STIMULATION-INDUCED EPILEPTOGENESIS:
KINDLING OF SPONTANEOUS MOTOR
SEIZURES IN RATS

by
LOUIS IRVING ROVNER
B.A., University of Nevada, 1974

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Department of Psychology

The University of British Columbia
2075 Wesbrook Place
Vancouver, Canada
V6T 1W5

Date August 21, 1976
When periodic electrical stimulation is applied to any of a number of brain sites there can be a progressive development and intensification of elicited motor seizures. This has been termed the kindling effect. Although it has been repeatedly suggested that the kindling paradigm could provide a valuable experimental model of clinical epileptogenesis, it was only recently found that kindling would eventually lead to a bona fide epileptic syndrome in rats, cats and baboons, characterized by spontaneous motor seizures. The purpose of the present study was to systematically describe the development of the epileptic syndrome in kindled rats.

In the two experiments animals were stimulated about 15 times per week for several months. Kindling progressed as others have reported, although with continued stimulations there were changes in the elicited seizures which had not been previously described. Almost all of the animals, regardless of whether they were stimulated in the amygdala, hippocampus, or entorhinal cortex, eventually displayed spontaneous motor seizures before the termination of the experiment. These seizures were found to persist in some animals for at least 7 months. Thus, it appears that the kindling paradigm may provide a valuable addition to the methods available for the experimental investigation of epilepsy and its genesis.
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INTRODUCTION

In cases where epilepsy is produced by serious head injury, months or even years may elapse before the first overt convulsion (Penfield, 1956). In such cases the initial motor seizures (MSs) may be relatively minor, but as time passes, subsequent seizures frequently become more and more severe (Jackson, 1870). Although there have been numerous studies of epilepsy itself, little attention has been given its progressive development, or epileptogenesis. Of the many laboratory models of epilepsy, few are suitable for the study of the development of the condition. Recently, however, Goddard, McIntyre, and Leech (1969) suggested that the kindling paradigm could be valuable in this respect. The purpose of the present studies was to examine the viability of kindling as a laboratory model of clinical epileptogenesis. Thus, this Introduction is comprised of a review of the kindling literature followed by a statement of the major purpose.

A. Kindling

1) Definition. There can be a gradual development and intensification of MSs when periodic electrical stimulations are applied to any of a number of forebrain structures, even at current levels which are initially too low to evoke any behavioural effects. With each successive stimulation MSs grow more severe. For example, if the rat amygdala is stimulated once a day at 50 μA, there is initially no behavioural response; however, with repeated stimulations the animal begins to display some minor behavioural symptoms which increase in severity and duration with
each successive stimulation. This development and exacerbation of MSs by periodic brain stimulation has been termed the kindling effect (Goddard, et al., 1969).

Kindling has been demonstrated in a variety of species even though the exact pattern of development may differ from one species to the next. Although kindling has been studied most often in rats, it has also been observed in monkeys (Goddard et al., 1969), rabbits (Tanaka, 1972), mice (Leech, 1972), cats (Wada & Sato, 1974), frogs (Morrell, Tsuru, Hoeppner, & Morgan, 1975), and baboons (Wada, Osawa, & Corcoran, 1975).

2) Progressive Development of Motor Seizures. The exact course of kindling is a function of the particular neural structure that is stimulated. Although most descriptions of kindling have been based on observations of the consequences of periodic stimulation of the rat amygdala, recent evidence has shown that kindling in the rat can take at least two general forms. Amygdaloid kindling is typical of the kindling which is observed when stimulation is applied to any of the structures related to the olfactory-limbic system, i.e., amygdala, olfactory bulb, hippocampus, etc. Usually a rat shows no convulsive response to the first few stimulations of any of the olfactory-limbic structures. However, with continued stimulations rats begin to display rhythmic jaw and ear movements, and subsequent MSs grow more severe until each stimulation elicits a bilateral clonic MS, characterized by facial and forelimb clonus, rearing, and a loss of equilibrium. Perhaps the most succinct method of describing this progression is in terms of Racine's (1972b) five MS classes: Class 1 - rhythmic jaw and ear movements; Class 2 - jaw and ear movements followed by head nodding; Class 3 - jaw, ear, and head movements
followed by forelimb clonus; Class 4 - jaw, ear, and head movements, forelimb clonus, followed by rearing; Class 5 - jaw, ear, and head movements, forelimb clonus, rearing, followed by loss of equilibrium (falling). Thus, the progressive development of MSs from stimulation to stimulation is reflected in the progression of symptoms during the course of an individual MS.

Although periodic stimulation of the anterior neocortex is also associated with a progressive intensification of elicited MSs, this kindling differs in several ways from that produced by olfactory-limbic stimulations (Racine, 1975). One major difference is that MSs are usually elicited by the first stimulation of anterior neocortex. These initial MSs consist of mild mouth movements and myoclonic forelimb contractions, but with repeated stimulations these MSs become more severe until eventually stimulations elicit MSs characterized by immediate loss of postural control and tonic extension of the body. In contrast, Racine (1975) found that stimulation of posterior portions of neocortex evoked no overt convulsive response whatsoever, even after 90 daily stimulations.

Periodic stimulation of paleocortical regions is associated with a progressive development of MSs like that associated with olfactory-limbic stimulation. The only difference is that there is an immobility of the animal following the first few stimulations. Subsequent stimulations then elicit the stereotyped olfactory-limbic MS progression. There is one exception to this course of MS development produced by paleocortical stimulation; stimulations to the cingulate or frontal-cingulate cortices at first elicit MSs of the neocortical type, which subsequently develop into subcortical-type seizures (Racine, 1975).
Although kindled MSs can be elicited from any of a number of different sites, most investigators have studied amygdaloid kindling, apparently because of the precedent established by Goddard et al. (1969). In one of their early experiments Goddard et al. found that of the many structures they tested, generalized MSs were elicited by fewer stimulations of the amygdala than of any other structure. In the rest of the experiments in this initial study they studied amygdaloid stimulation. Since this 1969 report has, in general, been the most influential paper in the kindling literature, it is not surprising that other investigators have followed the lead of Goddard et al. and have used amygdaloid stimulation almost exclusively.

3) Electrographic Factors in Kindling. The increase in the degree to which electrographic after-discharges (ADs) generalize from the site of stimulation to other structures seems to be the electrographic basis for the kindling phenomenon. Racine (1972b) stimulated the right amygdala of rats once per day. At first, ADs were usually elicited only at the site of stimulation, but subsequent stimulations evoked ADs which generalized to the contralateral amygdala. As the AD durations and wave forms in the contralateral amygdala grew longer and more complex, the elicited MSs became more intense. Racine, Gartner, and Burnham (1972) also found this generalization of ADs to precede the intensification of elicited MSs. In this more thorough study, the increasing duration and complexity of ADs in response to periodic amygdaloid stimulation were noted in several amygdaloid projection sites, including the contralateral amygdala, ventromedial hypothalamus, preoptic area, hippocampus, and frontal pole of the cortex. These findings are consistent with
numerous clinical reports (cf. Ward, Jasper, & Pope, 1969) which indicate that unless ADs become generalized, they are frequently not associated with MSs.

Insofar as the generalization of ADs appears to be the main electrographic correlate of kindling, it is not surprising that the elicitation of ADs at the site of stimulation is a necessary prerequisite for the kindling of MSs. In a study by Racine (1972b), daily amygdaloid stimulation was administered to rats at current intensities maintained just below their AD thresholds; no MSs were elicited during the six weeks of the experiment. However, generalized MSs were kindled in every subject stimulated at intensities just above the threshold. In fact, no one has ever demonstrated kindling without first eliciting ADs.

Racine (1972a) found that during the course of kindling there was a progressive reduction in the AD threshold; with each successive stimulation it was possible to elicit ADs at the site of stimulation with currents of progressively lower intensity. This raised the possibility that this threshold reduction might be the basis for the kindling phenomenon, or at least contribute to it; however, at least four independent lines of evidence demonstrate that this is not the case. First, periodic amygdaloid stimulation maintained below the AD threshold reduces the AD threshold without leading to the development of MSs (Racine, 1972); second, stimulation of posterior noecortex does not kindle MSs even though it reduces the local AD threshold (Racine, 1975); third, the reduction of the AD threshold in one brain site does not seem to affect the threshold in another (Racine, 1972b), whereas kindling of one site greatly influences the degree to which other sites can be kindled (Goddard et al., 1969;
McIntyre & Goddard, 1973); and fourthly, Pinel, Skelton, and Mucha (1976) demonstrated that when animals are kindled at high current intensities, the AD threshold is actually raised during the course of kindling.

Pinel et al. (1976) viewed the reduction of AD thresholds and kindling of MSs as independent but successive stages in epileptogenesis. According to this view a minor neural irritant of insufficient severity to trigger ADs may eventually reduce the AD threshold to a point where ADs are generated. Then each time an AD is elicited more and more neural structures are recruited, and this generalization is reflected in the development and intensification of MSs.

4) Permanence. Perhaps the most striking feature of kindling is its permanence. Goddard et al. (1969) rekindled rats after a 12 week stimulation-free period and found a savings of about 90% in the number of stimulations required to elicit generalized MSs.

Tress and Herberg (1972) measured kindling and its permanence in a different manner, but also found the increased seizure susceptibility to be relatively enduring. Rather than record the progressive increases in severity of MSs elicited by periodic stimulations of constant intensity, they measured the progressive reduction in current intensity required to produce MSs of constant severity. Each rat was initially stimulated at 10 μA and if no MS was elicited the intensity was increased in small increments every 10 sec until a MS was evoked. Both hypothalamic and septal stimulation reduced the MS thresholds effectively over a 14-day period. The thresholds were still significantly reduced following a 14-day stimulation-free period.

