & Goddard, 1973). There is, however, one potential difference between those other studies and the present one: The rats in the present experiment displayed a greater number of drop days during secondary-site kindling than during primary-site kindling, but this effect has not been reported in previous studies. The reason for this discrepancy is unclear. It is possible that this effect is common to transfer experiments but has simply not been observed before. Because most

kindling transfer experiments are curtailed once secondary-site kindling reaches criterion in individual rats, the number of elicited convulsions could have been insufficient to reveal the sort of variance that was observed in the present experiment. It is perhaps notable that days off have been observed during rekindling of the primary-site after the completion of secondary-site kindling (e.g., McIntyre & Goddard, 1973). An alternative possibility is that the procedures used in the present experiment differed from those of previous transfer studies. The only notable difference between the testing procedures used in the present experiment and those employed in previous transfer experiments is that the rats in the present experiment received many sham-stimulation trials. Why this procedural difference would selectively affect secondary-site kindling is unclear.

The results of the present experiment corroborate one of the conclusions drawn from the results of Experiment 5: That the effects of the stimulation environment on kindled convulsions are excitatory in nature. Rats that received secondary-site kindling in the same environment in which they had received primary-site kindling displayed significant transfer, whereas those rats that received secondary-site kindling in an environment different from the one in which they had received primary-site kindling did not.

Just as the results of Experiment 5 offered a solution to a long-standing mystery in the kindling literature, so too do the results of Experiment 6. One peculiar aspect of the interhemispheric-transfer of amygdalar kindling is that, in rats, it occurs even after total forebrain bisection (i.e., bisection of the corpus callosum, anterior and posterior commissures, massa intermedia, and the habenular and hippocampal commissures; McIntyre, 1975), or brainstem

bisection (i.e., bisection of the midbrain to the pons; Chiba & Wada, 1995)[11]. If the present

analysis of the transfer of kindling is correct, performing bisections should have little effect, if

any, on transfer simply due to the fact that both hemispheres of the rat are privy to the same

sensory information and both learn about the stimulation environment during primary-site

kindling.

Discussion of Line 3

The general purpose of this third line of experiments was to establish that conditioned

effects play a role in two of the defining features of kindling: its permanence and its transfer

between brain sites. Experiments 5 and 6 did just that: They demonstrated that effects

conditioned to the stimulation environment during kindling are a major determinant of both the

permanence and the transfer of kindling.

The permanence of the kindling phenomenon and its ability to transfer between brain

sites have been held up as evidence of its power to affect the wiring of the brain in a diffuse and

enduring manner. The results of line 3 in no way negate that claim. They do show, however,

that the animal's experience of the kindling stimulations is a major conduit through which

kindling exerts its effects on the organism.

One value of the experiments of line 3 is that they provide explanations for at least two

long-standing puzzles in the kindling literature. First, Experiment 5 suggested that the

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[11] But note that the interhemispheric-transfer of dorsal-hippocampal kindling is attenuated, but

not obliterated, by forebrain bisection (McIntyre, 1995).

paradoxical increases in post seizure inhibition that occur when the interstimulation interval is lengthened from 1.5-hr to 24-hr (Mucha & Pinel, 1977) are the result of excitatory effects conditioned to the temporal cues during kindling. Second, Experiment 6 suggested that interhemispheric transfer can still occur after forebrain (McIntyre, 1975) or brainstem (Chiba & Wada, 1995) bisection because it is the learning about the stimulation environment during primary-site kindling that accelerates subsequent secondary-site kindling, and such learning is common to both hemispheres with or without commissural neurotransmission.

GENERAL DISCUSSION

The general purposes of this thesis were to establish the reliability, generality, nature, and theoretical significance of our previously observed effects of the stimulation environment on the convulsions and interictal behaviour of basolateral amygdala (BA) kindled rats (Barnes et al., 2001). To achieve these general purposes, this thesis sought answers to the following three questions. First, are the previously observed effects of the stimulation environment on the convulsions and interictal behaviour of BA-kindled rats the result of Pavlovian conditioning? Second, is such conditioning associated with the kindling of brain structures other than the BA? Third, do conditioned effects contribute to the defining features of the kindling phenomenon? The results of the three lines of experiments in this thesis provided answers to each these questions.

