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DIVERSITY OF KINDLING EFFECTS: EEG MANIFESTATIONS IN CATS DURING KINDLING IN THE HIPPOCAMPAL FORMATION

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Abstract. The formation and subsequent development of after-discharges (ADs) and spontaneous interictal spikes was investigated in a group of ten kindled cats. The electrodes used for electrical stimulation and EEG recording were located in various parts of the hippocampal formation: in the hippocampal gyrus, dentate gyrus, subiculum and entorhinal cortex. The animals were stimulated once daily with 1 s trains of 50/s electric pulses. The choice of stimulating electrodes, stimulating currents and, in some cases, monitoring of the stability of stimulating conditions was aided by recording hippocampal field potentials evoked by intrahippocampal electrical stimulation. A subgroup of 5 cats was stimulated at AD threshold or near threshold currents of constant intensity. The following patterns of AD development were observed: (i) a long lasting initial phase of stable, a few second-long ADs with subsequent development, culminating in prolonged ADs and complex partial or secondary generalized tonic clonic seizures, after 80-120 days of kindling; (ii) stable pattern of brief ADs (0.3-1.5 s duration) during 120 days of kindling; (iii) gradual development of initially brief ADs (about 5 s) into longer lasting ADs (about 35 s) associated with complex partial seizures, during 220 days of stimulation. Spontaneous interictal spikes developed in this subgroup after 16-15 days of kindling. The second subgroup of 5 animals was subjected to stimulation with gradually increasing AD subthreshold currents. In two animals of this subgroup spontaneous EEG discharges of isolated spikes and clusters of high frequency spikes appeared in the stimulated hippocampal gyrus after 10 days of kindling. Apart from the diversity of after-discharge development patterns the results indicate relative independence of the processes underlying formation of the spontaneous interictal spikes and after-discharges.

INTRODUCTION

The kindling effect (17), is usually defined as a progressive change in brain functions obtained in the course of repeated stimulation of some brain structures using constant intensity stimuli (electrical or chemical), resulting in the appearance of generalized epileptic seizures. The exact nature of the kindling effect is as yet unknown. Kindling is being used as an experimental model for the studies of epileptogenesis and memory processes (25, 29).

Repeated local electrical stimulation of some, especially limbic structures, evokes a number of progressive effects accessible to electrophysiological investigations. Long term enhancement of the amplitudes of evoked potentials may be achieved by brief, high frequency bursts of after-discharge subthreshold stimulating pulses (4, 5). Persistent reduction of local after-discharge (AD) threshold even during AD subthreshold stimulation, was reported by Racine (32). However, the induction of after-discharges in the stimulated brain region appears to be a necessary prerequisite for the AD threshold reduction and AD formation in distant structures and seizure development (33).

Along with the development of after-discharges and epileptic seizures, the functional reorganization of nerve cells is manifested by the appearance in EEG records of spontaneous, interictal (between seizures) spikes (IIS).

The generally accepted criterion of the established kindling effect is the appearance of prolonged after-discharges and secondary generalized tonic-clonic seizures. Once established, this effect becomes a permanent feature of brain response to stimulation (50).

The susceptibility to kindling varies among the different limbic structures. While the amygdala belongs to the most susceptible regions, the hippocampus was found least sensitive (17). The reported values of amygdaloid kindling rates are between 2 and about 200 days to criterion. The differences of kindling efficacy seem to result mainly from the differences in kindling procedures and differences among species or animal strains. In the animals stimulated once daily with trains of pulses of 1-2 s duration, the mean rates of kindling ranged from 7.0 to 18 days in rats (17, 20, 33, 36), 25-30 days in cats (31, 49), while in monkeys generalized seizures appeared after 196 days of kindling (48). The corresponding mean kindling rates obtained during stimulation of the hippocampal formation were : 27.4-77 days during stimulation of the hippocampal gyrus in rats (17, 33, 37) and 51.8 days in cats (38), and 20.3-37 days in rats (17, 42) during stimulation of the entorhinal cortex (the authors stimulated region of the angular bundle containing in fact heterogenous fiber groups).

The kindling effect results from the local as well as distant effects of electrical stimulation caused by activation of nerve cells and nerve fibers: ascending, descending and fibers of passage. After extensive destruction of cells in the amygdala by the intraamygdaloid injections of kainic acid there was a great acceleration of kindling effects from this region (19). In this type of lesions afferent fibers as well as fibers of passage are spared. On the other hand, in the hippocampal formation of rats, significant slowing of the entorhinal kindling was observed after intradentate application of colchicine, a neurotoxin causing selective destruction of granule cells (15, 42).

The relatively slow progress of the hippocampal kindling can be regarded an unexpected finding since self-sustained after-discharges and spontaneous spikes could be readily induced, both in situ and in hippocampal tissue slices (1, 3, 9, 16, 39, 41). In this investigation, the attention was concentrated on relation between development of spontaneous EEG interictal spikes and development of after-discharges, and on patterns of AD development in cats stimulated in various regions of the hippocampal formation (according to the terminology introduced by Lorente de Nó (22)): in the hippocampal gyrus, dentate gyrus, subiculum and entorhinal cortex. The process of kindling was monitored by recording field potentials in limbic structures, evoked by the intrahippocampal electrical stimulation (report in preparation).