The progressive reductions in AD thresholds which may occur during
the course of kindling have also shown to be relatively permanent. Racine (1972a) found that rats' AD thresholds, which had been significantly reduced by 60 daily amygdaloid or hippocampal stimulations, were still significantly reduced after a 40-day stimulation-free period although there was a slight trend toward recovery. In a similar investigation Pinel et al. (1976) found no evidence of any recovery following a 7-day stimulation-free period.

5) Inter-Stimulation Interval. The interval between stimulations has proven to be a critical factor in determining the rate of kindling. Goddard et al. (1969) stimulated groups of rats of intervals of 5 min, 10 min, 20 min, 8 hr, 12 hr, 24 hr, or 7 days. The fewest number of stimulations were required for kindling when the intervals were 24 hr or greater, whereas animals stimulated at intervals of 10 min or less did not kindle at all. Because the 24-hr interval was deemed most effective by Goddard et al. and because such a schedule fits well into general laboratory routine, in most kindling experiments stimulations have been administered at 24-hr intervals. However, recent data published by Racine, Burnham, Gartner, and Levitan (1973) indicate that although more stimulations are required, rats can be effectively kindled at intervals as short as 1 hr.

6) Current Parameters. Various current parameters have been shown to influence the course of kindling. Current intensity, for example, has proven to have an appreciable effect on the rate of kindling. In general, kindling has been found to progress more rapidly when subjects are stimulated at higher intensities. The study by Racine (1972b) suggested that this relation is largely a result of the fact that ADs are
elicited more frequently at higher current intensities. Racine applied
daily amygdaloid stimulation to three groups of rats, one group at a
high current intensity (1000 μA), one at an intensity just above the AD
threshold, and a third at intensities that were continually adjusted to
be maintained just below the progressively decreasing AD thresholds. He
found that animals did not kindle in the condition where ADs were not
elicited, and that there was no significant difference between the kindling
rates of the two groups stimulated at intensities above the AD threshold.
Thus, Racine concluded that current intensity had no effect on kindling
independent of its effect on the elicitation of ADs. In a more recent
report, however, Pinel et al., (1974) found that kindling progressed more
rapidly at high intensities (500 μA) than at intensities just above the
AD threshold. They also found that once rats were kindled, there was
much less day-to-day variability in the ADs and MSs elicited by stimulation at the
higher intensity. Thus, current intensity does seem to have some effects
on kindling independent of its effects on the reliability with which ADs
are elicited.

Goddard et al. (1969) found that variations in current parameters
other than intensity had little effect on kindling. Variations in stimulus
frequency between 25 and 150 Hz (sine wave, 50 μA) had no effect on
kindling rate. Similarly, they found that 1-msec, rectangular pulses
kindled rats as effectively as 60 Hz, sine wave stimulations. They also
found that stimulation durations of 1 sec and 60 sec (50 μA to 10 mA)
were equally effective in kindling.

7) Strain and Sex. Strain and sex differences are two variables
which have been shown to influence kindling. Goddard et al. (1969)
found that albino rats of the Holtzman (Chicago) strain kindled more rapidly than albinos of the Wistar strain or the Royal Victoria strain of hooded rats, and that the latter two strains, once kindled, displayed MSs of shorter duration. However, in the Racine, Burnham, Gartner, and Levitan (1973) study, the Royal Victoria hooded rats and Sprague-Dawley albinos kindled faster than the Wistar albinos.

Pinel (1975), at the Vancouver Symposium on Kindling, described a study conducted in his laboratory in which the course of kindling was compared in male and female rats. Although there were no differences in their kindling rates, the day-to-day variability of MS durations was greater in the female rats. This variability appeared to be related to the estrous cycle; MSs were longer during the estrogen surges of the pre-estrous period. Ovariectomized females did not display this variability.

8) Effects of Pharmacological Agents on Kindled Seizures and Kindling. The kindling paradigm has been frequently used to assess the convulsive and anti-convulsive properties of drugs. These kinds of studies are of two basic types: those which measure the effects of pharmacological agents on kindled MSs and those which examine the effects of the agents on the kindling process itself. The most systematic study of the former type was recently published by Babington and Wedeking (1973). They determined the effects of a number of centrally-active drugs on MSs elicited by stimulation in kindled rats. They found that antidepressive drugs had a selective effect, blocking MSs elicited from the amygdala more effectively than those elicited by cortical stimulation. Nonselective blockade of seizures elicited from these sites was exerted by both anti-anxiety and antiepileptic drugs, with antianxiety agents proving to be
more potent. The inhibitory and facilitatory effects of neuroleptic
drugs and stimulants, respectively, were slight.

Two recent studies of this same type have demonstrated the ability
of $\Delta^9$-tetrahydrocannabinol (THC) to block kindled seizures. Corcoran,
McCaughran, and Wada (1973) found that intraperitoneal injections of $\Delta^9$-THC
suppressed the ADs and MSs typically produced by amygdaloid stimula-
tions in kindled rats. Both $\Delta^9$-THC and its isomer, $\Delta^8$-THC, were found to
have similar effects on the ADs and MSs elicited by amygdaloid stimula-
tion in kindled Senegalese baboons, *Papio papio* (Wada, Osawa, & Corcoran,
1975). However, Fried and McIntyre (1973) demonstrated that rats can
become tolerant to the anti-convulsive effects of $\Delta^9$-THC on kindled
seizures.

Other investigators have studied the effects of drugs on the pro-
gressive intensification of seizures from stimulation to stimulation. In
this type of investigation drugs are usually administered each day prior
to the stimulation and the degree to which the drugs inhibit or facilitate
kindling is then determined. Wise and Chinerman (1974) demonstrated that
diazepam and phenobarbitol, but not diphenylhydantoin, block the
kindling of MSs but not the decrease in the local AD threshold produced
by periodic amygdaloid stimulation. Arnold, Racine, and Wise (1973)
found that atropine, a cholinergic blocking agent, produced similar effects,
but that two drugs with anti-catecholaminergic effects, reserpine and
6-hydroxydopamine, facilitated kindling. Wada, Wake, Sato, and Corcoran
(1975) reported that intraperitoneal injections of either $\Delta^9$-THC or
$\Delta^8$-THC administered before each of 25 daily amygdaloid stimulations re-
tarded kindling in cats.
Perhaps the most thorough investigation into the effects of drugs on kindling was recently reported by Racine, Livingstone, and Joaquin (1975). In this study they found that the effects of procaine HCl, diphenylhydantoin, and diazepam on kindling depended on the site of electrical stimulation. Intraperitoneal injections of procaine HCl and diphenylhydantoin administered before each stimulation increased the rate of amygdaloid kindling, whereas diazepam had the opposite effect. However, the effects of procaine HCl and diphenylhydantoin on kindling from neocortical sites were quite different. Both of these drugs effectively blocked the ADs and MSs typically elicited by neocortical stimulation, even when the rats were stimulated at current intensities as high as 2000 μA. Diazepam, on the other hand, had no effect at all on the course of kindling from neocortical sites. Thus, the effects of drugs on kindling are a function of the site of stimulation.

9) Mechanisms. It is now widely believed that the progressive increase in the degree to which local ADs will generalize to other areas of the brain with repeated stimulation is the basis of the kindling phenomenon (Racine, 1972b; Racine, Okujava, & Chipashvili, 1972; McIntyre & Goddard, 1973). Thus, attempts to explain the kindling process have centred around the increases in the efficiency of the synaptic transmission which are presumed to underlie this increase in generalization. Racine, Okujava, and Chipashvili (1972), for example, have suggested that kindling may be the result of an increase in the efficiency of synaptic transmission between limbic structures. They found that rats which were simultaneously stimulated in both amygdalae or both hippocampi kindled more rapidly than subjects who were stimulated at a single site. Animals
which were stimulated in the left and right amygdalae on alternate days also kindled more quickly than those receiving an equal number of stimulations restricted to a single site. Subjects stimulated in the hippocampal commissure had the fastest kindling rate of all. It appeared that kindling progressed at a rate proportionate to the number of limbic connections that were potentiated. Further support for this assumption of Racine et al. was provided by Douglas and Goddard (1975). They found that the monosynaptic perforant-path EPSP in the hippocampus produced by stimulation of the ipsilateral entorhinal cortex was lastingly potentiated by daily entorhinal stimulation.

Racine, Gartner, and Burnham (1972) found that kindling rats with amygdaloid stimulation produced appreciable increases in the amplitudes of late components of responses recorded in amygdaloid projection pathways evoked by single pulses or pulse trains applied to the amygdala. Since the largest increases in amplitude were seen in late components presumably transmitted over multisynaptic pathways, they suggested that the change produced by kindling involves a facilitation of synaptic transmission which is widespread. This view that widespread synaptic changes form the basis of kindling receives additional support from studies which demonstrated that following the kindling of one site, MSs are more readily elicited from other sites (Racine, 1972b), even after the original site has been lesioned (McIntyre & Goddard, 1973).

The neuroanatomical correlates of kindling remain unknown. Two recent attempts to identify these correlates were unsuccessful. A thorough electron-microscope examination of amygdaloid tissue revealed no significant differences between animals kindled with amygdaloid stimulation
and unstimulated controls (Goddard & Douglas, 1975). Racine, Tuff, and Zaide (1975) kindled rats with anterior neocortical stimulation and prepared the tissue around the electrode tips for histological examination with a modification of the Golgi-Cox staining technique. They found no significant differences between this tissue and comparable tissue taken from unstimulated controls.

The fact that the rate of kindling seems to be primarily determined by the frequency at which ADs are elicited, rather than the passage of time per se, has ruled out the possibility that gross physiological changes (such as gliosis, edema, and vascular changes) produced by the mere presence of the electrode could be an important factor in kindling. Moreover, kindling does not seem to result from the stimulation-produced accumulation of epileptogenic metal ions at the electrode site. Goddard et al. (1969), in addition to employing the stainless steel electrodes which are commonly used and known to deposite metal ions, stimulated other rats with nichrome or platinum electrodes and found that the rates of kindling produced by stimulation through these different electrodes were indistinguishable.