Are the previously observed effects of the stimulation environment on the convulsions and interictal behaviour of BA-kindled rats the result of Pavlovian conditioning? The results of Line 1 indicated that they are. Experiment 1 supported the view that the effects of the stimulation environment are the result of Pavlovian conditioning by showing that a discrimination-reversal procedure could diminish the effects of the stimulation environment on convulsions and interictal behaviour. Although Experiment 1 clearly showed that the effects of the stimulation environment on interictal behaviour could be diminished, the discrimination-reversal procedure seemed to have little impact on the effects of the stimulation environment on convulsions. However, in Experiment 2 pre-exposure to the stimulation environment influenced the elicitation of BA-kindled convulsions.

Is conditioning associated with the kindling of brain structures other than the BA? The results of Line 2 confirmed that it is. In Experiment 3, rats that were kindled from the anterior

neocortex (AN) displayed convulsions that were topographically different from those of BA-kindled rats and a different pattern of conditioned effects: AN kindling led to more wet dog shakes and less, rather than more, severe convulsions in the stimulation environment. Interestingly, the number of wet dog shakes displayed by the AN-kindled rats in the stimulation environment was inversely correlated with the severity of their elicited convulsions. In Experiment 4, rats received stimulations to one of three sites in the hippocampal complex: the perirhinal cortex (PRh), ventral hippocampus (VH), or dorsal hippocampus (DH). The PRh-kindled rats displayed rapid kindling and a comparably swift emergence of interictal defensiveness conditioned to the stimulation environment. In contrast, the VH- and especially the DH-kindled rats displayed much slower kindling and slow or no conditioning, respectively. No effects of conditioning on the convulsions, comparable to those associated with BA or AN kindling, were observed. Thus, the findings of Line 2 unequivocally established that the site of kindling influences the nature of the conditioned effects of kindling.

Do conditioned effects contribute to the defining features of the kindling phenomenon? The third line of experiments showed that they contribute to the permanence of kindling and the transfer of kindling between brain sites. In Experiment 5, BA-kindled rats that were rekindled after a 100-day stimulation-free period in the same environment in which they had initially been kindled displayed near perfect retention of kindling; whereas, rats that were rekindled in a different environment displayed little retention. In Experiment 6, rats with an electrode in each BA were first kindled through one electrode and then kindled through the second electrode. Rats that were kindled through the second electrode in the same environment in which they had been kindled through the first electrode, displayed transfer; whereas, rats kindled through the second electrode in a different environment from the one in which they had been kindled through the first electrode, displayed a suppression of transfer.

The results of the three lines of experiments composing this thesis not only establish the nature of the conditioned effects of kindling. Together, they provide incontrovertible evidence that such effects are a general and reliable component of the kindling phenomenon.

The relevance and implications of the results of this thesis are discussed in the remaining sections of the General Discussion. They are discussed in the following five subsections: (1) prevalence of conditioning in kindling experiments, (2) theoretical significance of the conditioned effects of kindling, (3) relevance of the conditioned effects of kindling to the clinical epilepsies, (4) relevance of the conditioned effects of kindling to disorders other than epilepsy, and (5) conclusions and future directions.

Prevalence of Conditioned Effects in Kindling Experiments

To what degree are the conditioned effects observed in this thesis representative of the prevalence of conditioned effects in other kindling experiments? This is a critical question. If conditioned effects are present in the majority of kindling experiments, then those effects need to be taken into account when evaluating the results of those experiments. Although it is impossible to provide a precise assessment of the prevalence of conditioned effects in kindling experiments, the results of the present thesis suggest that such effects are likely pervasive, for three reasons. First, as much as my objectives permitted, the experiments in the present thesis attempted to adhere to widely used kindling protocols. The fact that such procedures were able to produce reliable conditioned effects is consistent with the idea that conditioned effects are common to many kindling experiments. Second, the results of Line 2 demonstrated that conditioned effects appeared during the kindling of four of the five brain sites that were sampled;