MATERIAL AND METHODS

The experiments were performed on 10 adult, male and female cats with implanted intracerebral electrodes for stimulation and recording. The bipolar or multipolar electrodes were made of straight stainless-steel wires, 300 or 400 μm in diameter. Individual wires were insulated except at the conically sharpened tips. Two wires glued together with one tip protruding with respect to the other by 1 to 2 mm composed a bipolar electrode. A multipolar electrode was a bunch of three to five wires with the distances between uninsulated tips of 2-3 mm. The electrodes were implanted under pentobarbital anesthesia, 40 mg/kg, i.p. They were soldered to a 20 pin miniature connector and fixed to the skull with dental acrylic.

Using the stereotaxic coordinates of Snider and Niemer (40) the electrodes were aimed at the following subcortical structures: ventral hippocampal formation (stereotaxic coordinates : A6, L11-L14, H down to −5), posterior hippocampal formation (A1, L11, H +2), amygdala (A11, L11, H-6), and entorhinal cortex (A7, L12-L15, H: the electrode was moved down to the cranial bone and than withdrawn about 1 mm). In some cats the electrodes were also implanted in the septal region and in the midbrain reticular formation. In addition, the bipolar electrodes were implanted in the neocortical regions: sensorimotor, auditory or visual. The majority of the electrodes were implanted in the right hemisphere. However, each animal had at least one electrode implanted in the left symmetrical ventral hippocampal formation. Proper localization of the electrodes was aided during implantation by recording injury discharges during penetration of the hippocampal formation by the electrodes and by recording evoked hippocampal field potentials to entorhinal and intrahippocampal electrical stimulation. The actual localization of electrodes was verified histologically after completion of the experiments. 50 μm coronal brain sections were stained according to Klüver-Barrera method.

After a variable period of recovery of at least 1 month, cross relations between the implanted limbic structures were tested in each animal using evoked potentials technique in order to find the optimal bipolar derivation for low threshold kindling. Evoked potentials were recorded using a recording system composed of a wide band AC amplifier (band pass 0.5 Hz-10 kHz), average response computer ANOPS 10 and an XY plotter. The potentials were evoked by pseudorandom pulses or by brief AD subthreshold trains of 10/s electric pulses in order to minimize kindling effect. During stimulation and recording sessions background EEG activity was monitored on the oscilloscope.

Kindling was performed using mono or biphasic (in different animals) rectangular pulses. With the exception of one cat C14, the pulses were delivered by the battery powered, optically-coupled stimulus isolation unit with output resistance of 1 Megohm. The cat C14 was stimulated with the aid of a constant voltage stimulus isolation unit. The animals were stimulated once daily, six or seven times a week, using 1 s trains of 50/s pulses. The intensity of stimulating currents was adjusted individually in each animal. Monophasic pulses were of 1 ms duration. A biphasic stimulus was composed of the rectangular pulse of 0.5 ms duration followed immediately by another one of equal amplitude and reversed polarity.

In a subgroup of 5 cats (C7, C11, C13, C15), the intensity of stimulation was kept constant on a predetermined level throughout the whole kindling period. Except for the cat C14, the animals were stimulated with biphasic pulses. The cats C11, C13 and C15 were kindled with AD threshold currents, while in the cats C7 and C15 ADs developed in response to near AD threshold currents.

In the second subgroup of 5 animals, initially-low, AD subthreshold currents were increased in daily 5-50 μA steps, up to the AD threshold, in order to test the possible development of spontaneous EEG spikes in the absence of after-discharges. The initial values of stimulating currents were established in relation to the thresholds of evoked potentials found during preparatory phase of the experiment. These animals were also used for studies of the kindling-related changes of limbic field potentials evoked by hippocampal stimulation. In no case did the stimuli evoke after-discharges during evoked potential testing.

Background EEG activity and effects of electrical stimulation were recorded during kindling on a 16 channel EEG apparatus.

RESULTS

Development of after-discharges

In five animals stimulated with constant current intensities, kindling related development of after-discharges showed significant variations. In two animals, (C11, C14) the pattern of AD progress was similar (Fig. 1). It ended in the secondary generalization of seizures (GS) in the cat C14 after about 2.5 months of stimulation. In the cat C11 kindling was stopped at the stage of prolonged after-discharges and complex partial seizures, after a similar period of time. The stimulating electrodes were located in these animals within the region of dentate and hippocampal (CA3, CA1) gyri. It took about 120 days to reach the stage of secondary generalization of seizures in the cat C15, stimulated in the region of lateral entorhinal and perirhinal cortex. The preliminary period of kindling in this animal (lasting about 70 days) was characterized by the presence of brief (1-2 s) ADs, composed of spikes recorded from the hippocampal gyrus and spikes and bursts of spikes (up to 20/s) in the hippocampal gyrus and amygdala.

In the cat C7 ADs were of short duration (Fig. 2*A*). The only change in AD distribution during about 120 days of AD presence was a transient appearance of single spikes in the ipsilateral amygdala during 20-30/s oscillations seen in the stimulated hippocampal gyrus and entorhinal cortex, after about 2 months of kindling. Widespread enhancement of evoked potentials in comparison to control conditions was observed in the ipsilateral amygdala, posterior hippocampal formation and contralateral hippocampal gyrus after 19 days of kindling.