B. Rationale

From the outset investigators studying the kindling phenomenon have emphasized its potential as a laboratory model of clinical epileptogenesis (Goddard et al., 1969; Morrell, 1973). There are striking similarities between the kindling phenomenon and the progressive development and subsequent intensification of epileptic symptoms in humans; in untreated clinical cases, as in kindled animals, there is often a delay
before the initial manifestation of overt epileptic symptoms, followed by a progressive intensification of symptoms within a particular attack as well as from one fit to the next (Jackson, 1870). Until recently, however, there appeared to be reasons for doubting the suitability of kindling as a model of epileptogenesis. Spontaneous MSs, those not elicited by the stimulation, were not mentioned in any reports of kindling prior to 1974. In fact, Goddard et al. explicitly stated that they had never observed a spontaneous MS to occur in any of the rats, cats, or monkeys kindled during the course of the experiments published in their 1969 paper. This failure to observe spontaneous seizures is of critical importance since epilepsy, by definition, is a spontaneously-recurring, self-sustained, paroxysmal dysfunction of the brain. It is difficult to see how an experimental paradigm which had not been shown to produce an epileptic state could seriously be considered as a model of clinical epileptogenesis.

However, there have been several recent reports of spontaneous MSs in kindled rats (Pinel, Mucha, & Phillips, 1975; Pinel, Phillips, Mucha, & Deol, 1973), cats (Wada, Sato, & Corcoran, 1974), and baboons (Wada & Osawa, 1976; Wada, Osawa, & Mizoguchi, 1975). In the Wada et al. (1974) study the cats were stimulated daily until generalized MSs were elicited (15 to 36 stimulations, $\bar{X} = 25.5$) and then each subject received 13 to 60 additional stimulations. Of the five cats in the study, three eventually displayed spontaneous MSs during the course of stimulations. During the course of the experiment they noticed, as others had before (cf. Wada & Sato, 1974), that between stimulations the ongoing electrographic activity was frequently punctuated by interictal discharges (IIDs). There appeared to be a marked increase in the prevalence of these IIDs in
the period before the first spontaneous MSs were observed. Although they reported that one subject experienced spontaneous seizures for 53 days after the stimulations were curtailed and then developed status epilepticus, they provided no data to illustrate the time course of spontaneity in the other cats.

In view of the number of investigators who had kindled rats and had never observed spontaneous MSs in those kindled animals, the reports of Pinel et al. (1973, 1975) were particularly surprising. The assumption inherent in all of the earlier kindling studies employing rats was that the kindling process was complete once generalized MSs (Class 5) could be reliably elicited; thus, most of the studies were curtailed at this point. However, in the Pinel et al. investigation the rats were stimulated at least once a day for several months. This led Pinel et al. to conclude that spontaneity is the ultimate manifestation of kindling, and that previous investigators had in fact been restricting their investigations to the early phases of epileptogenesis. Pinel et al. observed three changes in their animals in the days before they first displayed spontaneous MSs. First, they observed the same increase in the rate of IIDs that had been observed to precede spontaneity in the cat (Wada et al., 1975). Secondly, although stimulations had reliably elicited generalized MSs previously, the animals occasionally showed an attenuated response, or even no response at all, to the stimulation. And thirdly, the rats began to display myoclonic jerks in conjunction with IIDs of particularly high amplitude.

Wada et al. (1976) stimulated the left amygdalae of Sengalese baboons (Papio papio) each day until generalized MSs were elicited (100 to 135 stimulations). While subsequently stimulating the contralateral
amygdalae of these animals, spontaneous recurrent MSs were observed in two of the four subjects. Abrupt increases in the incidence of IIDs were seen throughout the brain about 24 hr before the occurrence of spontaneous MSs. The persistence of the epileptic syndrome was not studied, as the animals were employed in a postkindling photosensitivity experiment and then sacrificed.

Spontaneous MSs have only been observed in a single kindled animal who was stimulated at a site other than the amygdala. Wada et al. (1975) kindled two Sengalese baboons (Papio papio) with electrical stimulation of the prefrontal cortex administered twice a day. Although one of these animals died of an illness apparently unrelated to kindling, the other subject displayed 12 spontaneous MSs during the course of the experiment after about 280 stimulations had been administered. Fewer IIDs were observed in this animal than had been seen in baboons receiving amygdaloid stimulation (Wada et al., 1976) and IIDs were more numerous in the contralateral prefrontal cortex than at the site of stimulation. Information was not provided concerning IID increases before the onset of spontaneous MSs or the permanence of the epileptic syndrome.

The observations of Wada et al. and Pinel et al. of spontaneous seizures in kindled animals are of obvious critical importance to researchers studying the kindling process as a model of clinical epileptogenesis. In none of these studies, though, were the spontaneous seizures or the events which led up to them described or investigated systematically. However, now that it is clear that kindling will eventually culminate in an epileptic syndrome it is possible to explore this development in a more systematic fashion.
Thus, the general purpose of this thesis was to confirm the reports of Pinel et al. (1973, 1975) that periodic electrical stimulation of the brain can eventually lead to the development of spontaneous seizures in rats, and to provide a systematic description of the entire course of kindling from the first stimulation to the eventual display of spontaneous MSs, with a particular emphasis on the events later in the course of kindling which have not been described by other investigators. The intention of the thesis was to deal with some basic issues related to the epileptogenetic effects of repeated electrical brain stimulation, and thus to provide a data base for those researchers who want to use the kindling paradigm as a model of clinical epileptogenesis.
EXPERIMENT 1

In Experiment 1 the development of spontaneous MSs in rats kindled with amygdaloid stimulation was investigated. In many respects this study was similar to the study of spontaneous MSs in kindled rats by Pinel et al. (1975); however, it included three important methodological innovations. First, since only four animals completed the Pinel et al. experiment and only two of those displayed spontaneous MSs, it was difficult to estimate the consistency with which this method can produce an epileptic syndrome. In Experiment 1 the sample sizes were sufficiently large so that it was possible to estimate the proportion of kindled animals that would eventually become "spontaneous." Such information should make a practical contribution to the design of future investigations of spontaneous kindled seizures by providing researchers with a basis for determining the most appropriate sample sizes. From a more theoretical viewpoint, determining whether spontaneity is the inevitable result of kindling or whether it is simply an anomaly which occurs in a few animals should prove to be an important step in developing an understanding for the kindling phenomenon.

Secondly, in the present experiment electrographic activity was monitored from the contralateral amygdala as well as from the site of stimulation, whereas Pinel et al. recorded from only the latter site. Wada et al. have routinely recorded from multiple sites in their experiments on amygdaloical kindling in cats and baboons and have found that the development of spontaneous electrographic spiking is particularly striking in the contralateral amygdala. Thus, it was possible for the first time to record the electrographic antecedents of spontaneous kindled
seizures in the rat from the contralateral amygdala.

The third methodological improvement was that the progressive intensification of elicited MSs was carefully documented. Most investigators have assumed that the progressive intensification of elicited MSs was complete in the rat once Class 5 seizures were reliably elicited; in fact, Class 5 MSs have frequently been referred to as "full" MSs (cf. Racine, 1972b). Thus, in the Pinel et al. study there was no systematic attempt to document change in the MS pattern once the stimulation was reliably eliciting Class 5 MSs. Although they became aware during the course of their investigation that there were changes in the MSs which occurred after the development of Class 5 MSs, they were unable to provide accurate retrospective descriptions (Pinel, Phillips, Mucha, & Deol, 1973). Thus, in Experiment 1, the pattern of each MS was carefully observed and recorded.

**METHODS**

Two bipolar electrodes constructed of insulated nichrome wire (diameter = 0.01 in) were implanted in each of the 45 male, 350 to 450 g hooded rats (Canadian Breeding Laboratories, La Prairie, Quebec) which served as subjects. The electrodes were aimed at the right and left amygdalae of each subject (coordinates: 1.5 mm posterior to Bregma, 4.2 mm on either side of the sagittal suture, and 8.8 mm ventral to the dura). The electrode connectors were housed in pedestal caps (MS363, Plastic Products Co., Roanoke, Virginia) which were in turn secured to the skull in the standard fashion with stainless steel screws and dental acrylic.
After 10 days of post-surgical recovery, 33 of the subjects were stimulated (1-sec, 60-Hz, 400-μA, RMS, sine-wave current) through the left electrode at intervals of no less than 2 hr and no greater than 48, about 15 times per week. All experimental subjects were maintained on this stimulation schedule for 134 days. Of the original 33 experimental subjects, one animal was found to have a faulty electrode and 14 either died or dislodged their electrodes during the course of the experiment, and thus none of the data of these 15 rats were subjected to analysis. The 12 unstimulated controls were handled as often as the 18 surviving experimental subjects. On Day 135 all of the animals were sacrificed and perfused with buffered formalin. Several of the brains were shipped for Golgi analysis to Madge and Arnold Scheibel of the University of California, Los Angeles, School of Medicine. The other brains were sectioned and stained with cresyl violet in order to verify the electrode placements. All of the electrodes were found to terminate in the amygdaloid complex.

Pre- and post-stimulation electrographic activity was monitored from both electrode sites about three times per week through #363-open "L" connectors (Plastic Products Co.) which conducted the signals to a Grass model 78B polygraph. Electrographic activity was monitored for 60 sec prior to the stimulation and for 30 sec following the cessation of the AD. Switching from the recording to the stimulation mode and back again was done automatically by a device which also isolated the polygraph amplifiers during current delivery to reduce post-stimulation interference in the recording channels to about 1 sec. A permanent record of motor activity was obtained during recording sessions by displaying the movement artifact generated in a single insulated wire, housed in the connector but
not connected to the animal, on a channel of the polygraph. Movement of the wire by the subjects produced changes in potential proportional to the magnitude of the motor activity.