only dorsal-hippocampal kindling failed to produce conditioned effects. However, the results of Line 2 also demonstrated that the nature of those conditioned effects are a function of kindling site and that only some kindling sites (i.e., basolateral amygdala and anterior neocortex) produce significant conditioned effects on convulsions. So although conditioned effects are likely to be pervasive in kindling experiments, the affected behaviours will be a function of the brain site that is kindled. Third, although robust kindling-induced conditioned effects were observed in the present thesis, they likely underestimate the impact of conditioning in most kindling experiments. In each of the experiments discussed in this thesis, subjects were required to learn a discrimination between a CS+ and a similar CS-. The advantage of this discrimination paradigm is that it permits within-subjects comparisons. However, the disadvantage is that the results of these experiments likely underestimate the magnitude and rate of development of conditioned effects in conventional kindling experiments, in which there is no requirement for the subjects to discriminate between two similar CSs. For conditioned effects to emerge in the standard kindling experiment, subjects need to learn only the predictive relation between one of the many obvious antecedent cues (e.g., environmental cues, temporal cues, experimenter cues, procedural cues, etc.) and the subsequent stimulation and convulsion. Furthermore, the potential for such conditioned effects to generalize between various test situations--in any kindling experiment--cannot be ruled out.

Although the three lines of experiments in the present thesis focused on effects conditioned to the stimulation environment during kindling, a fourth ongoing line of research in our laboratory has focused on the potential for other stimuli to serve as CSs during kindling. We have demonstrated that flavour cues and discrete light and sound cues can serve as CSs in the conditioning of flavour aversions (Wig, Barnes, & Pinel, 2002) and defensive behaviour (unpublished data), respectively, by BA kindling. But the experiments that are particularly

relevant to the present discussion are our studies of the potential for temporal cues to serve as CSs (Barnes, Magyar, Pinel, & Takahashi, 2004).

As with environmental stimuli, temporal cues are a part of many kindling experiments. The time of day at which the animal is stimulated, the order in which events occur from the time an animal is taken from its cage to the time it receives a stimulation, and the temporal intervals between each of those events are reliably repeated on each trial. In two experiments, we showed that temporal cues could serve as CSs in the conditioning of interictal behaviour by BA kindling. In both experiments, rats received one BA stimulation at one time of day (the CS+ time) and one sham-stimulation at another time of day (the CS- time), on each of 53 or 73 days. The only difference between the two experiments was that the second employed a longer preadministration interval and included a peak-procedure test (e.g., Holder & Roberts, 1985): At the CS+ time, the rats were placed in the test chamber as usual, except they did not receive a stimulation after the preadministration interval. In the first experiment, as kindling progressed the rats displayed more freezing at the CS+ time than at the CS- time (Barnes et al., 2004). In the second experiment, rats also began to display more freezing at the CS+ time than at the CS- time, and during the peak-procedure test freezing gradually increased over the preadministration interval, and peaked at the time at which they had previously received a stimulation, at which point normal activity quickly resumed--this peak in freezing was only present at the CS+ time (Barnes et al., 2004).

In light of the aforementioned findings, it would seem virtually impossible to design an experiment in which the occurrence of the stimulations is not predicted by either environmental or temporal cues. Moreover, it seems unreasonable to argue that effects conditioned during kindling would not affect behavioral tests administered during or after kindling (e.g., tests of amnesic effects, tests of anticonvulsants), even if such testing took place outside of the

experimental environment or at another time of day. For instance, if an animal is always kindled during the light phase of a light-dark cycle, the conditioned effects of kindling would likely influence the results of any subsequent or concurrent behavioral testing conducted during the light phase. But even if behavioral testing were conducted in the dark cycle, and kindling in the light cycle, conditioned effects could still influence such testing. This is because the animal doesn't just learn that stimulations are administered during the light phase of a light-dark cycle, it also learns that they are not administered during the dark phase. Accordingly, because conditioning involves learning about probabilities and not instances (Gallistel & Gibbon, 2002), conditioned effects cannot be definitively ruled out of any kindling experiment.

Theoretical Significance of the Conditioned Effects of Kindling

Conditioned effects may be integral to the kindling phenomenon, but will an understanding of the conditioned effects of kindling offer anything useful to the kindling researcher? Or are these conditioned effects merely a nuisance factor? I believe that understanding the conditioned effects of kindling will be necessary for a complete understanding of the kindling phenomenon for at least three reasons. First, the conditioned effects of kindling offer explanations to long-standing puzzles in the kindling literature. Second, if learning does play a role in kindling, then insights into the mechanisms of kindling could be gleaned from the even larger literature on the mechanisms of learning and memory. And third, an appreciation of the role of conditioned effects in kindling could generate new testable hypotheses on the mechanisms of kindling.