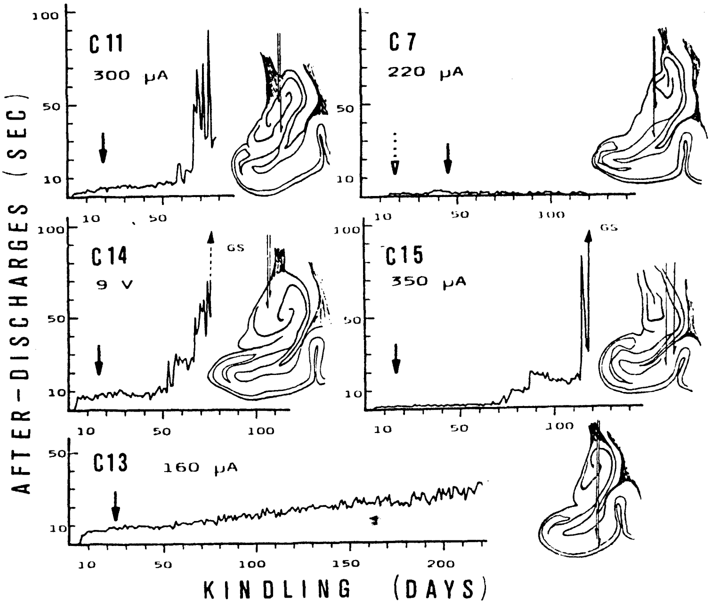

Fig. 1. Patterns of after-discharge development in the course of kindling in various regions of the hippocampal formation in a group of five cats. Black triangles indicate secondary generalization of seizures (GS). Arrows indicate formation of the spontaneous interictal spikes. Two arrows in the cat C7 indicate slow, gradual "maturation" of spikes.

In the cat C13, stimulation of the lateral entorhinal cortex caused gradual progress of the AD duration from about 5 to 30-35 s during kindling period of 220 days. After 200 days of kindling, the thresholds of evoked potentials recorded in the hippocampal formation after hippocampal stimulation in the kindled region did not exceed the thresholds found before kindling, indicating stable stimulation conditons. This animal did not reach the stage of secondary generalization of seizures. Distribution and EEG pattern of after-discharges on the 7th, 14th and 57th day of kindling in this animal is shown in Figs. 3*A* and 3*B*. Spread of the ADs to cortical and subcortical structures remained basically the same as that seen on the 57th day of stimulation.

Early EEG after-discharge patterns in the animals stimulated initially with the AD subthreshold currents included brief (2-5 s) episodes of 8-12/s oscillations or 3-4/s spikes observed in the hippocampal formation and amygdala. In three out of 5 cats the first ADs evoked after 15-47 days of kindling lasted 26-53 s and were associated with complex partial seizures accompanied with twitches of facial muscles and salivation. Description of the behavioral seizures and analysis of the EEG patterns of after-discharges may be found in another paper (27).