On those occasions when electrographic activity was monitored, the durations of AD from both amygdalae were recorded, as well as the duration and intensity of MSs. The intensity of each MS was rated according to the aforementioned five-class scale of Racine (1972b): (1) facial movements only, (2) facial movements and head nodding, (3) facial movements, head nodding, and forelimb clonus, (4) facial movements, head nodding, forelimb clonus, and rearing, (5) facial movements, head nodding, forelimb clonus, rearing, and falling. On occasions when electrographic responses to stimulation were not monitored only the MS class was recorded.

Once a spontaneous MS was observed in an animal, that subject was observed irregularly for 2 or 3 hr per day, in addition to the routine observation during the stimulation sessions. The incidence and patterns of MSs were recorded during these additional sessions, and electrographic activity was occasionally monitored.

RESULTS AND DISCUSSION

During the course of the experiment both the electrographic and behavioural aspects of the elicited seizures increased progressively in severity, and 16 of the 18 experimental subjects that completed the experiment eventually displayed spontaneous MSs.

The progressive intensification of elicited seizures is summarized in Figures 1, 2, and 3 which illustrate the progressive increases in AD
duration, MS class, and MS duration, respectively. The mean responses to stimulation administered during the first 60 days of the experiment are illustrated in the left panel of each figure. The right panel of both Figures 1 and 3 illustrates the mean responses of subjects during the recording sessions in the 3- or 4-week period before each displayed its first spontaneous MS. For example, the subjects' average response, observed during the recording session immediately preceding each animal's first spontaneous MS, is plotted in both figures above "Day -1." Figure 2 differs in that the right panel illustrates the subjects' average class of elicited MSs for the last 10 days before each animal displayed spontaneity. The averages were determined and plotted in this fashion so that any systematic changes occurring just before the development of spontaneity would be evident.

AD Duration. Figure 1 illustrates the progressive changes in the durations of ADs recorded from the site of stimulation and from the contralateral amygdala in the 16 experimental animals that eventually displayed spontaneous MSs. There was a sharp increase in the duration of ADs recorded from both sites in the first 8 days (18 stimulations) of the experiment (both sign tests, x=0, N=16, p<0.001). However, there was a subsequent partial, but significant decrease in the duration of ADs recorded from the site of stimulation (sign test, x=2, N=16, p=0.002) and from the contralateral amygdala (sign test, x=5, M=16, p=0.105) between Days 8 and 28. Thereafter, there were no systematic changes in AD durations; thus, on Day 60 the ADs recorded from both sites were significantly longer than the ADs elicited by the first stimulation (both sign tests, x=0, N=16,
Fig. 1. Progressive changes in the mean duration of ADs recorded from stimulated and contralateral amygdalae (Experiment 1). The left panel illustrates the changes in AD duration over the first 60 days of the experiment. The right panel illustrates the mean duration of ADs elicited during the ten recording sessions before each animal displayed its first spontaneous MS. Thus, the point above Day -1 indicates the mean duration of ADs elicited during the last recording session before each animal displayed its first spontaneous MS. There were 2 or 3 days between each of these last ten recording sessions.
p<0.001). Many investigators have reported that the durations of ADs recorded from the site of stimulation increase monotonically during the course of kindling (cf. McIntyre & Goddard, 1973). However, the marked increase and subsequent partial decline observed in the present experiment has been reported previously (Racine, 1972b) and is almost identical to the function reported by Pinel, Phillips, and Deol (1974), who used methods comparable to those employed here.

In response to the first stimulation, ADs were recorded from the site of stimulation in all 18 experimental subjects and from the contralateral amygdala in 7. By Day 7, however, ADs were invariably recorded from both amygdalae in all of the experimental subjects. In none of the seven cases in which contralateral ADs were elicited by the first stimulation were they longer than those recorded from the site of stimulation, but by Day 13 (27 stimulations) the mean ADs recorded from the contralateral amygdala were slightly, but consistently, longer and remained so through Day 60 (except for Day 29). On an average day during this period (Day 13 to Day 60), six of the animals displayed ADs which were longer on the contralateral side, in four the durations were longer at the site of stimulation, and in six cases the durations were equal. Thus, although the mean durations of the ADs from the contralateral side were consistently longer than the means from the site of stimulation, on no day was this difference significant (sign test, x=4, N=10, p=0.377).

The right panel of Figure 1 clearly indicates that there were no systematic changes in AD durations during the ten recording sessions before each animal first displayed spontaneity. For example, the durations of all ADs recorded during the last session before each individual animal
displayed a spontaneous MS (Day -1) were not significantly different from those recorded on Day 60 (stimulated side, sign test, $x=7$, $N=14$, $p=0.605$; contralateral side, sign test, $x=6$, $N=14$, $p=0.395$). Thus, the development of spontaneity does not seem to be related in any obvious way to changes in the duration of elicited ADs.

Class of Elicited MSs. Figure 2 illustrates the progressive increase in the severity of the class of elicited MSs. Each point in Figure 2 represents the mean MS class on a particular day. For a day when more than one stimulation was administered to each subject, the score for each subject was the average MS class observed during that day. The first stimulation of the experiment generally elicited no response, however startle responses were observed in several subjects and one animal displayed mild clonic facial movements. By the third or fourth stimulation mild overt convulsive responses were elicited in most animals and these increased in severity with each successive stimulation. Class 5 MSs had been elicited in all of the subjects by the end of the second week. This is the point at which most investigators have assumed the intensification of elicited MSs to be complete. However, the left panel of Figure 2 clearly shows that the MSs continued to grow more intense even though the rate of change was not as great as that associated with the earlier phases of kindling. No systematic changes in the class of elicited MSs were observed after Day 37 (80 stimulations).

In order to illustrate the progressive intensification of elicited MSs during the later stages of the experiment it was necessary to add several classes to Racine's five-class scale. The pattern of MSs elicited
Fig. 2. Progressive increase in the mean class of elicited MSs (Experiment 1). The left panel illustrates the increase in the mean class of elicited MSs over the first 60 days of the experiment. The right panel illustrates the mean class of MSs elicited during the ten recording sessions before each animal displayed its first spontaneous MS. Thus, the point above Day -1 indicates the mean class of MSs elicited during the last recording session before each animal displayed its first spontaneous MS. There were 2 or 3 days between each of these last ten recording sessions.
in each animal seemed to progress through three additional classes. When the rearing and loss of equilibrium characteristic of a Class 5 MS occurred more than once the MS was labelled Class 6. Thus, a Class MS was comprised of jaw and ear movements, head nodding, forelimb clonus, and a multiple sequence of rearing and falling. The next pattern of MS that emerged (Class 7) was strikingly different from any of the MSs that had preceded it. Up to this point each new MS pattern could be characterized simply by adding an additional behaviour pattern to the end of the pattern at the previous level. However, in this case the pattern was completely different and seemed to be identical to the running fits typically elicited in rats by audiogenic stimulation (Collins, 1972). Immediately upon delivery of the current the animals ran rapidly in circles, violently jumped around, or rolled over repeatedly. These three behaviours occurred either separately or in combination and were almost always accompanied by loud squealing. These Class 7 MSs usually lasted for about 30 sec. A Class 8 MS was essentially the same as a Class 7, but it culminated in a curious pattern of body tonus: each animal reared and supported himself against a wall of the stimulation chambre, balancing on its tail and one hindlimb. The MS ended after this 5-sec period of tonus. Three animals in which Class 8 MSs had been elicited exhibited a stereotyped reaction to the stimulation on several occasions. Following stimulation to the left amygdala they walked in slow clockwise circles and were hyperreactive to handling for several min. However, since this response was observed in only three animals, it was not included in the overal classification system.

The right panel of Figure 2 illustrates the mean class of MSs elicited during the 10 days before spontaneous MSs were first observed
in each individual subject. There did not appear to be any striking change in the elicited MSs during this period. Although the mean class of MSs on the last day before spontaneity was higher than it had been at any other period of the experiment, the MSs elicited on this day were not significantly more intense than those elicited on Day 60 (sign test, $x=5$, $N=11$, $p=0.50$).

Over the last 10 days before spontaneity the stimulations elicited MSs that were remarkably stereotyped in individual subjects. This observation is inconsistent with the report of Pinel et al. (1975) that their two epileptic subjects occasionally failed to respond to the stimulation, or responded with MSs of low intensity and unusually short duration, in the period just before their first spontaneous MSs were observed. It is possible that those two subjects were exceptional cases; however, the present data suggest a more parsimonious interpretation. In the present study, although the subjects were very consistent in their responses to the stimulations prior to spontaneity, the responses to stimulations in the period when animals were displaying spontaneous MSs were frequently extremely variable. Thus, the instability of the pattern of elicited MSs may be a feature of the period of spontaneity itself, rather than being an antecedent. Just such an hypothesis has been proposed previously by the authors of the original report (Pinel et al., 1973).

Duration of Elicited MSs. The progressive increases in the duration of elicited MSs are illustrated in Figure 3. Although the first stimulation elicited a MS in only one animal, by Day 11 MSs were elicited in all subjects and the MS durations did not appear to change
Fig. 3. Progressive changes in the mean duration of elicited MS (Experiment 1). The left panel illustrates the changes in the duration of elicited MSs over the first 60 days of the experiment. The right panel illustrates the mean duration of elicited MSs elicited during the ten recording sessions before each animal displayed its first spontaneous MS. Thus, the point above Day -1 indicates the mean duration of MSs elicited during the last recording session before each animal displayed its first spontaneous MS. There were 2 or 3 days between each of these last ten recording sessions.
systematically after this point. This striking increase in the durations of elicited MSs during the early phase of kindling has been frequently reported (cf. Pinel et al., 1974).