Potential Solutions to Kindling-Related Puzzles

One value of the experiments in the present thesis was that they provided explanations for at least three long-standing puzzles in the kindling literature. First, Experiment 3 demonstrated that the severity of AN-kindled convulsions was negatively correlated with the expression of wet dog shakes in the stimulation 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 because there were more wet dog shakes in the stimulation environment and the convulsions elicited in the stimulation environment by AN stimulation were weaker than those elicited in the sham stimulation environment. This finding provided a potential explanation for the well-documented day-to-day variation in AN-kindled convulsions (e.g., Burnham, 1978; Seidel & Corcoran, 1986): Such variability may be a consequence of variations in the prevalence of wet dog shakes. Second, Experiment 5 suggested that the paradoxical increases in postconvulsion-inhibition that occur when the interstimulation interval is increased from 1.5-hr to 24-hr (Mucha & Pinel, 1977) are the result of excitatory effects conditioned to temporal cues during kindling. And third, Experiment 6 suggested that the reason that interhemispheric transfer still occurs after forebrain (McIntyre, 1975) or brainstem (Chiba & Wada, 1995) bisection is because both hemispheres learn about the stimulation environment during primary-site kindling, and that such learning accelerates subsequent secondary-site kindling--even in the absence of commissural pathways.

Implications for an Understanding of the Kindling Phenomenon

The kindling phenomenon has been studied as a form of neural plasticity since its initial discovery (Goddard et al., 1969), and it has been shown to have many parallels with certain types of learning and memory (see McIntyre et al., 2002). The finding that contextual and temporal conditioned stimuli can modulate convulsions and interictal behavior, as they can do to other forms of learning and memory (Bouton, Nelson, & Rosas, 1999; Murnane, Phelps, & Malmberg, 1999), constitutes yet another parallel. In so doing, the present findings support the premise that kindling constitutes a useful physiological model of certain types of learning and memory. Moreover, the discovery that conditioned effects contribute significantly to the effects of kindling may contribute to the search for its neural mechanisms--in the same way that the discovery that conditioned effects contribute to drug tolerance has focused attention on the role of the hippocampus and amygdala in the development of drug tolerance (Mitchell, Basbaum, & Fields, 2000). Clearly, the key to discovering the mechanisms underlying the kindling phenomenon lies, to a large degree, in an understanding of the interactions of the subjects with the cues that predict each stimulation and not solely in the unconditioned consequences of the brain stimulations and convulsions.

Conditioning-Related Speculations About the Mechanisms of Kindling Epileptogenesis

The results of the present thesis suggest that excitatory effects conditioned to the stimulation environment play an important role in the kindling of some sites. But what are those excitatory effects? The following are some speculations concerning the nature of those excitatory effects and the role they might play in the kindling phenomenon.

Brain stimulations elicit a variety of unconditioned responses (URs). For example, stimulation of the amygdala in the awake rat elicits various autonomic responses that are associated with fear, such as increases in arterial pressure and heart rate (Iwata, Chida, & Ledoux, 1987), and freezing (Davis & Whalen, 2001); and administering amygdalar stimulations to humans elicits feelings of fear and anxiety as well as fear-related autonomic responses (Chapman et al., 1954). Thus, the first amygdalar stimulation administered to a rat elicits a UR that consists of several behavioural and physiological changes that are, under more natural conditions, elicited by a threatening stimulus, such as a cat, or by a CS previously associated with a threatening stimulus. For example, a fear-inducing stimulus, whether unconditioned or conditioned, will increase activity in the amygdala (Ledoux, 2000)[12]. A likely determinant of the nature of the conditioned response (CR) which develops in response to brain stimulations is the nature of the CR that would develop upon activation of the brain structure in a natural setting. Although the literature on physiological conditioning suggests that the CR would be compensatory to the disrupting effects of the brain stimulation (i.e., that it would counter the increases in amygdalar activity), it is difficult to apply those same principles in the context of invasive brain stimulations. That is, a CR that is adaptive in a natural setting might not be in the context of invasive brain stimulations; there is no reason to believe that the intracerebral

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[12] The nature and direction of the activation of a brain structure is obviously more complicated. Some amygdalar nuclei respond to a CS with an increase in activity whereas others respond with a decrease (Dolan, 2000), and both aversive and rewarding stimuli are associated with amygdalar activation (Everitt, Cardinal, Hall, Parkinson, & Robbins, 2000). In the present analysis, I assume that an aversive stimulus produces a general increase in amygdalar activity.

application of an exogenous stimulus (e.g., a kindling stimulation) is a situation for which an adaptive response could have evolved.