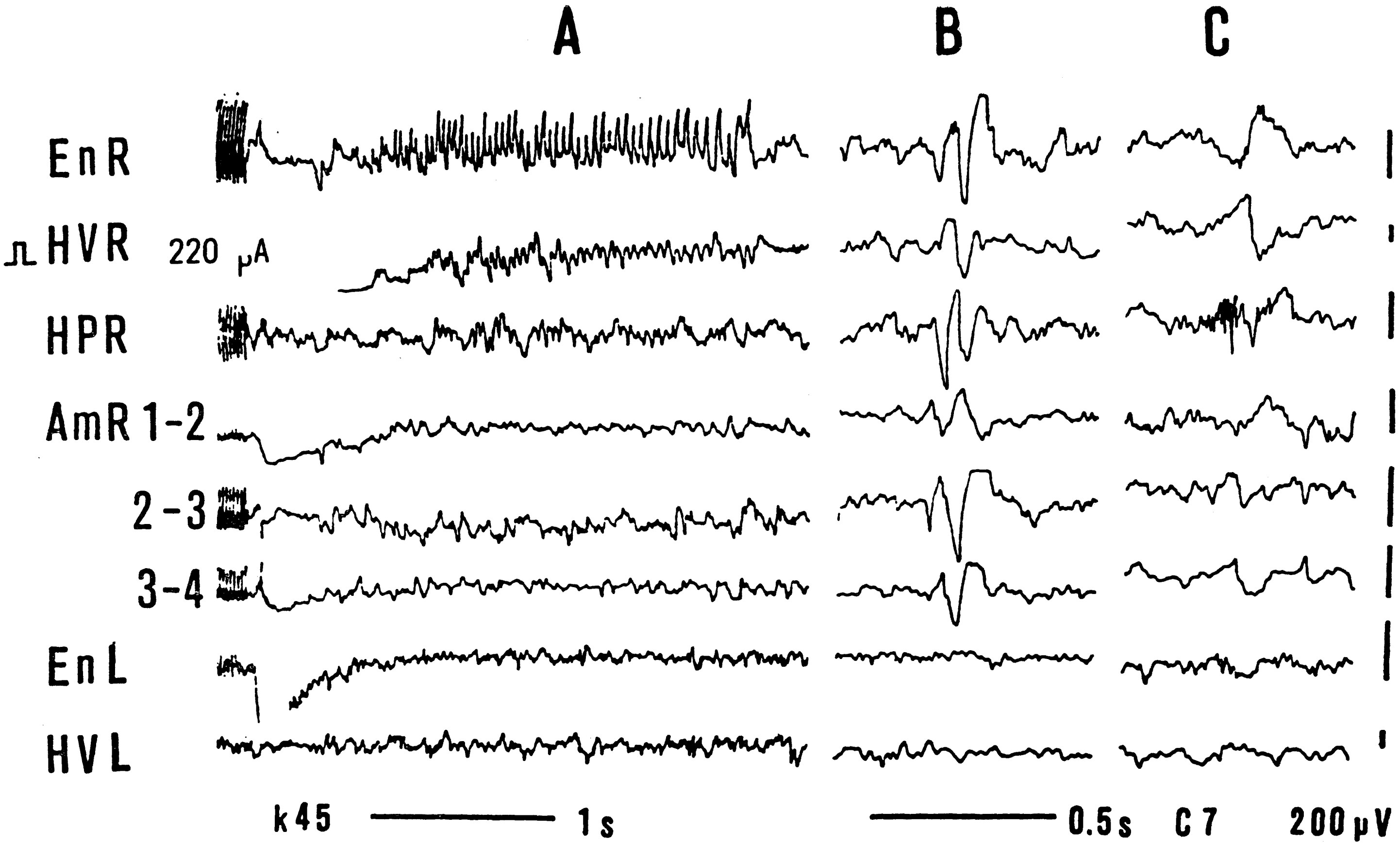

Fig. 2. EEG patterns of after-discharges (A) and spontaneous interictal spikes (part *B* and C) observed in the cat C7 after 45 days of kindling. Abbreviations in this and other figures: EnR, EnL: right and left entorhinal cortex; HVR, HVL: right and left ventral hippocampal formation (in this animal the electrodes were localized in the region of dentate gyrus and subiculum on the right side and in the left hippocampal gyrus); HPR, right posterior hippocampal formation (in this animal CA3 region); AmR, right amygdala (three records obtained with the aid of a four contact electrode covering vertical distance from the ventral limit of the lateral nucleus to the level of globus pallidus).

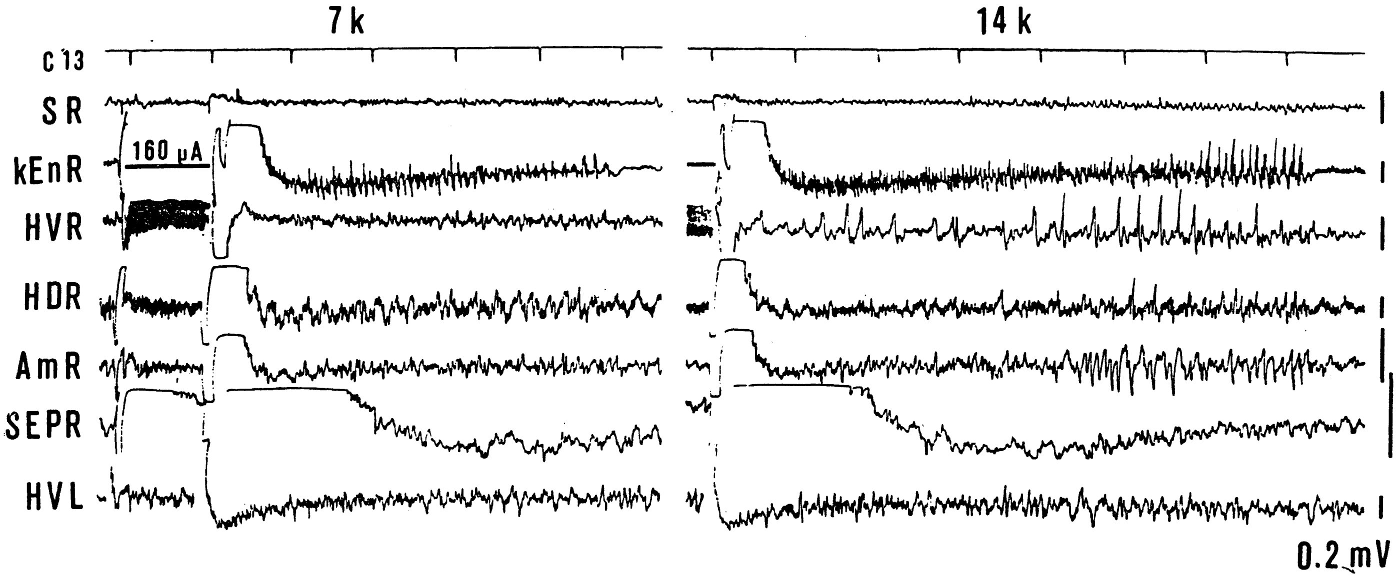

Fig. 3*A*. EEG patterns of after-discharges recorded on the 7th and 14th day of kindling in the cat C13. SR, right sensorimotor cortex; SEPR, right septal region. Time marker indicates 1 s intervals.

