Hippocampal evoked field potentials and interictal spikes in hippocampally kindled cats

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Abstract. Kindling–related changes of the hippocampal evoked field potentials and patterns of the spontaneous interictal spikes were investigated in 10 hippocampally kindled cats. A complex potential waveform was recorded by macroelectrodes placed in the CA3 region of the hippocampal gyrus and hilus of the gyrus dentatus, close to the granule cell layer, after stimulation of the entorhinal cortex. After high intensity repetitive (10/s) stimulation a late component could be recorded with the latency of about 30–40 ms, in addition to the early response originating in the gyrus dentatus. Probably this component developed during kindling into a delayed, high amplitude spike. After application of the double shock test, post–stimulus facilitation of the spike response was observed within time limits of 20–100 ms. Another observation was a widespread, ipsilateral and bilateral long–term enhancement of the amplitudes of field potentials evoked by entorhinal and intrahippocampal stimulation. It was the most common effect observed during kindling. Widespread synchronized discharges of hippocampal spikes and localized clusters of brief irregular spikes were the most significant features of spontaneous interictal spikes. The paroxysmal discharges of spikes could be evoked by ipsi or by contralateral stimulation of the afferent pathways projecting to the kindled hippocampus, rather than by direct electrical stimulation of the kindled hippocampal gyrus.

Key words: kindling, evoked potentials, waveforms, components, after–discharges, interictal spikes
INTRODUCTION

The evidence on long-term potentiation of synaptic transmission (LTP) is rapidly accumulating (Bliss Lynch 1988, Racine and de Jonge 1988). However, it is not clear what is exactly the cause of transition from the normal patterns of electric activity of cellular populations into paroxysmal discharges characteristic of a formed epileptic focus developed in a process of kindling. The term kindling refers to epileptogenic effects of prolonged, systematic electrical stimulation of neuronal structures using weak, originally ineffective currents, resulting in the development of pathological patterns of spontaneous bioelectric activity, development of after-discharges (AD) and appearance of the epileptic seizures.

The recent evidence shows that the LTP by itself can not be responsible for the development of kindling effect (Racine et al. 1983, Racine and de Jonge 1988). After initial growth the LTP may even decline on advanced stages of kindling. There is also as yet no conclusive evidence that the kindling effect is mediated through impairment of inhibitory processes. On the contrary, in the experimental model of hippocampal epileptogenesis, at last in the region of gyrus dentatus, an increase of the GABA mediated inhibition was reported as a result of kindling. Significant increase of the number of benzodiazepine receptors was found in the hippocampus after dentate kindling in rats (Tuff et al. 1983). The evidence was presented in another study on rats that benzodiazepine receptors were localized in the region of cell bodies and dendritic trees of the granule cells in the gyrus dentatus (Valdes et al. 1982). Furthermore, a paradoxical increase of the number of GABAergic cells was observed in the gyrus dentatus and CA3 hippocampal region in seizure sensitive gerbils in comparison to seizure-resistant animals (Peterson and Ribak 1987).

Also results of the electrophysiological tests indicate a possible increase of GABA-mediated inhibition as a result of kindling. Double pulse stimulation of the perforant path and recording differences in the evoked field potentials in the gyrus dentatus as a measure of excitability changes have shown an increased paired pulse depression in cats after amygdalar kindling (Tuff et al. 1983).

In this research, to further test the alterations of functional properties of hippocampal cellular populations, evoked field potentials were recorded during hippocampal kindling. The attention was focused upon: (1) reorganization of the evoked potential patterns in the transverse (lamellar) neuronal circuit activated by stimulation of the entorhinal cortex; (2) hippocampal responses mediated presumably by the longitudinal system of association fibres, and (3) in the patterns of spontaneous interictal spikes (IISs). EEG manifestations of the development of kindling effects on cats during hippocampal kindling were described in another paper (Sobieszek 1989).