Subsequent amygdalar stimulations administered in the presence of the CS (e.g., the stimulation environment) will activate the amygdala coincident with its activation by the CS. Thus, the progressive lowering of the afterdischarge threshold that occurs with the repeated administration of subthreshold stimulations (Pinel, Skelton, & Mucha, 1976) might not be the direct result of the brain stimulations, but rather the result of progressive strengthening of a CR that involves amygdalar excitation.

Once afterdischarges come to be elicited by the brain stimulations, the CR is likely to change as a function of the prolonged activation of the stimulation site. The subsequent spread of the afterdischarge and the development of kindled convulsions might result from the recruitment of brain areas that are involved in the sensory and mnemonic processing of the CS-- both ipsilateral and contralateral to the site of stimulation.

As kindling progresses, different sorts of conditioned effects might develop as a function of the alterations in the severity of the behavioural and physiological consequences of the convulsions. For example, kindled convulsions are followed by postictal (after seizure) hypoactivity that is mediated by seizure-induced increases in endogenous opiates (Cottrell & Bohus, 1987). The repeated elicitation of endogenous opiates could produce a conditioned compensatory response (CCR) to the stimulation environment or other antecedent cues, comparable to what occurs with the repeated administration of exogenous opiates (Siegel et al., 1982). In the context of brain stimulations, such a CCR might increase rather than decrease the animal's susceptibility to the convulsive effects of the brain stimulations.

Amygdala-kindling has been shown to increase interictal defensive behaviour in cats (Adamec, 1975) and rats (Kalynchuk et al., 1999; Pinel et al., 1977). It could be that these

defensive behaviours are also a function of a kindling-induced CCR. For example, opiate withdrawal is known to create increases in defensive behaviour in rats (Covington & Miczek, 2003) and such defensiveness is elicited by cues associated with drug administration (McNally & Akil, 2001). In Experiments 1, 3, and 4 of the present thesis, rats displayed more defensive behaviour in the stimulation environment than in the sham stimulation environment--this could be the result of a CCR. In the context of demonstrations of amygdala-kindling-induced increases in interictal defensiveness, the CCR might be conditioned to a variety of CSs during kindling, and that CCR could be elicited by any one of those CSs present during defensive-behaviour testing (e.g., time of day, removal from home cage, handling by experimenter, etc.) and thus produce increases in defensive behaviour.

Although most kindling experiments are curtailed once fully generalized convulsions are reliably elicited--after about 15 stimulations in amygdala-kindled rats, it has been demonstrated that if kindling is continued (for about 250 stimulations in the rat), convulsions begin to recur spontaneously (Pinel & Rovner, 1978; Wada et al., 1975; Wada et al., 1974). It is tempting to speculate that conditioned effects are responsible for the elicitation of kindling-induced spontaneous convulsions. But such speculations are not consistent with the failure of previous efforts to elicit kindled convulsions with a CS (e.g., Janowsky et al., 1980). Given the results of the present thesis, wherein convulsions were modulated rather than mediated by conditioned effects, it is easier to envision how conditioned effects might provide the "extra push" needed to elicit a spontaneous convulsion.

Relevance of the Conditioned Effects of Kindling to the Clinical Epilepsies

Do the conditioned effects observed in the present thesis have any counterparts in the human epilepsies? For some forms of epilepsy, like reflex epilepsy, it is easy to see how conditioned effects might be important.

Reflex Epilepsy

Reflex, or sensory-evoked, epilepsy is an epileptic syndrome in which seizures are evoked by identifiable exogenous triggers. Between 5% (Symonds, 1959) and 6.5% (Servit et al., 1963) of all epileptics have reflex epilepsy. Potential seizure triggers include the following: a variety of visual and auditory stimuli (e.g, flashing lights, startling noises); somatosensory stimuli (e.g., rubbing, tapping); either the anticipation of, or the act of, eating; specific sorts of body movements (Forster, 1977); specific musical themes; language-related activities (e.g., reading or writing); and decision-making (e.g., playing chess)[13].

