The kindling of motor seizures from hippocampal commissure in the rat

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The hippocampal commissure was stimulated in 35 rats in order to find conditions which favor triggering of motor seizures on the first trial. Strain and supplier of rats, size and orientation of electrodes, distance between electrode poles, area of the commissure stimulated, ongoing behavior, pattern of electrical activity in the hippocampus, and state of arousal were varied. In each case, motor seizures developed only after many daily stimulations, as reported for other limbic sites. However, stimulation of corpus callosum or cingulum yielded motor seizures on the first trial. It is concluded that previous reports of first-trial motor seizures triggered from the hippocampal commissure (Racine et al., 1972) must have resulted from stimulation which encroached upon these more dorsal structures. The results suggest that kindling may not be restricted to the strengthening of limbic-limbic connections but may also require modification of circuits lying more distal to the limbic system.

Kindling is said to occur when electrical (Goddard, McIntyre, & Leech, 1969) or chemical (Voss & Wise, 1975) stimulation is applied repeatedly to a focal brain area and the response to the stimulation changes with repetition until eventually the animal responds with a behavioral convulsion. Gaito (1974) has recently reviewed some of the literature on this kindling effect, pointing out that the alterations in response are probably due to a lasting modification of neural circuitry. It is possible that investigation of the kindling effect will provide insight into mechanisms by which connectivity in the adult mammalian brain, and consequently behavior, may be modified. It is already known that the process of kindling from repeated stimulation of the amygdala leaves a long-lasting aftereffect on the ability of rats to learn a conditioned emotional response (McIntyre & Molino, 1972).

In an early mapping experiment, Goddard et al. (1969) indicated two systems in the rat brain from which kindling can be obtained. One, perhaps centered on the amygdala and involving many limbic and associated areas, required many daily repetitions of stimulation before motor seizures could be triggered. When triggered from this system, the seizures were of relatively long latency and were primarily clonic in form. The other system involved an area of anterior neocortex and underlying corpus callosum, from which motor seizures could sometimes be triggered immediately upon the first application of stimulation. When triggered from this second system, the convulsions were of short latency and were

tonic-clonic in form.

More recently, Racine, Okujava, and Chipashvili (1972) reported that electrical stimulation of the hippocampal commissure, a part of the limbic system, could trigger motor convulsions on the first stimulation trial. Such stimulation would cause bilateral synchronous driving of the hippocampus, and these authors argued that the observation of a Trial 1 motor seizure implies that kindling results from the strengthening of limbic-limbic rather than limbic-motor connections.

The logic would appear to be that focal stimulation and epileptiform seizure activity within the limbic system is not able to drive motor convulsions until enough connections have been made to a critical output pathway which, when activated, is able to drive the motor system without further modification. If this critical output pathway could be stimulated directly, motor seizures would be seen immediately, no kindling being necessary. This is the situation seen in anterior neocortex which is near or involves motor cortex, and Racine et al. (1972) appear to suggest that a parallel example exists in the limbic system which can be driven directly by stimulation of the hippocampal commissure. If this were true, it would greatly simplify the search for the elements modified by kindling, since they must lie between the point of kindling stimulation and the output pathway.

However, it is surprising that the hippocampal commissure provides such easy access to motor seizures. Hippocampal seizure activity does not usually produce motor manifestations until it has been repeated many times. Also, some authors have suggested that the major effect of hippocampal function in behavior is to inhibit motor activity.

We thought that additional experiments on this system would be fruitful, but our initial attempts to trigger the Trial 1 motor seizure by stimulating the

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hippocampal commissure were not successful. Correspondence with Racine convinced us that first-trial convulsions were possible, and we conducted a series of exploratory studies to find the appropriate experimental conditions. Variables that were manipulated include: strain and supplier of rat, location within the hippocampal commissure, size of electrode wire and distance between electrode poles, orientation of electrode poles across or parallel to the commissural fibers, and intensity or duration of stimulation.

It is important not to confuse the issue with false assumptions about current intensity, or long durations (minutes) of stimulus duration. In the amygdala, for which the most detailed information is available, extremely high amplitudes of stimulation will not trigger motor convulsions on the first trial, even though the stimulation triggers extensive epileptiform afterdischarge throughout the limbic system (Goddard, 1972; Goddard et al., 1969). The amygdala, being a relatively large structure, permits this type of experiment without danger that areas outside of the limbic system would be stimulated by current spread. There is one situation in which first-trial motor seizures can be triggered from the amygdala, namely long trains of 3-Hz bursts of high-frequency pulse packets (Racine, Burnham, & Gartner, 1973). In this situation, the motor seizure does not appear until after at least 1 min of recruiting, and it usually takes 3-4 min of recruiting. This finding is in agreement with the argument that the amygdala drives motor convulsions only via a very large synaptic distance which must be primed before it can operate. This type of stimulation, therefore, would not be appropriate for testing the hippocampal commissure Trial 1 hypothesis. Neither would extremely high intensities of current be appropriate, since the hippocampal commissure is a small structure lying directly below anterior cortex.