MATERIAL AND METHODS

The experiments were performed on 10 adult, male and female cats in a process of hippocampal kindling. Each cat had permanently implanted electrodes for stimulation and recording. Implantation was performed under pentobarbital anaesthesia, 40 mg/kg, i.p. The electrodes were made of straight stainless steel wires of 300–400 μm in diameter. The wires were insulated except at the conically sharpened tips. Two to five wires glued together formed a bipolar or multipolar electrodes. The 400 μm wires were used in some bipolar, subcortical electrodes. The vertical distances between uninsulated tips of the electrodes were in the range of 1–3 mm. The electrodes were implanted in a conventional way into various limbic and neocortical structures in both hemispheres using stereotaxic coordinates of Snider and Niemer (1964). Reference electrode for unipolar recording was implanted into the bone overlying the frontal sinus. The electrodes were soldered to a 20 pin miniature connector and fixed to the skull with dental acrylic.

After the implantation and recovery from surgery (at least 1 month), the animals were subjected to electrical kindling in various regions of the hippocampal formation. After Lorente de Nó (1934), the entorhinal cortex is regarded in this study as part of the hippocampal formation.

Kindling was performed by 1 s train of 50/s electric pulses applied once daily. The intensities of stimulating currents differed in different animals. They were either sufficient to produce initially only local, a few seconds long after–discharges, or were set at near threshold or subthreshold intensity. The details of implantation and kindling procedures are described elsewhere (Sobieszek 1989). The localization of electrodes was verified histologically after the completion of the experiments. The 50 μm coronal brain sections were stained according to Klüver–Barrera method.
Evoked potentials were recorded with the aid of a recording system composed of the AC wide band amplifiers (band pass: 0.5–10 kHz), average response computer ANOPS 10 and XY plotter. Stimulating system included programmed square pulse generators and constant current, battery powered, optically coupled stimulus isolation units with output resistance of 1 Megohm. The background EEG activity was monitored during recording sessions on an oscilloscope monitor screen.

RESULTS

Three classes of the hippocampal field potentials were tested: hippocampal responses to entorhinal stimulation, hippocampal responses to intrahippocampal stimulation and spontaneous interictal spikes. The experiments were performed on three subgroups of animals.

Effects of kindling on hippocampal field potentials evoked by entorhinal stimulation

The effects of hippocampal kindling on hippocampal evoked field potentials elicited by stimulation of the entorhinal cortex were tested in four animals. The patterns of evoked potentials present Fig. 1, 2 and 3.

Figure 1A illustrates the relation between the composition of the hippocampal field potentials and changes of stimulation parameters: different intensities of stimulating currents and application of repetitive (10/s) vs. pseudorandom stimulation. The intensity of stimulation was expressed as multiples of threshold currents sufficient to produce reliable hippocampal evoked potentials. With the increase of the stimulus intensity to 2 times threshold current and after the application of repetitive, 10/s stimulation, the late component could be observed with the latency of about 25–30 ms in addition to the early components present during low intensity stimulation.

Fig. 1A Relation between patterns of the average evoked field potentials (n = 32 sweeps) recorded from the left ventral hippocampus and parameters of entorhinal stimulation in cat C2. Abbreviations in this and other figures: ENL, left entorhinal cortex; HVL, left ventral hippocampus; T, threshold of the early biphasic response; PR, pseudorandom stimulation. B. Superimposed individual evoked potentials recorded from ventral hippocampus before kindling (control) and after reaching the kindling stage characterized by prolonged after-discharges and secondary generalized seizures (kindling) in cat C5.
Complex waveforms containing five distinct components were observed after 3.5 times threshold stimulation of the lateral entorhinal cortex in another animal (Fig. 1B). The localization of recording and stimulating electrodes in this animal was comparable to that shown in Fig. 1A, that is in the CA3, CA4 hippocampal subfields in close vicinity of the granular cell layer in the gyrus dentatus. Application of the double pulse stimulation disclosed another property of this response, namely, a changed pattern of the field potential evoked by the second stimulus in control (prekindling) conditions. Small component of the early potential (component 2) recorded at the latency of about 20 ms after the application of the first stimulus was replaced by a high amplitude spike in the second response. The fourth component (latency about 30 ms), most pronounced in the first response, lost its distinctiveness in the second evoked potential. These properties were lost in records made after kindling had reached the stage of prolonged after-discharges and secondary generalized, tonic–clonic seizures. Stimulation of the entorhinal cortex at that stage using the same stimulus parameters showed a consistent reorganization of the evoked response pattern. Namely, the basic patterns of the first and second evoked potential became similar. In both responses the early biphasic wave was followed by spiky component with the latency of about 30–40 ms.