Shortly after Pavlov's characterization of the conditioned reflex, many researchers attempted to condition epileptic seizures but with little success (see Servit et al., 1963, for a review). The general conclusion was that although behavioral and EEG changes can be conditioned (Forster, Chun, & Forster, 1963), a focal afterdischarge cannot. Nevertheless, subsequent research has demonstrated the efficacy of operant conditioning (reviewed in Engel, Troupin, Crandall, Sterman, & Wasterlain, 1982; Mostofsky & Balaschak, 1977; Sterman, 2000),

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[13] Seizures elicited by the performance of a particular cognitive task are also classified as "secondary psychogenic seizures" (see Fenwick, 1981).

extinction, habituation (see Mostofsky & Balaschak, 1977), and inhibitory Pavlovian conditioning (Efron, 1957) as treatments for reflex epilepsy.

Forster (1977, p. 301-302) concluded that reflex seizures could not be a Pavlovian phenomenon because such conditioning would necessitate repeated temporally contiguous presentations of the triggering stimulus (the CS) and the seizure (the US). However, that analysis has proven incorrect in at least three respects. First, as the discovery of conditioned taste aversions has demonstrated, temporal contiguity is not necessary for conditioning to occur; only contingency is (Garcia, Kimeldorf, & Koelling, 1955). Second, conditioned effects can emerge after a single conditioning trial (e.g., Bevins & Ayres, 1994; Kirsch & Boucsein, 1994; Luxton, Parker, & Siegel, 1996; Madden et al., 2001; Solvason et al., 1992). And third, strong backward excitatory conditioning occurs in many situations (Spetch, Wilkie, & Pinel, 1981). Given the results of the present thesis, a role for conditioned effects in reflex epilepsy needs to be reconsidered.

Nonreflex Epilepsies

If conditioned effects do play a role in reflex epilepsy, it becomes easier to speculate on how conditioned effects might play a role in other epilepsies. The occurrence of spontaneous seizures is the hallmark of epilepsy. The general conception of epileptogenesis is that it results from abnormal brain discharges caused by damaged tissue or physiological changes in the brain (Fenwick, 1991). But the word "spontaneous" is deceptive, for it implies that seizures are without precipitants--which is clearly not true. The human literature indicates that a wide variety of environmental and situational factors are capable of increasing the likelihood of a seizure; and, by one estimate, such factors are obvious in approximately 40% of epileptic patients (Servit

et al., 1963). For example, stress seems to be a relatively potent precipitant of seizures (Feldman & Paul, 1976; Grant, 1985; Stevens, 1959; Temkin & Davis, 1984)--in patients with limbic foci but not necessarily in patients with cortical foci (Semenov & Kamenskaya, 1973)-- and relaxation techniques are an effective way of reducing the likelihood of the occurrence of a seizure (Antebi & Bird, 1992; Grant, 1985; Yardi, 2001). Indeed, many patients come to this realization independently and learn to avoid stressful situations or practice methods of relaxation (Antebi & Bird, 1992; Cull, Fowler, & Brown, 1996; Spector, Cull, & Goldstein, 2000). Even when there is a clear epileptogenic lesion, most epileptologists will concede the existence of single or multiple precipitating factors within a given epileptic patient (Servit et al., 1963). So reflex epilepsy seems to be a special case of epilepsy, wherein there is a single identifiable exogenous stimulus capable of eliciting a seizure.

In other forms of epilepsy, even when it is not immediately obvious that external or internal stimuli are precipitators of a seizure, environmental or behavioural precipitants are likely to exist. Reflex epilepsy could simply be a special case of epilepsy in which the time between the triggering stimulus and seizure onset is quite short. Recent research on seizure prediction in human epileptics is suggestive: By applying nonlinear analyses to patient electroencephalograms (EEGs), Lehnertz and colleagues (e.g., Elger & Lehnertz, 1998; Lehnertz & Elger, 1995) demonstrated the existence of a preictal (before seizure) state that begins at least 25 minutes prior to seizure onset, and that is characterized by idiosyncratic EEG changes--leading them to propose that the seizure is merely the climax of a larger state change. Identifying those stimuli that are antecedent to the preictal state might reveal the existence of exogenous or endogenous triggers, and those triggering stimuli could be the subsequent targets of behavioural interventions--much as the precipitating stimuli of reflex seizures have been.