Manipulation of electrode type and stimulus parameters within reasonable range and bracketing those used by Racine et al. (1972) were not successful. We considered the possibility that the ongoing behavior of the animal or ongoing activity within the hippocampus could provide a gating mechanism which, in one state, would block access to the motor system and, in the other state, provide easy access to the motor system. Two lines of rationale were used. The first was based on reports (Arnold, Racine, & Wise, 1973; Pinel, Phillips, & MacNeill, 1973) that states of high arousal interfere with kindling or the triggering of kindled convulsions from the amygdala. The second was based on Vanderwolf's (1969) observation that rhythmic slow activity (RSA) in the hippocampal EEG of rats is present when and only when the animal engages in excursive movements (voluntary behavior).

The experiments were not designed to evaluate

subtle effects of these variables on kindling rate, but concentrated on finding conditions which favor the triggering of motor seizures on the first trial of hippocampal commissure stimulation. If such first-trial convulsions are not possible within reasonable limits of stimulation intensity, then it must be assumed that the modification of neural circuits necessary for the kindling of motor convulsions are considerably more widespread than envisioned by Racine et al. (1972).

METHOD

Subjects

Twenty-nine male hooded Royal Victoria rats (24 from Canadian Breeding Farms and 5 from Les Elevages Medicos Inc., Quebec) and 13 male albino Wistar rats (Woodlyn Farms, Ontario) were used. The animals weighed from 265 to 373 g at the time of surgery. They were housed individually in stainless steel cages and maintained on a light-dark cycle of 14 and 10 h.

Surgery

Sodium pentobarbital was used for anesthesia, and all rats were injected (IM) with 60,000 IU of Bicillin after surgery. Bipolar nichrome electrodes were implanted in the hippocampal commissure in all rats. Fifteen rats were implanted with an additional electrode pair in the contralateral hippocampus. Size, distance between, and orientation of electrode poles were varied. As in the study by Racine et al. (Racine, personal communication), the poles of the electrodes were separated by 0.10-0.20 mm in 28 rats. Fourteen rats were implanted with electrodes in which the poles were separated by only the factory-applied insulating varnish. The poles of the electrodes were oriented in an antero-posterior direction in all but seven rats, in which the poles were oriented medio-laterally. Diameter of the electrode wires was 0.25 mm in 35 cases and 0.127 mm in the remaining 7 cases. The hippocampal electrodes had one pole cut 0.40-0.90 mm shorter than the other pole in order to enhance the recording of hippocampal EEG rhythms.

Stimulation Parameters

Stimulation consisted of diphasic pairs of 1-msec rectangular pulses with a frequency of 50 Hz. The hippocampal commissure was stimulated at an intensity of 400 μ A peak to peak, constant current. Duration of stimulation was 5 sec, except in seven animals which received 1 sec stimulation for one to five trials and then 5 or 10 sec for the remainder of the experiment. The hippocampus was stimulated at intensities ranging from 100 to 400 μ A and for durations of 1 to 5 sec. Stimulation was monitored and the EEG recorded in all experiments. The leads to the polygraph and the ground lead from the animals were disconnected by a relay during stimulation.

Procedure

Stimulation began 14 to 22 days after surgery. The 27 rats implanted with one electrode were stimulated once every 48 h in the hippocampal commissure until several motor seizures had occurred. Each rat was brought directly from its home cage and stimulated following 1 to 2 min of baseline EEG recording. Two rats in this group were terminated on Trial 47 as motor seizures had not yet occurred. For statistical purposes, they were considered to have kindled on Trial 48.

Fifteen other rats, which had an additional electrode in the hippocampus, were placed together on a rest stand for at least 1/2 h each day. On Days 2 to 6 after surgery, each of these rats was also placed in the stimulation box for 5-10 min each day. The rats then began a water-deprivation schedule in which water was available 1 to 4 h each day and the relationship between behavior and hippocampal EEG was studied. Deprivation did not seriously

debilitate the animals and was sufficient to ensure reliable drinking on demand, permitting good control over hippocampal EEG. Since the correlations between behavior and hippocampal EEG were in agreement with Vanderwolf's (1969) analysis in the rat, they will not be reported in detail here. The procedures assured that the animals were calm, easily handled, and producing predictable patterns of EEG when stimulation trials were begun.