Figure 2 illustrates kindling–related reorganization of the evoked potential patterns in another animal, during monopolar recording against a frontal reference electrode. The average evoked potentials were recorded from the ventral (kindled) and posterior (ipsilateral) regions of the hippocampal gyrus, amygdala and from the contralateral (symmetrical) ventral hippocampus. After 19 days of stimulation in the region of angular bundle, ipsi- and contralateral increase of the amplitudes of evoked potentials was observed with the development of late spike delayed by about 30 ms in the ventral hippocampus.

In the animal in which the recording electrode was localized in the medial part of the gyrus dentatus, the early biphasic wave complex observed after entorhinal stimulation was not followed by the delayed spiky component.
Figure 3 presents the results of analysis of the hippocampal response to entorhinal stimulation in cat C2 after the development of prolonged after-discharges and partial complex seizures in advanced stages of kindling. Part A illustrates superimposed individual potentials recorded from the hippocampal gyrus evoked by stimulation of the entorhinal cortex (localization of the electrodes shown in Fig. 1A) using pulse pairs with the interstimulus interval of 35 ms. Application of this test showed the existence of the frequency potentiation effect, influencing both the early and late components of the evoked potential. Excitability curves for both components expressed by the ratio of their amplitudes (A2/A1 for the early component and A3/A2 for the delayed spike) as a function of interstimulus intervals are presented in Fig. 3B. The components A2 and A3 were used for estimation of the frequency potentiation of the delayed spike, since the amplitude of this spike evoked by the first stimulus could not be measured accurately at short interstimulus intervals. The intervals were within the range of 20–200 ms. The most effective intervals were in the range of 30–100 ms with the maximum effectiveness for the delayed spike (A3/A2) shifted toward shorter intervals (about 30 ms). Figure 3C shows a delayed spike in the right ventral hippocampal field potentials recorded from the left entorhinal cortex and right ventral hippocampus elicited by stimulation of the contralateral (left) ventral hippocampus. D, Superimposed individual field potentials evoked in the left ventral hippocampus by repetitive, 10 s stimulation of the ipsilateral entorhinal cortex. The responses were recorded during 30 min period following prolonged (about 7 s) after-discharge and partial complex seizures evoked by kindling stimulation of the right posterior hippocampus.
gyrus and field potentials in the left entorhinal cortex evoked by stimulation of the left hippocampal gyrus. Kindling in this animal was performed by stimulation of the right posterior hippocampal region. Figure 3D presents superimposed individual records of the hippocampal field potentials evoked by trains of 10/s pulses applied to the ipsilateral entorhinal cortex during the postseizure period. Enhancement of this response was observed after prolonged after-discharges and partial complex seizures evoked by kindling stimulation of the contralateral posterior hippocampal region.

Effects of kindling on hippocampal field potentials evoked by intrahippocampal stimulation

Evoked field potentials elicited by intrahippocampal stimulation were tested in four cats. In all of them the potentials were composed of the early and late components, delayed by about 30 ms. This late component showed evident signs of frequency potentiation. However, augmentation could be also observed after prolonged, repeated stimulation with pseudorandom pulses (varying interstimulus intervals: 0.3–10 s).
Figure 4A shows average evoked potentials elicited by pseudorandom and 10/s stimulation, as well as localization of the stimulating and recording electrode (posterior hippocampal region) in the kindled cat C2. The incremental type of responses of the delayed component after repetitive stimulation illustrates superimposed individual records in Fig. 4B (before AD). Transient suppression of this component was observed after prolonged after-discharges (lasting about 70 s) and partial complex seizures.