Interictal Psychopathology

Although the reasons are unclear (Devinsky, 1991), there is a relatively higher incidence of psychopathology, such as affective and anxiety disorders, amongst patients with intractable temporal-lobe epilepsy. The common interpretation has been that such psychopathology is the unconditioned result of focal brain pathology and the occurrence of seizures; little consideration has been given to the role of behavioural, social, and environmental factors (Whitman & Hermann, 1989), even though the greatest correlates of psychopathology in epileptic patients are socioeconomic variables--medication and neurobiological variables are not significant predictors of mood and anxiety disorders in epileptics (Hermann, 1991).

In particular, the role of cues that are regularly associated with seizures in the development and expression of abnormal levels of anxiety and affect has received scant attention. In light of the present finding of a role for conditioned effects in amygdala-kindling-induced defensive behaviour and the finding that amygdala-kindled rats model interictal psychopathologies (Kalynchuk, 2000), it is pertinent to ask if conditioned effects are capable of mediating psychopathology in persons with epilepsy. Such hypotheses have been advanced. Hermann (1979) suggested that the depression and anxiety disorders of human epileptics could result from the repeated exposure to unpredictable and uncontrollable aversive events (i.e., the seizures) and that the emergence of psychopathology is a result of learned helplessness. Engel et al. (1984) proposed that interictal behavioural disturbances could be manifestations of withdrawal from the endogenous opiates released during seizures. Related to their hypothesis is the possibility that the occurrence of seizures in one setting could condition CCRs to that setting, and that in the absence of a seizure, that setting could elicit an aversive state of withdrawal.

Relevance of the Conditioned Effects of Kindling to Disorders Other than Epilepsy

In addition to epilepsy, kindling has also been used to model the development of several forms of psychopathology, most notably the affective disorders. Indeed, the application of the kindling model to affective disorders has led to some new treatment options for patients with bipolar disorder. Kindling has also been used to model anxiety syndromes such as post-traumatic-stress disorder, obsessive-compulsive disorder, and panic-anxiety disorders.

Kindling has been studied as an analogous model of affective disorders because of its progressive nature. Similar to kindling, repeated bouts of mania or depression increase the risk and severity of subsequent bouts (Cutler & Post, 1982). Furthermore, it has been noted that drug sensitization, such as occurs with the repeated use of cocaine, also shares a kindling-like mechanism (Post, Weiss, & Pert, 1988); and because drug sensitization is mediated by conditioned effects (Post, Weiss, Pert, & Fontana, 1992), Post and colleagues (1986) proposed that affective episodes initially elicited by external events or personal crises subsequently become conditioned to cognitions or physiological changes associated with those affective episodes, such that those cognitions or physiological states come to be effective triggers of affective episodes. Accordingly, the conditioned effects observed in the present thesis provide another way in which kindling is analogous to the affective disorders.

Many patients with panic-anxiety disorder initially display only cue-elicited panic attacks and then later begin to have spontaneous attacks (Post & Weiss, 1998)--similar to the transition between stimulation-elicited convulsions and spontaneous convulsions (e.g., Pinel & Rovner, 1978). Similarly, in obsessive-compulsive disorder, initially minor obsessions and rituals progress to the point of being increasingly severe and crippling; and in post-traumatic-stress disorder, individuals who have experienced prior traumatic events are more likely to develop the

disorder after a subsequent traumatic event (Post & Weiss, 1998). Moreover, patients with anxiety disorders become increasingly agoraphobic, which can result in complete withdrawal and incapacitation (Post & Weiss, 1998). There is good evidence that conditioned effects play a role in anxiety disorders (Bouton, Mineka, & Barlow, 2001), though how large a role is debatable (Rachman, 1991). Accordingly, as in the case of the affective disorders, the conditioned effects of kindling provide another way in which kindling is analogous to the anxiety disorders.