Stimulation was delivered once each day. Six animals were stimulated in the hippocampal commissure when hippocampal RSA (rhythmic slow activity; Vanderwolf, 1969) was *not* present, either because the animals were sitting quietly or because they were drinking. Seven rats were stimulated while hippocampal RSA was present, either because the animals were exploring a novel aspect of the test situation or were attempting to gain access to water that was covered by a transparent glass beaker.

Three rats in the RSA group were stimulated while deprived and four while nondeprived (water was made available 1 h before testing). Long exposure (30-45 min) to the box was also tested, since Racine et al. (1972) often left their animals in the stimulation box for long periods (Racine, personal communication) and because conditions producing high arousal, such as footshock, have been shown to suppress the subcortical triggering of motor seizures (Pinel et al., 1973). Two animals in which RSA could not be recorded were placed on ad lib food and water and stimulated once each day following the procedure used in the animals without hippocampal electrodes.

Typically, stimulation was discontinued when four to six motor seizures had been observed and a 7- to 10-day rest interval with ad-lib food and water begun. Deprivation was then reintroduced and stimulation delivered through the hippocampal leads. Bipolar stimulation was tested first in five rats using 5-sec trains of 400- μ A pulses, and more extensive testing of monopolar stimulation was done later in these and eight other rats. Monopolar stimulation through the dorsal and ventral poles was alternated. Again, stimulation was delivered either in the presence or in the absence of hippocampal RSA. Each trial was recorded on videotape for later behavioral analysis of the motor seizure.

Histology

At the completion of the experiment, the animals were perfused with 10% Formalin and the brains removed 1 to 4 h later. After additional fixation in Formalin, the brains were frozen sectioned at 40 μ and alternate sections stained with Weil and thionin.

RESULTS

Implants in the hippocampal commissure ranged along its entire antero-posterior length, with the majority being located at the level of the corpus subfornicale (Figure 1A). Histological reconstruction was adequate to clearly resolve the location of both poles of the electrode in 22 cases and one pole in the remaining 20 rats.

Kindling in the Hippocampal Commissure

Of 35 rats in which the electrodes were histologically verified to be in the hippocampal commissure, not one displayed a motor seizure on the first trial. All rats did display electrographic epileptiform afterdischarge on this first trial, although it was usually brief ($\bar{x}$ = 9.6 sec, range 2-16 sec). Motor seizures developed after an average of 18 (range = 6-48) stimulation trials. Development followed a pattern similar to that described for other limbic sites (Goddard et al., 1969). Other than a brief vigorous shiver of the back muscles

Figure 1. Sections redrawn from original histological material. (A) Location of electrodes aimed at the hippocampal commissure. Filled circles indicate sites from which motor seizures were triggered in 6 to 48 trials. Open circles indicate sites from which motor seizures were triggered on the first trial. (B) Location of electrode poles on the contralateral side of the brain stimulated following kindling in the hippocampal commissure. Filled circles indicate poles from which motor seizures were not triggered. Open circles indicate poles from which motor seizures were triggered.

which was invariably observed, there were few signs of convulsive behavior on Trial 1. By Trial 10, arrest, rhythmic nodding of the head, and jaw movements were observed in many animals. With continued stimulations, bilateral clonic motor seizures appeared and, by this time, the epileptiform afterdischarges had increased to larger amplitude and longer duration ($\bar{x}$ = 46.0 sec, range = 15-123 sec).

Two rats, one with an electrode in the cingulum and one in the corpus callosum (Figure 1A), responded with a motor seizure on the first stimulation trial. Both convulsions were relatively brief (7 sec) and were of a mixed tonic-clonic form. Of the remaining five rats, two had their electrodes located in the corpus callosum and three in the lateral septum (Figure 1A). These animals kindled in 8 to 42 trials.

Analysis of variance showed no difference in the number of trials to first motor seizure with separated vs. nonseparated electrode poles ($p > .20$), Royal Victoria vs. Wistar strains ($p > .20$), anterior-posterior vs. medial-lateral orientation of electrode

poles ($p > .20$), or stimulation while hippocampal RSA was present vs. absent ($p > .20$). In the latter experiment, RSA was judged to be present immediately before stimulation on an average of 61.2% (range = 30%-88%) of the trials in the RSA group and *not* to be present on an average of 91.4% (range = 75%-100%) of the trials in the non-RSA group.

Hippocampal Stimulation

A total of 136 stimulation trials were delivered to the hippocampal leads and a total of 38 motor seizures observed. Motor seizures were triggered by bipolar stimulation on 10 of 12 trials, by monopolar stimulation of the ventral pole on 3 of 68 trials, and by monopolar stimulation of the dorsal pole on 25 of 56 trials.