The right panel of Figure 3 illustrates the mean durations of MSs elicited during the ten recording sessions before each animal's first observed spontaneous MS. It is obvious that there were no systematic changes in the durations of elicited MSs during this time. For example, during the session before the first spontaneous MS was observed in each animal (Day -1) the elicited MSs did not differ significantly in duration from those elicited on Day 60 (sign test, x=6, N=16, p=0.227). Thus, changes in the duration of elicited MSs do not seem to be related in any obvious way to the development of spontaneity.

Interictal Discharges. The development and proliferation of IIDs (spike-shaped discharges with more than twice the amplitude of the background EEG) was indicative of the imminent development of spontaneous MSs. Because electrographic activity was not monitored every day, it is difficult to specify exactly when IIDs developed in each animal, but by the recording session on Day 29, IIDs were first observed in 8 of the 16 experimental animals who eventually displayed spontaneity. Although IIDs were observed in some of these animals as early as Day 19 and in others as late as Day 43 (X=59.6 stimulations, range = 41 to 93 stimulations), they were observed in each of these 16 subjects. IIDs were first observed at the site of stimulation in three animals, whereas they were first observed in the contralateral amygdala in seven. In the remaining six experimental subjects which eventually became spontaneous, the development of IIDs
seemed to progress at the same rate in both amygdalae. IIDs eventually
developed in both amygdalae in all animals.

Figure 4 illustrates the mean incidence of the IIDs observed during
the 60-sec prestimulation period before each of the last ten recording
sessions preceding each animal's first observed spontaneous MS. It is
clear from Figure 4 that IIDs were observed in both amygdalae during
this period and that there was a striking increase in the incidence of
IIDs in the animals' contralateral amygdalae in the last three of these
sessions (sign test, x=0, N=7, p=0.008). Thus, these findings are con­
sistent with the observations of both Pinel et al. (1975) and Wada et
al. (1974); the present results agree with those of Pinel et al. that IIDs
can be recorded from the site of stimulation before spontaneity, and they
also agree with the report of Wada et al. in that the increase in IIDs
may be more striking at the contralateral sites. In fact, in the present
experiment this increase on the contralateral side seemed to be a par­
ticularly reliable sign that an animal would soon display spontaneous MSs.

The two animals which survived the entire experiment, but did not
eventually display spontaneous MSs, displayed spontaneous IIDs relatively
early in the experiment (Days 19 and 22). These IIDs were first observed in
the left amygdala of one rat and in the right amygdala of the other, and
eventually became bilateral in both animals. Thus, it does not appear
that the early development of IIDs necessarily signals the early develop­
ment of spontaneous MSs.

In the past many investigators have referred to the focus estab­
lished at the site of stimulation as the "primary focus" (cf. Arnold et
al., 1973). Insofar as seven of the subjects which eventually became
Fig. 4. Incidence of interictal discharges recorded from stimulated and contralateral amygdalae (Experiment 1). Fig. 4 illustrates the mean incidence of interictal discharges observed in both amygdalae during the 60-sec prestimulation period preceding each of the last ten stimulations before each animal displayed its first spontaneous MS. Thus, the point above Day -1 indicates the mean incidence of interictal discharges observed during the last recording session before each animal displayed its first spontaneous MS. There were 2 or 3 days between each of these last ten recording sessions.
spontaneous in the present experiment first displayed spontaneous IIDs in the contralateral amygdala, it is clear that epileptogenic foci may be initially established in areas other than the site of stimulation.

Spontaneous MSs. Periodic stimulation produced the epileptic syndrome with considerable reliability; spontaneous MSs were eventually displayed by 16 of the 18 experimental subjects although none were ever observed in any of the unstimulated controls. The animals varied considerably in the rate at which these spontaneous MSs developed during the course of the experiment. Spontaneous MSs were first observed in each subject after an average of 79 days (170 stimulations); however, this number ranged from 41 days (88 stimulations) to 132 days (293 stimulations).

The spontaneous MSs were similar in both pattern and duration to those elicited by the stimulations. The classes of these spontaneous MSs ranged from 1 to 7, and although some were abbreviated, in many cases their durations were the same as those elicited by electrical stimulation. Even though the animals were not observed continuously, most subjects were observed to have at least three spontaneous MSs. Although only one spontaneous MS was observed in each of three subjects, 30 were observed in one rat.

The electrographic activity associated with a spontaneous Class 7 MS is illustrated in Figure 5. The fact that the MS began several seconds before there was any obvious spiking recorded from either of the two amygdalae suggests that the abnormal discharges responsible for triggering this particular seizure originated in some other structure. This confirms
Fig. 5. Electrographic and motor activity of a subject during a spontaneous Class 7 motor seizure (Experiment 1).
the view stated earlier that the focus for the epileptic discharges is not necessarily at the site of stimulation.

Thus, the present results confirm the report of Pinel et al. (1975) that an epileptic syndrome can be generated in rats with long-term, periodic electrical stimulation of the amygdala; however, these results extend or qualify those of Pinel et al. in several important ways. The fact that 16 of the 18 experimental subjects displayed spontaneous MSs before the arbitrary termination of the experiment suggests that spontaneity may be the inevitable result of repeated amygdaloid stimulation of the rat rather than being an anomaly observed in a small portion of kindled animals. The impression of Pinel et al. that the development and proliferation of IIDs preceded the first manifestation of spontaneous MSs was also confirmed. However, as Wada et al. (1975) had observed in the baboon, the proliferation of IIDs before spontaneity was found to be more striking on the contralateral side. The pattern of development of elicited MSs reported by Pinel et al. and many other investigators was confirmed in the present experiment, but there were several important changes in these elicited MSs which occurred in the later stages of kindling which were documented here for the first time. The observation of systematic and stereotyped changes in the form of elicited MSs led to the addition of three classes to Racine's original five-class scale of kindled-MS development.
EXPERIMENT 2

The primary purpose of Experiment 2 was to determine whether periodic stimulation of structures other than the amygdala would eventually result in an epileptic syndrome in rats. Previous demonstrations that kindling results in spontaneous MSs employed only amygdaloid stimulation (Pinel et al., 1973, 1975; Wada et al., 1974, 1976), with the exception of a single baboon who became spontaneous after periodic stimulation of the prefrontal cortex (Wada et al., 1975). Thus, in Experiment 2 the development of spontaneous MSs was studied in rats receiving amygdaloid, hippocampal, caudate, or entorhinal stimulation. Goddard et al. (1969) found that periodic electrical stimulation applied to any one of these four structures would lead to the development and progressive intensification of elicited MSs. If the development of an epileptic syndrome can be viewed as a natural extension of the kindling of elicited MSs, then stimulation to any of these four sites should eventually lead to the development of spontaneous MSs. Although Goddard et al. found that the rates of the development of elicited MSs were different depending upon which of the above structures was stimulated, elicited MSs seemed to develop in the same fashion regardless of the site of stimulation.

The second purpose of Experiment 2 was to determine how long the epileptic syndrome, once induced, would persist. If this syndrome in kindled animals is comparable to clinical cases of epilepsy, one would expect it to be relatively enduring. From a practical point of view, the longer the syndrome lasts, the more opportunity for investigation it
affords. Wada et al. (1974) reported that one of their cats seized spontaneously for 53 days before developing status epilepticus, but did not provide similar data for their other subjects. Pinel et al. (1975) found that the epileptic syndrome persisted for 2 months in one of their rats, and for only 1 week in the other. In the present experiment, when subjects displayed spontaneous MSs they were no longer stimulated and changes in the incidence of spontaneous MSs were systematically assessed.

METHODS

One bipolar electrode was implanted in each of the 72 male, black-hooded subjects (Canadian Breeding Laboratories, La Prairie, Quebec) which weighed between 285 and 530 g at the time of surgery. The surgical procedures were the same as those employed in Experiment 1, except for the electrode placements. Equal numbers of subjects (n=18) had electrodes implanted at the following coordinates: amygdala, P 1.5, L -4.2, V 8.8; hippocampus, P 4.0, L -4.9, V 4.1; entorhinal cortex, P 3.7, L -4.0, V 8.5 (17°); caudate, A 1.9, L -3.2, V 4.7. Following a 10-day period of postsurgical recovery, stimulations commenced for the experimental animals in each group. As in Experiment 1, the experimental subjects were stimulated (1-sec, 400-μA, 60-Hz, sine-wave current) about 15 times per week at intervals of no less than 2 hr and no greater than 48; however, pre- and post-stimulation electrographic activity was monitored less frequently. Since the development of elicited seizures had been studied in detail in Experiment 1, and the early phases of kindling have been subjected to reasonably intensive investigation by other authors, electrographic
activity was monitored only every 2 or 3 weeks. On these occasions, pre-stimulation electrographic activity was monitored for 2 min rather than 1 min in order to provide a more reliable assessment of the incidence of IIDs. Since the development of IIDs was the most obvious antecedent of spontaneity in Experiment 1, when IIDs began to punctuate the records of an individual animal in the present experiment, the animal was observed for 1 or 2 hr each day in addition to the observation which routinely occurred during stimulation sessions. When three spontaneous MSs of Class 5 intensity or greater were observed in a given animal, 10 additional stimulations were administered to that animal at the usual intervals. Then that animal was observed for 1 hr per day for the next 35 days, during which the incidence and class of its spontaneous MSs were recorded.

The remaining 12 rats served as unstimulated controls. Their electrographic activity was monitored prior to the beginning of the experiment in order to ensure that it was normal and thereafter they were periodically observed in order to determine whether any were displaying spontaneous MSs. Although all of the control animals survived the experiment, 33 of the experimental animals either died or dislodged their electrodes during the course of the experiment and none of their data were subjected to analysis. Of the 27 experimental animals which survived the experiment, 8 had electrodes in the amygdala, 5 had hippocampal electrodes, and there were 7 in each of the entorhinal and caudate groups.