Conclusions and Future directions

The present thesis presented experiments from three ongoing lines of research on the effects of Pavlovian conditioning on the ictal and interictal effects of kindling. These studies made three important points: (1) that the effects exerted by the stimulation environment on both the convulsions and interictal behaviour of BA-kindled rats are a result of Pavlovian conditioning, (2) that the topography of the conditioned effects of the stimulation environment on kindled convulsions and interictal behaviour is a function of kindling site, (3) and that two of the defining aspects of kindling--its permanence and its capacity to transfer between brain sites-- are greatly influenced by effects conditioned to the stimulation environment.

Why is it important to consider conditioned effects in the study of kindling? There are at least four important reasons. First, my research is showing that conditioned effects are likely a major component of virtually all conventional kindling experiments, and thus a complete understanding of kindling is not likely to emerge without considering them--the experiments on the permanence and transfer of kindling illustrate this point. Second, considering conditioned effects may resolve some long-standing kindling-related puzzles. For example, why do AN-

kindled convulsions show such a remarkable degree of within- and between-subjects variability (Burnham, 1978; Seidel & Corcoran, 1986)? I believe that the answer may lie in the neurobiological mechanisms that underlie the conditioned wet-dog-shakes observed during AN kindling (Barnes et al., 2003). Third, an appreciation of the fact that conditioning plays an important role in kindling supports the view that kindling may constitute a useful model of certain types of learning and memory, and as such may contribute to the search for its neural mechanisms--in the same way that the discovery that conditioned effects contribute to drug tolerance has focused attention on the role of the hippocampus and amygdala in the development of drug tolerance (Mitchell et al., 2000). Fourth, it is unlikely that kindling will provide valid insights into the mechanisms of epilepsy or the other disorders for which it is a model without a complete understanding of the role of conditioning in kindling.

Finally, the results of the present thesis provide strong support for a caution recently issued by Döbrössy and Dunnett (2001): In the development of neuroplastic therapeutic protocols, it is a mistake to focus on molecules, neurons, and synapses at the exclusion of the patient and his or her experiences; these can have a substantial influence on the development and survival of neuroplastic change.

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APPENDIX A

Counterbalancing Measures Employed in Experiment 1. Immediately following the last

postsurgical handling session, the rats were randomly divided into two equal groups of 18 rats

each. The rats in the first group, group A, were stimulated in the first of the two test chambers

and sham stimulated in the second; whereas the rats in the second group, group B, were sham

stimulated in the first of the two test chambers and stimulated in the second. Immediately

following their final stimulation of the kindling phase, each of the two groups of rats (i.e., groups

A and B) was subdivided into two equal subgroups--to create four subgroups of 9 rats each. The

switch test was conducted the following day, the switch-test day. The rats in one of the

subgroups from each of the two groups (i.e., groups A and B) received a test stimulation in their

CS- environment (switch), whereas the other rats received a test stimulation in their CS+

environment (nonswitch).

The day after the kindling-phase switch tests, each of the four subgroups of rats, which

had been constituted solely for the purpose of the kindling-phase switch tests, was further

randomly divided into one subgroup of 5 rats and one subgroup of 4 rats--to create eight

subgroups. Then, using the method illustrated in the accompanying figure, two of the subgroups

with 5 rats (one of the subgroups from each of groups A and B) and two of the subgroups with 4

rats (one of the subgroups from each of groups A and B) were combined to form one new group

(n=18), the interchange group, while the remaining rats composed a second new group (n=18),

the no-interchange group. The rats in the no-interchange group were tested as before: During the

reversal phase, they received 45 stimulations in their original CS+ and 45 sham stimulations in

their original CS-. The rats in the interchange group had their original (i.e., kindling phase) CS+

and CS- interchanged: During the reversal phase, they received 45 stimulations in their original

CS- and 45 sham stimulations in their original CS+. Immediately following their final stimulation of the reversal phase, each of the two groups of rats (i.e., the no-interchange and interchange groups) was randomly subdivided into two equal subgroups--to create four subgroups of 9 rats each. The switch test was conducted the following day, the reversal-phase switch-test day. The rats in one of the subgroups from each of the two groups (i.e., the no-interchange and interchange groups) received a test stimulation in their CS- environment, whereas the other rats received a test stimulation in their CS+ environment.

Kindling Phase   Reversal Phase