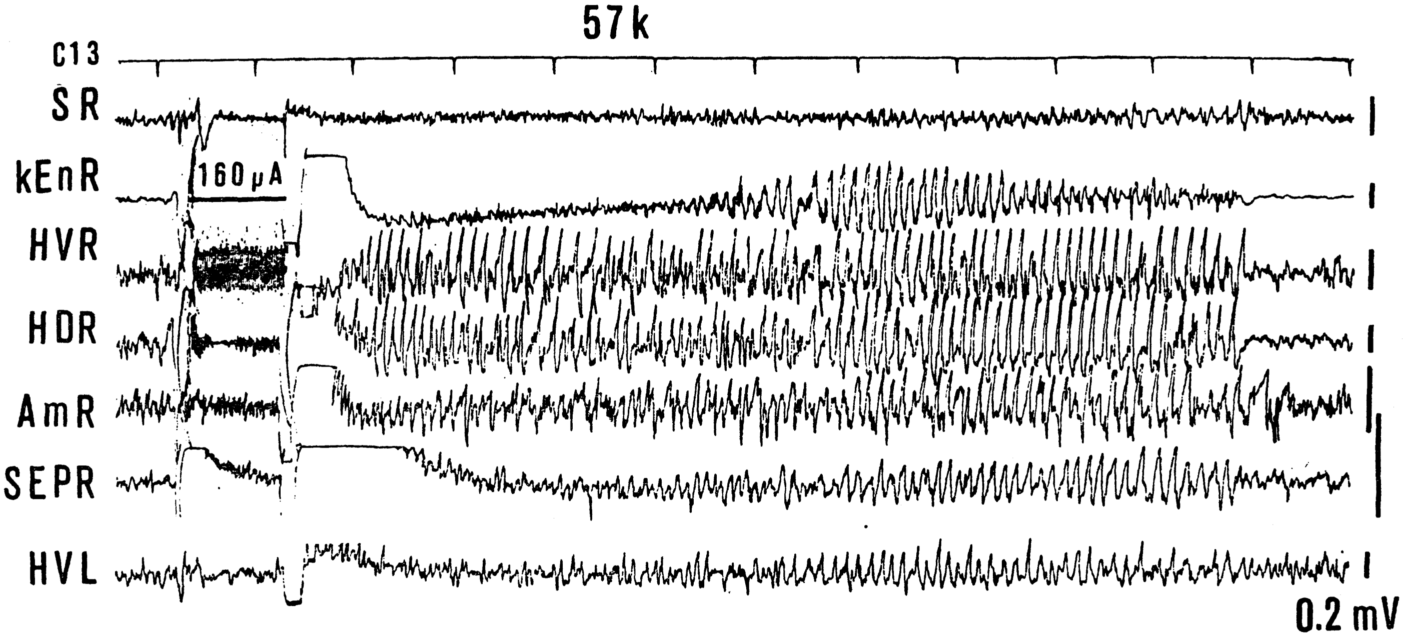

Fig. 3*B*. EEG after-discharge pattern *in* the cat C13 on the 57th day of kindling.

Development of the spontaneous interictal spikes

The appearance of spontaneous interictal spikes (IIS) in EEG record is generally accepted as an indication of the epileptogenic reorganization of neuronal functions. Recognition of the spontaneous interictal spikes at the early stage of kindling required special caution because of the well-known fact that paroxysmal EEG patterns could be readily evoked in the hippocampal formation. The waveforms, which could be accepted as IISs on the basis of follow-up observations, fulfilled most or all of the following criteria: (i) the IISs developed in the course of kindling, that is, differed from the EEG patterns observed during control EEG recording, before kindling; this condition alone was not satisfactory, especially because some IISs seemed to develop from EEG patterns hidden in the background EEG activity during prekindling period; (ii) the IISs included short-lasting components which could not be faithfully reproduced by the EEG pen recorder; (iii) the IISs involved synchronously various regions of the stimulated hippocampal formation or hippocampal formation and ipsilateral amygdala; (iv) high amplitude components of the IISs were clearly isolated from the background EEG activity and (v) the waveforms of the presumed IISs and their distribution observed before electrical kindling stimulation resembled the waveforms immediately following hippocampal stimulation, which were the first indicators of IIS formation.

Detection of the spontaneous interictal spikes in an EEG record made 24 h after preceding kindling stimulation was accepted as a criterion of formed epileptic focus.

The development of spontaneous interictal spikes required 10 to 25 days of kindling (mean = 16.6 days). In the cats C11 and C14, spontaneous IISs were detected on the 18th ad 16th day of kindling respectively. They were random large-amplitude complexes of spikes and slower waves involving ipsilateral hippocampal formation and amygdala.

In the cat C7, the first acceptable indications of spontaneous IISs were observed on the 18th day of kindling. They were of the type illustrated in Fig. 2*B*. The IISs seemed to have developed from the EEG wave pattern involving hippocampal formation and amygdala, hidden in the background EEG activity before kindling, especially during slow wave sleep. Another wave-pattern which developed in a process of kindling was composed of clusters of high frequency spikes (Fig. 2*C*), superimposed usually on slower waves. The IISs, at first infrequent, increased in number after about 60 days of kindling. The frequency of their appearance was at that time close to that presented in Fig. 4. This figure shows distributions of the IISs (of the type presented in Fig. 2*B*) observed before and after stimulation of the hippocampus on kindling days 124-128. At that time stimulation ceased to evoke detectable ADs. This type of IIS distributions is representative of a relatively early stage of epileptogenesis.

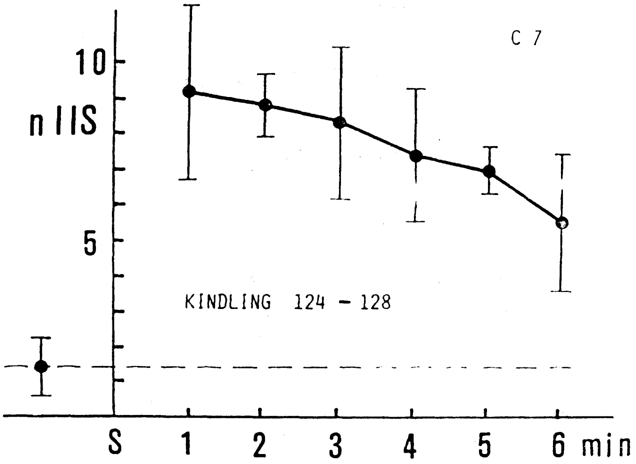

Fig. 4. Distribution of the spontaneous interictal spikes (nIIS) of the type illustrated in the Fig. *2B*, recorded on kindling days 124-128 in the cat C7. Presented are means and standard deviations of the number of spikes before and during 6 min period following electrical stimulation (S).