At the end of the experiment several experimental and control subjects were sacrificed, perfused with 8% buffered formalin, and their brains were removed and shipped to UCLA for Golgi analysis by Madge and Arnold Scheibel. The other animals were kept for 6 additional
months of unsystematic observation, after which they were sacrificed and perfused with 8% buffered formalin. Their brains were then removed, sectioned, and stained with cresyl violet in order to confirm the electrode placements. All of the electrode tips were found to be positioned in the target structures.

RESULTS AND DISCUSSION

Periodic stimulation of each of the electrode sites led to a progressive increase in the severity of the electrographic and behavioural responses similar to that observed in Experiment 1. All of the experimental animals that completed the experiment eventually displayed spontaneous MSs and continued to display them 35 days following the cessation of stimulations. Some subjects continued to display spontaneous MSs until they were sacrificed 7 months after their last stimulation. As in Experiment 1, IID s were recorded from the site of stimulation in the period before each animal in the amygdala group displayed its first spontaneous MS, and the same was true of subjects in the hippocampus group. However, spontaneous IID s were recorded in only a few subjects from the entorhinal cortex and were never recorded from the caudate, although all of the subjects in each of these two groups eventually displayed spontaneous MSs.

The progressive intensification of elicited seizures is illustrated in Figures 6, 7, and 8 in a manner similar to the way that this information was presented in Experiment 1. In each of these figures, Panel A illustrates the mean responses to stimulations elicited during the first 64
days of the experiment, and Panel B illustrates the mean responses elicited in the weeks prior to each subject's first observed spontaneous MS.

AD Duration. Although there were a few exceptions early in the experiment, the stimulations consistently elicited ADs in each animal. Panel A of Figure 6 illustrates the progressive increases in the durations of ADs observed in the four experimental groups over the first 64 days of the experiment. The ADs elicited on Day 64 were significantly longer than those elicited on Day 1 (F(3,69) = 13.98, p<0.001). However, the overall difference in AD durations between the groups for the same period was not significant (F(3,23)=2.31, p=0.104). Although the mean hippocampal ADs tended to be longer than the others for the first 64 days, the later differences were less striking. Racine (1972b) also found that the ADs elicited by hippocampal stimulation were initially longer than those elicited by amygdaloid stimulations; however, in his experiment he found that by the time Class 5 MSs were elicited in his rats amygdaloid ADs were of far greater duration.

There seems to be one major difference in the course of development of ADs between the animals in Experiment 1 and those receiving amygdaloid stimulation in the present experiment. Whereas the durations of the ADs elicited in the subjects in Experiment 1 were asymptotic by about Day 11 and then declined somewhat, the durations of amygdaloid ADs in Experiment 2 increased monotonically until Day 64.

An analysis of the data summarized in Panel B of Figure 6 indicated that there were no systematic changes in the durations of ADs elicited over the last four recording sessions before each animal first displayed
Fig. 6. Progressive changes in the mean duration of ADs (Experiment 2). Panel A illustrates the changes in AD duration of the four groups over the first 64 days of the experiment. Panel B illustrates the mean duration of ADs elicited during the four recording sessions before each animal displayed its first spontaneous MS. Thus, the points above Recording Session -1 indicate the mean durations of ADs of each group elicited during the last recording session before each animal displayed its first spontaneous MS. There were 2 or 3 weeks between each of these last four recording sessions.
spontaneous MSs. Although the durations of ADs elicited in the subjects in the four groups differed significantly \( (F(3,23)=6.73, p=0.002) \), largely because of the differences between the hippocampal and caudate animals, there were no significant changes during this period \( (F(3,69)=0.11, p=0.95) \). Thus, as in Experiment 1, the development of spontaneity does not seem to be related in any obvious fashion to changes in the durations of ADs.

Class of Elicited MSs. The first stimulation of the amygdala, hippocampus, or entorhinal cortex produced no obvious motor response other than the occasional Class 1 MS; whereas the first caudate stimulation invariably elicited MSs in all subjects, and in all but one case they were of Class 3 intensity or greater. However, thereafter a similar progressive increase in MS class was observed in all groups (Panel A of Figure 7). Thus, by Day 64 the classes of elicited MSs were significantly higher overall than they were on Day 1 \( (F(3,69)=53.96, p<0.001) \). Although MSs elicited in most of the subjects which were kindled with amygdaloid, hippocampal, or entorhinal stimulation progressed through the eight-class scale devised in Experiment 1, none of the animals in the caudate group ever experienced an elicited MS of greater severity than Class 6. Although the progressive increase in the class of MSs elicited by amygdaloid stimulation was similar to that observed in Experiment 1, the "circling behaviour" displayed by three animals in Experiment 1 was never observed in the present experiment.

There were no significant differences in the rate of kindling that were attributable to the site of stimulation \( (F(3,23)=0.53, p=0.66) \). This is surprising indeed in view of the report of Goddard et al. (1969) that
Fig. 7. Progressive increases in the mean class of elicited MSs (Experiment 2). Panel A illustrates the increases in MS class of the four groups over the first 64 days of the experiment. Panel B illustrates the mean class of MSs elicited during the four recording sessions before each animal displayed its first spontaneous MS. Thus, the points above Recording Session -1 indicate the mean classes of MSs of each group elicited during the last recording session before each animal displayed its first spontaneous MS. There were 2 or 3 weeks between each of these last four recording sessions.
A

- AMYGDALA
- HIPPOCAMPUS
- ENTORHINAL CORTEX
- CAUDATE

MS CLASS

DAYS

1 10 32 64

B

RECORDING SESSIONS PRECEDING SPONTANEITY
there were large differences in the rates of kindling produced by periodic stimulation of the four sites stimulated in the present experiment. Although there is other evidence which suggests that some limbic structures differ in their susceptibility to kindling (Racine, 1972b; Racine, Okujava, & Chipashvili, 1972), the differences found in those studies were not nearly as large as those reported by Goddard et al. Since kindling is a function of the number of ADs elicited rather than of the number of stimulations administered (Racine, 1972b), the different rates of kindling in the Goddard et al. study may primarily reflect the different AD thresholds of the structures. This view is quite plausible in light of the finding that at least three limbic structures (the amygdala, hippocampus, and septal area) have different AD thresholds (Racine, 1972a). Racine, Burnham, Gartner, and Levitan (1973) have previously suggested that the low current intensity (50 μA) employed by Goddard et al. may have failed to reliably trigger ADs in all of their subjects; however, this is impossible to determine, since Goddard et al. did not monitor the electrographic effects. Thus, previous observations that the stimulation of various structures results in different rates of kindling may be due to either different AD thresholds in some structures or to their differential susceptibility to kindling, or perhaps a combination of both. However, in the present experiment it was clear that the current intensity employed here (400 μA) reliably elicited ADs at all four sites, and there was no evidence that these sites were differentially sensitive to kindling.

The progressive changes in the patterns of MSs elicited by periodic stimulations of the amygdala, hippocampus, and entorhinal cortex were indistinguishable. However, the patterns of MSs associated with periodic
caudate stimulations differed in three important respects. First, as mentioned previously, all but one of the seven caudate animals displayed a Class 3 or 4 MS at the first stimulation. Secondly, their elicited MSs were never of greater intensity than Class 6. Thirdly, in a form similar to MSs elicited by stimulation of the cingulate cortex (Racine, 1975), they immediately fell over when stimulated and remained immobile for about 5 sec before displaying the stereotyped limbic MS pattern.

Panel B of Figure 7 illustrates the mean class of MSs elicited during each of the four recording sessions just before spontaneous MSs were first observed in the individual animals. Subjects in the four groups differed significantly in their mean classes of elicited MSs during this period (F(3,23)=3.41, p=0.04), apparently because caudate stimulation never elicited Class 7 or 8 MSs. However, there were no systematic changes in the classes of elicited MSs in the 6- to 8-week period before spontaneity (F(3,69)=0.58, p=0.63).

Duration of Elicited MSs. The progressive increase in the durations of elicited MSs over the first 64 days of the experiment is illustrated in Panel A of Figure 8. In those cases where the first stimulation elicited a MS, it was of relatively short duration; however, by the eighth stimulation all 27 of the experimental animals were displaying elicited MSs which monotonically increased in duration over the first 64 days of the experiment. Thus, on Day 64 the mean durations of elicited MSs were significantly greater overall than on Day 1 (F(3,69)=28.73, p<0.001) although the site of stimulation had no significant effect on the duration of MSs elicited during this period (F(3,23)=1.42, p=0.26).

Panel B of Figure 8 illustrates that the site of stimulation had
Fig. 8. Progressive changes in the mean duration of elicited MSs (Experiment 2). Panel A illustrates the increases in MS duration of the four groups over the first 64 days of the experiment. Panel B illustrates the mean duration of MSs elicited during the four recording sessions before each animal displayed its first spontaneous MS. Thus, the points above Recording Session -1 indicate the mean durations of MSs elicited during the last recording session before each animal displayed its first spontaneous MS. There were 2 or 3 weeks between each of these last four recording sessions.
no appreciable effect on the durations of MSs elicited in the period before spontaneity \( (F(3,23)=0.15, p=0.93) \). Moreover, there were no systematic changes in the durations of elicited MSs that occurred over this time \( (F(3,69)=0.88, p=0.46) \). Thus, the development of spontaneity appears to be unrelated to any changes that occur in the durations of elicited MS.