The electrode poles from which motor seizures were triggered were located in the cingulum, corpus callosum, or alveus. Electrode poles which did not typically yield MS were scattered throughout the hippocampus and dentate gyrus (Figure 1B).

The motor seizures triggered from the dorsal electrodes were violent and extensive. A single description of their form was not achieved through study of the videotapes. Tonic components, low body position, rapid clonus, and short latencies were often seen (Burnham, 1971; Goddard et al., 1969). Frequently, during stimulation, the animal would fall, jerking its forelimbs and turning its body to the side contralateral to the electrode. After landing on the ipsilateral side, the animal would occasionally roll several times during stimulation.

DISCUSSION

The results of the present experiments differ substantially from those reported by Racine et al. (1972). They report motor seizures triggered from the hippocampal commissure in an average of 2.4 (range = 1-8) stimulation trials with 5 of 8 animals exhibiting full motor seizures on the first trial. The present results yielded an average of 18 (range = 6-48) stimulation trials, with none of 35 rats stimulated in the hippocampal commissure showing first-trial motor seizures. However, 2 rats with more dorsal implants exhibited motor seizures on the first trial, and motor seizures could be triggered by stimulation through the hippocampal EEG lead when the dorsal pole was in alveus, corpus callosum, or cingulum. Monopolar stimulation left little doubt that the motor seizures were triggered from this dorsal pole. Prior kindling from the hippocampal commissure was not necessary for the effect, since motor seizures were seen in two similar cases in which the hippocampal commissure had not been stimulated. Previous reports of first-trial motor seizures from anterior cortical areas (Goddard et al., 1969; Racine, 1975) are in general agreement

with this anatomical interpretation. An experiment currently in progress has similarly found that 17 of 29 animals with electrodes implanted in these dorsal areas responded with a motor seizure on the first trial provided that the stimulation intensities were sufficient to trigger electrographic afterdischarge.

Previous descriptions (Goddard et al., 1969) identified two motor types: cortical and limbic. Motor seizures triggered from dorsal areas in the present studies did not easily fit either classification, but included both, being a mixed clonic and tonic-clonic form. The motor seizures kindled by stimulation of the hippocampal commissure were more typically limbic (clonic) in form. In view of the rapidity with which motor seizures appeared from stimulation of the dorsal areas, and the proximity of these areas to the hippocampal commissure, it seems likely that the results reported by Racine et al. (1972) arose through encroachment of the stimulation current into these dorsal areas rather than by simple stimulation of the hippocampal commissure.

Variables such as size, orientation of, or distance between electrode poles did not affect the probability of triggering first-trial motor seizures. Nor did differences in handling conditions, electrophysiological state in the hippocampus, or differences in arousal level resulting from different exposure times to the stimulation box. The present studies were not sufficiently extensive to determine if these variables have more subtle effects on kindling rates.

While two strains of rat were found not to differ in kindling rate in the present studies, Tbilisi rats such as those used by Racine et al. (1972) were not tested. However, Tbilisi rats were reported by Racine et al. (1972) to kindle from the amygdala in an average of 11.3 trials and Wistar albinos by Goddard et al. (1969) in an average of 15 trials. Nine Wistar albinos which were tested at the same time as the Wistars used in the present studies required an average of 14.4 trials before MS were observed from amygdala. These results suggest that if strain is related to the difference in results, then it must be due to very marked strain differences in the hippocampal commissure which are not found in the amygdala.

The present experiments agree with the Racine et al. (1972) general conclusion that simultaneous stimulation of both hemispheres facilitates kindling in the limbic system. Their conclusion was based on stimulation via bilaterally implanted electrodes, unilateral stimulation in split brain animals and stimulation of the bilaterally projecting hippocampal commissure. In our study, also, the hippocampal commissure kindling rates were considerably more rapid than has been found in previous studies using unilateral stimulation of the hippocampus. However, the Trial 1 convulsions from hippocampal commissure stimulation were not observed, and the conclusion that kindling depends entirely on the strengthening of

limbic-limbic connections is brought into question. It seems more likely that kindling also involves the strengthening of connections between the limbic system and areas remote from the limbic system. Recent evidence by Wada and Sato (1974, 1975) indicates that one important link that is made during kindling from the cat amygdala involves the brainstem reticular formation. Until it can be shown that some locus in the limbic system can drive motor seizures promptly, without priming, recruiting, or kindling, it must be assumed that the limbic system does not have adequate connections to the motor system and that adequate connection must be established as part of the kindling process.

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