In the cat C15, random low-amplitude bursts of spikes could be observed in the posterior hippocampal formation already before kindling. A new pattern of kindling-related spikes was detected in the third week of kindling. However, a significant increase of the number of IISs was observed after about 3 months of stimulation.

In the cat C13, irregular bursts of high frequency spikes appeared in the entorhinal cortex during the fourth week of kindling.

In two animals from the group of cats stimulated with AD subthreshold currents, bursts of high-frequency spikes developed in the absence of after-discharges (Fig. 5). In the cat C5, they were detected in the derivation from the hippocampal gyrus of the posterior hippocampal region on the seventh day of kindling, after stimulation of the ventral hippocampal gyrus (hilus of the gyrus dentatus). Spontaneous IISs were found on the 10th day of kindling. The frequency of bursts found on kindling days 10-13 was in the range of 0-11/min; $x_{.50}$ = 3/min (N = 21 min of EEG record). Widespread distribution of the spontaneous spikes in the hippocampal gyrus, entorhinal cortex and amygdala was observed in the same animal on more advanced stages of kindling (Fig. 5, 60 days of stimulation), after development of after-discharges.

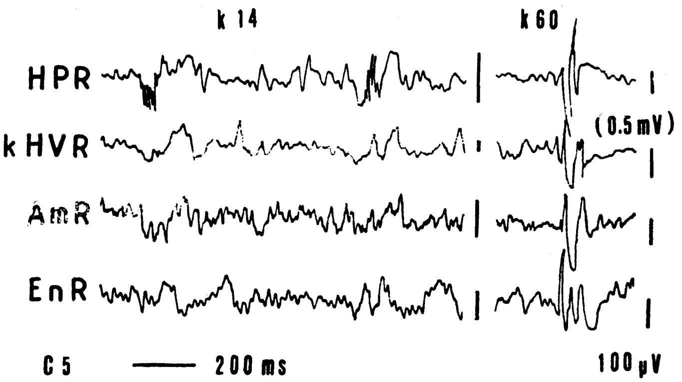

Fig. 5. EEG patterns of the spontaneous interictal spikes recorded on the 14th and 60th day of kindling in the cat C5. 0.5 mV calibration refers only to the EEG record KHVR made before electrical stimulation on the 60th day of kindling.

In the cat C4 from the same subgroup, after 10 days of kindling, spontaneous bursts of high-frequency spikes superimposed on top of the EEG spikes emerged from the background EEG activity recorded from the hippocampal gyrus of the posterior hippocampal formation. After 18 days of kindling, still without detectable after-discharges, spontaneous large-amplitude spikes appeared in the stimulated hippocampal formation. The number of spontaneous interictal spikes (of both types) found on kindling days 16-20 was $x_{.50} = 1$/min (range: 0-4/min, $N = 22$ min of EEG recording).

In general, the appearence of the IISs was state dependent. They were absent during movements, during hippocampal EEG theta activity (except in advanced stages of kindling with intensive spiking), and during REM sleep. They could be observed during quiet waking in animals with desynchronized EEG neocortical activity. Their number increased during quiet wakefulness with the fronto-parietal and occipital EEG spindle activity. They could be also observed during slow wave sleep.

DISCUSSION

Progressive intensification of electrophysiological and behavioral responses in the course of systematic, repeated stimulation of brain structures may be achieved using different stimulation paradigms.

The original procedure of kindling introduced by Goddard et al. (17) required repeated, once daily stimulation, using 1 s train of biphasic electric pulses of 1 ms duration, delivered at a rate of 62.5/s. Stimulus intensity was set below threshold for after-discharges and behavioral responses. The choice of stimulation frequency resulted from the observation that the "number of stimulation trials necessary to elicit the first convulsion decreased as the interval between trials approached 24 hours". It has also been found that the optimal pulse rate was between 60 and 150/s. With this stimulation paradigm the hippocampus was rated the least sensitive to kindling among the other tested limbic structures in rats (17). Criterion of the generalized motor seizures was obtained after 77 days of kindling, while the entorhinal cortex required 37 days of stimulation.

The results of the present investigation in cats confirm a relatively slow development of kindling effects during stimulation of the hippocampal formation. In addition, the results point to yet another fact, namely, that the process of hippocampal kindling in cats must not necessarily end in secondary generalized tonic-clonic seizures.

Development of after-discharges

The example of the cat C13 shows that the after-discharges in hippocampal formation may develop gradually and may stabilize at the stage of medium length ADs and complex partial seizures. In the cat C7, the ADs evoked by initially near-threshold stimuli did not develop into long lasting ADs and behavioral seizures.

The variable efficacy of kindling may result from interaction between enhancement of neuronal excitability and other factors. If we exclude genetically-predisposed seizure susceptibility, these factors are: (i) decreased effectiveness of stimulation due to deterioration of stimulation conditions at the electrode-tissue interface; (ii) possible operation of the endogenous anticonvulsant mechanisms, and (iii) dimensions of the stimulated brain region and position of the stimulating electrode with respect to cellular masses and fiber tracts, activated ortho- and antidromically.