**Interictal Discharges.** As in Experiment 1, the increases in the incidence of IIDs was the only event which seemed to indicate the imminent development of spontaneous MSs. However, this was the case only for the subjects in the amygdala and hippocampus groups. Figure 9 illustrates the mean number of IIDs in each of the four experimental groups recorded during the 2-min prestimulation period on each of the last four occasions before spontaneity when electrographic activity was monitored. Each subject receiving amygdaloid or hippocampal stimulation displayed IIDs before the development of spontaneous MSs. Subjects in both of these groups required an average of about 200 stimulations before their first IIDs were observed; however, it is difficult to do more than estimate this figure since electrographic activity was monitored so infrequently. On the other hand, no IIDs were ever recorded from those animals with electrodes in the caudate, and only three of the seven subjects with electrodes in the entorhinal cortex displayed IIDs before they exhibited spontaneous MSs.

Figure 9 illustrates the significant differences in the incidence of IIDs between the four experimental groups in the prespontaneity period \( (F(3,23)=3.36, p=0.04) \), the lack of overall difference between the incidence of IIDs recorded on Sessions -4 and -1 \( (F(3,64)=0.05, p=0.98) \), and the highly significant interaction effect \( (F(3,9)=2.50, p=0.02) \) which seemed
Fig. 9: Incidence of interictal discharges (Experiment 2). Fig. 9 illustrates the mean incidence of interictal discharges observed in each of the four groups during the 2-min prestimulation period preceding each of the last four stimulations before each animal displayed its first spontaneous MS. Thus, the points above Recording Session -1 indicate the mean incidence of interictal discharges of each group observed during the last recording session before each animal displayed its first spontaneous MS. There were 2 or 3 weeks between each of these last four recording sessions.
to result from the different trends observed in the amygdala and campus animals. The results of correlated t-tests indicated that the incidence of IIDs increased significantly in subjects in the amygdala group over this period (t(6)=2.71, p<0.01), but that there was no significant change in animals in the hippocampus group (t(3)=1.11, p=0.05). The apparently striking change in the hippocampus group was insignificant because of the high variability in this group.

The idea that the development and proliferation of IIDs is a critical step in the development of spontaneous MSs receives support from experiments on the kindling of MSs. The most popular view of the kindling of MSs is that when ADs are first elicited they become more generalized and then become associated with MSs. A simple extension of this logic can be used to account for the development of spontaneous MSs. According to this view, spontaneous IIDs at first remain relatively local and isolated, but each discharge facilitates the production of other discharges and their generalization to other structures until the point where they are sufficiently frequent and general to be associated with motor symptoms. In this context one might view as somewhat surprising the finding in Experiment 2 that many animals which displayed spontaneous MSs never displayed IIDs. However, in addition to the results of Experiment 1, several previous reports indicate that epileptogenic discharges associated with spontaneous MSs do not necessarily originate at the site of stimulation (Wada et al., 1974, 1975, 1976). Thus, it is most parsimonious at this time to assume that IIDs preceded the development of spontaneous MSs in all of the spontaneous animals in Experiment 2, but that they were not necessarily manifested at the site of stimulation.
Spontaneous MSs. As in Experiment 1, there was considerable variability in the rate at which individual subjects developed spontaneous MSs. The number of stimulations required to generate the epileptic syndrome in the 27 experimental animals ranged from 92 to 508, with a mean of 348. An analysis of variance indicated that there was no significant difference between the rates at which subjects in the four groups developed spontaneous MSs ($F(3,23) = 0.29, p > 0.05$).

In most cases, the spontaneous MSs were the same as those elicited by stimulation, except for the fact that spontaneous MSs displayed by animals in the caudate group lacked the initial 5-sec loss of postural control. Thus, it was impossible to differentiate between the four groups on the basis of the form of their spontaneous MSs.

Figure 10 illustrates the mean incidence of spontaneous MSs during the 35-day post-stimulation observation period in the subjects completing the experiment. Of the 27 subjects who displayed spontaneity, 16 did not survive the entire 35-day period, and their data were not subjected to analysis. Of those animals that did survive, four were in the amygdala group, three were in the hippocampus group, and there were two each in the caudate and entorhinal groups. The electrode placements had no significant overall effect on the incidence of spontaneous MSs during this 35-day period ($F(3,7) = 0.95, p = 0.47$). Even though spontaneous seizures were observed in most of the subjects on Day 35, there was a significant overall decrease in the incidence of spontaneous MSs over the 35-day period ($F(34,238) = 2.11, p = 0.001$). However several animals were observed for as long as 7 months following the cessation of stimulations and they continued to display spontaneous seizures until the experiment was arbitrarily terminated.
Fig. 10. Incidence of spontaneous MSs during poststimulation observations (Experiment 2). Fig. 10 illustrates the mean incidence of spontaneous MSs for each of the four groups which were observed during each animal's 35 daily poststimulation observation sessions. Each observation session was 1-hr long.
The results of Experiment 2 support the view that an epileptic syndrome is the inevitable culmination of the kindling process. Regardless of the site of stimulation, the gradual intensification of elicited seizures eventually led to the manifestation of spontaneous MSs in every subject who completed the experiment. This epileptic syndrome, once induced, proved to be relatively enduring, lasting in some cases for up to 7 months after the curtailment of stimulations.
GENERAL DISCUSSION

Spontaneous Seizures

The present experiments have established several important points concerning the spontaneous MSs which are eventually displayed by kindled rats.

1) Incidence. The present results confirm previous reports that periodic amygdaloid stimulation may lead to the development of spontaneous MSs (Pinel et al., 1973, 1975; Wada et al., 1974, 1976). Although these previous studies demonstrated that spontaneity could be induced by periodic amygdaloid stimulation, this is the first instance in which spontaneity was demonstrated in a reasonably large number of subjects. Hence, it provides the first information concerning the reliability with which periodic amygdaloid stimulation can induce an epileptic syndrome. The results of both Experiments 1 and 2 suggest that an epileptic syndrome may be the inevitable result of long-term, periodic, amygdaloid stimulation. In Experiment 1, 16 of the 18 experimental subjects displayed spontaneous MSs before the experiment was arbitrarily terminated. In Experiment 2, all of the amygdaloid subjects eventually displayed spontaneous MSs. The main difference between these experiments and others in which the development of spontaneity has not been observed in rats kindled with amygdaloid stimulation is that in the present case considerably more stimulations were administered over a longer period of time.

2) Placements. The present findings provide the first systematic evidence that an epileptic syndrome can be induced by the periodic stimulation of structures other than the amygdala. The only previous report of spontaneity resulting from the stimulation of a structure other
than the amygdala was published by Wada et al. (1975), but it was the report of only a single subject. In Experiment 2 it was found that long-term periodic stimulation of the hippocampus, caudate, or entorhinal cortex, as well as the amygdala, reliably leads to the development of spontaneous MSs. The number of stimulations which preceded spontaneity did not vary significantly between these sites, and the behavioural form of the spontaneous MSs was indistinguishable in all of the animals.

3) Permanence. The results of Experiment 2 provide the first systematic information regarding the permanence of the epileptic syndrome induced by kindling. Although other investigators have indicated that the syndrome persisted for some time after stimulations were curtailed (cf. Pinel et al., 1975; Wada et al., 1974) they did not observe its permanence in a systematic fashion. All of the subjects in Experiment 2 continued to display spontaneous MSs during the 35-day observation period following the termination of stimulations, and those animals which were observed irregularly for an additional 6 months continued to display spontaneous MSs throughout this period.

4) Antecedents. The results of the present experiments confirm, and in some cases qualify, the impressions of previous investigators that the development of IIDs seem to be an important event in the development of spontaneity. In animals kindled with amygdaloid stimulation there was a development of IIDs before spontaneity, and in Experiment 1 the proliferation of these IIDs was found to be particularly striking in the contralateral amygdala. IIDs were also observed in all of the hippocampus subjects who eventually displayed spontaneous MSs. However, although all of the entorhinal and caudate animals eventually became spontaneous,
only some of the entorhinal subjects and none of the caudate animals displayed IIDs at the site of stimulation. Since the development of spontaneous MSs in the amygdala animals seems to be associated with a proliferation of IIDs, and since the development of elicited MSs in the caudate and entorhinal animals appeared to progress in approximately the same manner, it is most reasonable at this time to assume that these animals were experiencing IIDs, but they were not being generated from the site of stimulation. If this were true it indicates that, in contrast to the assumption that seems to be implicit in some investigations (cf. Arnold et al., 1973), the epileptic focus is not necessarily at or near the site of stimulation. Supporting this view is the fact that in Experiment 1 IIDs were frequently recorded from the contralateral amygdala even when they did not occur at the site of stimulation. Moreover, it was possible for a spontaneous MS to begin without any abnormalities from either of the electrode sites (Figure 5).

It was striking that there were no systematic changes in the elicited seizures that seemed to indicate the imminent manifestation of spontaneous MSs. This absence of a relationship between changes in elicited seizures and the development of spontaneous seizures has two important implications, one theoretical and one practical. The theoretical implication of this lack of relationship is that the development of spontaneity does not simply result from an extension of the same processes which are responsible for the intensification of elicited seizures. The progressive development of elicited ADs and MSs appears to be complete long before the first manifestations of spontaneous motor symptoms. Moreover, there was no obvious relation between the development of elicited MSs
and the rate at which spontaneous MSs developed. For example, there was no significant correlation between the number of stimulations necessary to elicit a subject's first Class 5 MS and the number before its first spontaneous MS \((r(41)=0.118, p>0.05)\). Thus, whatever mechanisms underlie the intensification of elicited MSs they do not seem to be the same as those responsible for the later development of spontaneous MSs. If a theory is going to be developed to explain the entire course of kindling and its culmination in spontaneity, it appears that it will have to involve more than a single mechanism.

On the practical side, this lack of relation between the course of development of elicited seizures and the development of spontaneity has implications for the use of the kindling paradigm as a model of epileptogenesis, especially as an assay device for convulsant and anticonvulsant drugs. Investigators conducting studies of this type (cf. Racine, Livingston, & Joaquín, 1975) usually inject subjects with a given drug prior to each stimulation, and then observe the drug's effect on elicited seizures. However, these studies have usually been terminated when animals have displayed their first generalized elicited MSs, or shortly thereafter. Thus, if drugs do not block the development of elicited MSs to this point, it is assumed that they have no effect on epileptogenesis. In light of the present findings that kindling is far from complete when generalized MSs are first elicited, subsequent attempts to screen drugs for their antiepileptogenic effects should look at the entire course of epileptogenesis. It may be that a drug which is completely ineffective in blocking elicited seizures may block the development of spontaneous seizures.
Elicited Seizures

Although the primary purpose of the present experiments was to study the spontaneous MSs produced by stimulation of the amygdala or other sites, the progressive development of elicited seizures was also documented during the course of the experiment.

1) AD Duration. Two different patterns of the development of ADs were noted in the amygdaloid subjects. In Experiment 1 the durations of ADs increased dramatically over the first 18 stimulations, and then partially declined. However, in Experiment 2 the durations of ADs increased monotonically. Although on the basis of the present investigation it is impossible to determine which factors produced these two patterns, both the monotonic increase and the biphasic pattern of development have been previously reported (cf. Pinel et al., 1973; Racine, Burnham, Gartner, & Levitan, 1973).

2) MS. Duration. The monotonic increases of the durations of the elicited MSs in the amygdaloid subjects in both Experiments 1 and 2 were similar to numerous previous reports (cf. McIntyre & Goddard, 1973). In particular, the striking increase in the duration of elicited MSs during the early phase of kindling has been frequently observed (cf. Pinel et al., 1974).

3) MS Patterns. The progressive increase in the severity of MSs elicited with amygdaloid stimulation was the same (up to Class 5) as that observed by other investigators (cf. Racine, 1972b). However, with continued stimulations MSs continued to grow more severe, although the rate of change was slower than that observed in the earlier phases of kindling. The manifestations of these more severe elicited MSs included the
development of multiple sequences of rearing and falling, running fits, and running fits which culminated in a rigid body tonus. On the basis of these observations, three classes of MSs were added to Racine's original five-class scale. This highlights the fact that most previous kindling studies have been arbitrarily curtailed not only long before spontaneous MSs developed, but even long before the development of elicited MSs was complete.

4) Placement. The same general course of the development of ADs and elicited MSs that was observed in the amygdala animals was also seen in subjects receiving hippocampal, entorhinal, or caudate stimulation. There were, however, two exceptions. First, the durations of ADs in the hippocampal animals were consistently longer than those of the other subjects. Secondly, the classes of elicited MSs of the caudate animals ranged only from 3 to 6, and the MSs began with an immediate loss of postural control before the display of the usual limbic-seizure pattern. In form, these MSs closely resembled those elicited by stimulation of the cingulate cortex (Racine, 1973).

One of the most surprising observations in Experiment 2 was that there did not seem to be any significant differences in the rates of kindling of animals in the four experimental groups, even though striking differences has been reported by other investigators, namely Goddard et al. (1969). As suggested earlier, these large differences observed by Goddard et al. may have been due to either the differential sensitivity to kindling of these limbic structures, or to the fact that the AD thresholds in some structures may have been higher than the stimulation levels (50 µA) employed, or a combination of both. However, the relatively
high current intensity employed in the present study reliably elicited ADs at all four sites, and there was little evidence that these sites were differentially sensitive to kindling.

Aggressive Behaviour and Kindling

In both Experiments 1 and 2 many of the experimental subjects but none of the controls became extremely aggressive and hyperreactive. They were extremely resistant to handling, and some became quite adept at drawing the experimenter's blood. Although these behaviours were not objectively scored or systematically observed, they were obviously different from those of the control animals. This aggressiveness usually developed in the latter stages of kindling, and seemed to intensify as IIDs proliferated. It was first assumed that this behaviour was a result of the many stimulations and MSs these animals had experienced, but in Experiment 2 the caudate animals did not exhibit this hyperreactivity, even though they had as many stimulations and more elicited MSs than the other rats. Therefore, it would appear that the aggressive behaviour associated with kindling is a function of neural changes specific to the site of stimulation rather than to the stimulations or seizures per se. These observations are particularly interesting when viewed from the perspective of reports of a relation between epilepsy and aggression (cf. Kligman & Goldberg, 1975; Stevens, 1966), and reports of the successful treatment of aggressive disorders by excision of suspected epileptic foci (cf. Falconer, 1973; James, 1960; Heimberger, Whitlock, & Kalsbeck, 1966) and by anticonvulsant drugs (cf. Munroe, 1975). Although there has yet to be a convincing demonstration that epileptic discharges are causally related to
aggressive behaviour, the possibility cannot be ruled out. Because of the lack of a suitable animal model of the relation between epilepsy and aggression, research in this area has been limited to correlational studies (natural experiments). However, it is feasible that further study of the relationship between kindling and hyperreactivity might establish the kindling paradigm as a useful tool in studying epilepsy-related aggression.

**ECS Kindling and Spontaneous MSs**

The development of spontaneous MSs in animals kindled with local brain stimulation may be related in some way to the development of spontaneous seizures after repeated electroconvulsive shocks. The occurrence of spontaneous seizures following a series of electroconvulsive shocks has been widely reported in both the experimental and clinical literature (cf. Essig, Groce, & Williamson, 1961; Blumenthal, 1955; Pacella & Barrera, 1945). This is extremely interesting in view of a recent report that electroconvulsive shocks, when administered to rats at 3-day intervals, produce a progressive increase in the severity of the elicited MS pattern — essentially, ECS kindling (Ramer & Pinel, 1976).

**Other Methods of Producing Experimental Epilepsy**

In addition to the obvious similarities between the kindling of elicited MSs and clinical epileptogenesis, it is now clear that the kindling procedure can reliably produce a long-lasting and relatively stable epileptic syndrome in rats. Thus, it appears that kindling may be a valuable addition to the methods available for producing experimental epilepsy. Although experimental epilepsy had been produced in animals
with strychnine (Ralston, 1958), penicillin (Faeth, Walker, & Warder, 1956), mescaline (Crighel & Stoica, cited in Kreindler, 1965), alumina cream (Kopeloff, Burrera, & Kopeloff, 1942), local freezing of the cortex (Speransky, 1943), and ethyl chloride (Morrell & Florenz, 1958), in most cases more control is afforded by the kindling procedure. For example, there is more control over the site of application, and it would appear that gross cellular damage is minimal.

However, the main advantage of kindling procedure over the other methods is that it allows one to study the process of epileptogenesis itself in a step-by-step fashion. Other methods have several shortcomings in this regard; the development of epilepsy is largely out of the experimenter's control, and he has no way of characterizing this development. In addition, the reliability of these methods in producing the epileptic syndrome appears to be somewhat less than that of the kindling procedure. In fact, these other procedures are so unsuitable for studying epileptogenesis that such applications are extremely rare. However, with the kindling procedure the experimenter can control the frequency and intensity of stimulations, and can follow the development and intensification of elicited seizures as well as the development and proliferation of IIDs until the point at which spontaneous MSs are first displayed.

**Neuroanatomical Correlates of Epileptogenesis**

That very little is known about the neuroanatomical correlates of epileptogenesis has perhaps been due to the lack of suitable methods for its study. The usual method of studying these correlates is to examine the brains of epileptic animals or patients and then to try to infer the changes that
have led up to that pathological condition. Madge and Arnold Scheibel, for example, have examined the brains of human epileptics and found a number of changes in the hippocampal pyramidal cells and dentate granular cells, ranging from minor pathology along single dendrites to massive degenerative changes culminating in the death of many neurons (Scheibel, Crandall, & Scheibel, 1974). However, they pointed out that although their results seemed to indicate a systematic development of this pathology, the precise relation between those changes and epileptogenesis could not be determined until the pathology could be studied as it was developing. As a result of the interest of the Scheibels in epileptogenesis the brains of some of the animals in the present experiment have been sent to them for analysis, and their investigation is still in progress.

There have been two notable attempts to study the neuroanatomical correlates of kindling. Both an electron-microscope study (Goddard & Douglas, 1975) and a Golgi-Cox study (Racine, Tuff, & Zaide, 1975) failed to reveal significant differences in the brains of kindled rats and unstimulated controls. However, in both of these cases the stimulations had been curtailed relatively early in kindling, at a point at which one would expect that the neuroanatomical changes would not be so obvious as they would in the later stages of kindling.

In view of the striking pathology in human epileptic tissue it is likely that there are some obvious changes in the brains of rats which display spontaneous MSs. Once these changes are observed, the Scheibels, in collaboration with John Pinel of the University of British Columbia Department of Psychology, will conduct a study in which the genesis of
these final changes is traced in rats at various stages of epileptogenesis.

Thus, the kindling paradigm should be a valuable addition to the methods of studying epileptogenesis and its correlates, and the data provided by the present experiments should greatly facilitate its subsequent use in this regard.
REFERENCES


Cullen, N., & Goddard, G.V. Kindling in the hypothalamus and transfer to the ipsilateral amygdala. Behavioral Biology, 1975, 15, 119-131.


Appendix A

Analysis of Variance Tables for Experiment 2

Analysis of Variance Table for AD Duration (Panel A)

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* p<.05

Analysis of Variance Table for AD Duration (Panel B)

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### Analysis of Variance Table for MS Class (Panel A)

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### Analysis of Variance Table for IID Incidence

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* p<.05
### Analysis of Variance Table for Spontaneous MS Incidence (35 days)

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* p<.05

### Analysis of Variance Table for Spontaneous MS Incidence (Day 1 & 35)

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* p<.05