(i) The possibility of the gradual deterioration of stimulating conditions in the cat C7 could not be excluded, since brief ADs and IISs were the only indicators of stimulation efficacy. The evoked potentials were recorded during kindling on the 19th day of stimulation, and, since that time, evoked potential thresholds and impedance of the stimulating electrode were not measured in order to avoid application of additional current pulses and their possible modifying effect on the process of kindling. However, the presence of stable ADs during 120 days of kindling indicated that at that time the component of kindling-related response enhancement was not as strong as in other animals. In the cat C13, the resistance of the stimulating electrode and thresholds for intrahippocampal evoked potentials measured after 200 days of kindling did not exceed the prekindling values, thus indicating stability of stimulating conditions.

(ii) Although the possible operation of the endogenous anticonvulsant processes during kindling is overshadowed by the enhancement of neuronal excitability, a number of reports provide evidence in favor of their presence and complexity of action. The early and delayed effects of post-seizure suppression of subsequent seizures were found in the hippocampally kindled cats (37). Considered is the involvement of the GABAergic mechanisms. Paired-pulse depression in the dentate gyrus after double-pulse stimulation of the perforant path was significantly increased as a result of amygdala and dentate kindling (45). This depression is assumed to be an indication of the GABA-mediated recurrent inhibition of granule cells. Furthermore, kindling, as well as seizures evoked by electroshock, increased the number of hippocampal benzodiazepine receptors (46, 47). Autoradiographic studies demonstrated that increased radioligand binding involved the dentate gyrus: the region of granule cell bodies and dendritic trees (47). Although the results of investigations on endogenous ligands for the benzodiazepine receptors are inconclusive, the interpretation of this finding is that by increasing the number of benzodiazepine receptors kindling and seizures increase rather than decrease GABA-mediated inhibition due to augmentation of the affinity of the GABA-benzodiazepine receptor complex to endogenous GABA.

Other currently considered endogenous anticonvulsant mechanisms involve action of neuropeptides (2), biogenic amines and cyclic nucleosides (11, 14).

(iii) Whereas results of amygdaloid kindling in rats give an impression of great efficacy and reproducibility of the effects in terms of their time course and outcome (in the form of secondary generalized seizures), limbic seizures rather than secondary generalized, tonic-clonic seizures were observed by Wada et al. (48) during amygdaloid kindling in rhesus monkeys. The authors assumed decrease of seizure susceptibility along the phylogenetic scale from rats to monkeys. An alternative hypothesis for the effect of kindling in monkey brain, as proposed by Goddard et al. (17), assumes that kindling requires activation of “a critical mass of tissue which is relative to the size of brain being stimulated”.

The diversity of AD development patterns of the type described in this report points to yet another possible factor determining kindling efficacy: a stimulation selectivity effect. This effect would depend on the type and position of the stimulating electrode with respect to nerve cells and tracts of particular importance for the kindling process. The larger brain size of monkeys and cats in comparison to rats offers a better chance to selectively stimulate particular brain regions. The expression of stimulation selectivity, in association with the other factors, would be the development of spontaneous, focal interictal spikes (alone, or with) and the development of more or less local after-discharges with or without a tendency for generalization, as observed in some types of epilepsy in humans. Such a possibility is supported also by the results of investigations performed on rats and guinea pigs. Racine et al. (34) tested the hypothesis that development of ADs and motor seizures were dependent on increased strength of limbic-limbic connections. In rats, after the stimulation of the hippocampal commissure, a mean number of 2.8 ADs (range 1.8) was required to develop motor seizures in comparison to 23.6 ADs (range 8-42) necessary during stimulation of the posterior hippocampus. In guinea pigs, gradual AD development, intermittent pattern and no AD increase was found during 150 days of amygdala, hippocampal or neocortical kindling (26).

Development of spontaneous interictal spikes

The time necessary for development of IISs of 10 to 25 days (mean = 16.6 days) found in this investigation is comparable to the results obtained in hippocampally kindled cats by Sato (35). Faster rate of IIS development was found during amygdaloid kindling. Wada and Sato (49) reported that in cats spontaneous interictal discharges evoked by once daily stimulation begin to appear between the second and ninth day (mean = 5.3 days) of kindling.

Several factors require special consideration in the search for spontaneous bioelectrical indicators of kindling-related reorganization of hippocampal functions: (i) sharp waves and spikes comparable to the IISs in kindled animals may appear as a result of injury inflicted at the time of implantation (long recovery period after surgery may minimize this possibility); (ii) application of the moving electrodes for recording unitary activity in unanesthetized animals may promote injury discharges, (iii) sharp waves and spikes may represent normal EEG patterns in certain conditions in some species; and v) the animals may belong to seizure prone strains.

Most of the present knowledge on the interictal spikes or so called epileptiform discharges in the hippocampal formation is derived from the experiments on rat brain, in vivo and in tissue slices (1, 9, 12, 13, 16, 21, 33, 39, 41, 43, 44). Characteristics of some hippocampal EEG patterns in rats, considered to be physiological phenomena, meet some of the criteria regarded as typical for epileptic discharges, that is high signal to background EEG ratio and brief duration. These waveforms are: large amplitude sharp waves and clusters of high frequency spikes (6, 8, 43, 44).

Irregular sharp waves in rats may have amplitudes of several milivolts and 30-120 ms duration. They have been recorded from the various parts of the hippocampal formation, including CA3 and CA1 regions of the hippocampal gyrus, gyrus dentatus and subiculum. They appeared at the rate of 0.03-3/s. Their presence coincides with bursts of complex-spike hippocampal cells. It appeared that the sharp waves initiated in CA3 region periodically invaded the entire hippocampal formation (6, 8). Sharp waves in rats have been observed during drinking, quiet sitting, grooming and during slow wave sleep. They never appeared during behaviors accompanied by rhythmic slow EEG activity (6, 8, 43). It was suggested that this type of activity may express operation of the physiological mechanisms responsible for the formation of the long term potentiation effect (7).

The behavior of the sharp waves in rats corresponds in many respects with the behavior of spontaneous interictal spikes observed during the present investigation in cats. However, there were a few exceptions: (i) no IIS frequency increase was observed during grooming, eating and drinking, and (ii) IISs were also observed during hippocampal slow EEG rhythmic activity in cats in advanced stages of kindling.

The results of studies of the spontaneous interictal spikes in rats reported by Lopes da Silva et al. (21) seem to point out to the possibility that some IISs (or their components) could be modified sharp waves. Kindling was performed by electrical stimulation of the dorsal hippocampus, in the region of Schaffer collaterals. The spikes (called by the authors spontaneous interictal transients; SIT) were recorded from the CA1 region. The type I spikes could be observed already during the first week of kindling in a period following electrical stimulation of the hippocampus. They had about 1 mV peak amplitude and 80 ms duration. Their detection sometimes required the application of the averaging procedure. Spikes of the type II could be found around the second week of kindling. They had larger amplitudes and shorter durations (about 20 ms) and could contain population spikes. In comparison to the type I spikes, they showed polarity reversal inndicating that generation of the large depolarizations shifted to the region of cell bodies.

Clusters of high frequency spikes may be recorded both in vivo and in hippocampal tissue slices (1, 9, 16, 39). The recent experiments on tissue slices strengthen the assumption about their origin in the CA3 hippocampal region (18, 39). It was postulated that bioelectric seizures in CA1 cells, in response to CA3 interictal bursts of spikes, could be the main mechanism responsible for the initiation of self-sustained afterdischarges and behavioral seizures (28). Taking into account available evidence, it seems justified to assume that clusters of high frequency spikes described in the present report, developed in the hippocampal gyrus after stimulation with the AD subthreshold currents, expressed unmasking of intrinsic properties of the hippocampal cells as a result of kindling.

There is increasing evidence indicating that there is no simple relation between the development of IISs and after-discharges. Spikes and high-amplitude sharp waves have been observed in a period following electrical stimulation in amygdala kindled rats stimulated with AD subthreshold currents (13). The evidence was presented that IISs may in fact express states of depressed rather than increased excitability (12, 13). In addition, the results of tissue slices experiments suggest that the mechanisms underlying generation of ADs and formation of IISs may be to some extent independent.

Induction of the spontaneous bursts of population spikes in hippocampal tissue slices has been achieved by high potassium, low chloride or absence of the magnesium ions in artificial cerebrospinal fluid, by electrical stimulation, or by pharmacological means (1, 3, 9, 16, 18, 30, 39, 41). The results obtained so far show that the induction of interictal-like spikes and self-sustained, repetitive after-discharges (bioelectric seizures), usually in the CA3 b/c pyramidal cells, required involvement of N-methyl-D-aspartate (NMDA) receptors (1). The two modes of activity: random bursts of spikes and repetitive (ictal-like) evoked or spontaneous discharges of spikes reacted differentially to the NMDA receptor antagonists (1) and to the $GABA_B$ receptor agonist, baclofen (41). APV (2-Amino-5-Phosphonovalerate) blocked stimulus train-induced repetitive discharges of spikes but not random spontaneous bursts after their induction by the NMDA (1). Spontaneous episodes of the repetitive self-sustained (ictal-like) discharges of spikes replaced random spikes after application of baclofen. The authors concluded that the presence of interictal activity may indicate decreased probability of occurrence of bioelectric seizures (41).

Different kindling procedures, kindling rates and possible implications for the development of kindling effects

The interpretation of the experiments on mapping relative seizure sensitivy in rat brain presented by Goddard et al. (17) has been questioned in that it was not stimulation itself but afterdischarges which were critical for the AD and motor seizure development (33). It was also found that the interstimulus intervals as short as 1 hour (36) and stimulation frequencies as low as a fraction of 1 Hz could be very effective in producing escalation of the ADs and seizures. First trial generalized seizures in rats were evoked after stimulation with 2 or 3/s, 20 ms trains of 400/s electric pulses (35).

Prolonged after-discharges and generalized seizures could develop already on the first day (mean = 2.9 days) during once daily stimulation of amygdala in rats, using 60s trains of high intensity, low frequency (0.875-10/s) pulses. The animals were considered fully kindled, since after the development of generalized seizures they responded persistently with generalized seizures to the test trains of 1 s duration of 60/s pulses (10).

Prolonged after-discharges, behavioral seizures and EEG spikes were obtained on the first day of hippocampal stimulation in rats subjected to stimulation procedure with rapidly recurring seizures (22, 24). The animals were stimulated once every 5 min, using 10 s trains of 10/s AD suprathreshold pulses. Daily kindling session lasted usually 3-9 h.

At present there is no ready answer to the question whether appearance of spontaneous IISs and prolonged after-discharges and seizures evoked as a result of fast kindling procedures have the same meaning as IISs and ADs slowly developing during "classical" kindling. It appears that the direct application of electric stimuli to the brain tissue may activate more or less selectively, and on more or less widespread scale different component processes contributing to the overall kindling effect, depending on the electrode localization and stimulation parameters. However, this is the slowly developing response to kindling, which provides straightforward evidence about the initiation as well as consolidation of endogenous processes leading to the epileptogenic reorganization of brain function.

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