Figure 5 illustrates evolution of the evoked potentials in a process of kindling in two animals stimulated with after-discharge subthreshold currents. Figure 5A illustrates the effect of kindling on composition of the evoked potentials recorded from symmetrical, right and left ventral hippocampal regions after 10/s stimulation of the right posterior hippocampus. Evoked potentials recorded after 47 days of kindling stimulation of the posterior hippocampus showed an increase of amplitudes and formation of the delayed components, particularly in the right hippocampus. Similar effect was observed in the posterior hippocampus and entorhinal cortex in another animal after ipsilateral kindling stimulation of the ventral hippocampus (Fig. 5B).

**Interictal spikes**

The patterns of the spontaneous interictal spikes (IISs) were analyzed in four animals with long-lasting after-discharges and partial complex seizures in advanced stages of kindling. Their waveforms were recorded on an XY plotter after preliminary inspection on the oscilloscope monitor screen.

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**Fig. 5A, average evoked field potentials (n = 64) recorded from symmetrical regions of the right and left ventral hippocampal regions evoked by 10 / s stimulation of the right posterior hippocampus using current pulses of 160 μA intensity. They were recorded before (control) and after 47 days of after-discharge subthreshold kindling in the right posterior hippocampus.**

**Fig. 5B, average evoked field potentials recorded form the right posterior hippocampus and entorhinal cortex after 10 / s stimulation of the ipsilateral ventral hippocampus. The potentials were recorded before (control) and after 20 days of kindling in the right ventral hippocampus.**
The individual IIS waveforms differed greatly in their details. However, some of their features: distribution and temporal relation between components showed a relative stability in a particular animal. This stability could be lost in brain states characterized by disorganization of background EEG activity e.g. during postsiezure period following long-lasting after-discharges. Figure 6 and 7 (on the left side) present graphically superimposed individual waveshapes of the IISs recorded from two cats (C7 and C12) kindled in the region of hippocampal gyrus. The zero time reference point was chosen at the onset of the raising phase of the most stable IIS waveform selected from the collection of spontaneous IIS transients recorded simultaneously from different hippocampal derivations. These particular waveforms are indicated on the graphs by arrowheads.

The Fig. 6 presents the IIS patterns recorded after 210 days of kindling in cat C7. Kindling in this animal was first performed in the right ventral hippocampus and later in the symmetrical region of the left hemisphere. The presented waveforms are representative for a class of IISs triggered by the spike discharge in the left hippocampal gyrus. Population spikes of about 5 ms duration may be also observed superimposed on slower waves in the right hemisphere.

In another animal, a 10 ms spike originating in the entorhinal cortex initiated IISs recorded from the ipsilateral ventral and posterior hippocampal gyrus with a delay of 5–10 ms.

In two other animals the first signs of IIS formation were clusters of irregular spikes superimposed on slower waves.

Figure 7 presents "average" IIS recorded in cat C12 with a single epileptic focus. Stimulating electrode was localized in this animal in the right ventral hippocampal gyrus and gyrus dentatus. However, the IISs: clusters of irregular spikes could be first observed in the posterior hippocampal region. After delays of several to about 20 ms, the discharges of population spikes and slower waves were observed in the ipsilateral (kindled) ventral hippocampus, entorhinal cortex, amygdala and in the contralateral hippocampal gyrus. The superimposed traces are representative for a sample of 50 traces observed during quiet wakefulness and slow wave sleep. It can be seen that the latencies of evoked field potentials (right side of Fig. 7) elicited by stimulation of the posterior hippocampus are comparable with the latencies of early components of the IISs in relation to spikes first recorded in the posterior hippocampal region. However, the waveforms of evoked potentials did not resemble in all respects the naturally occurring IISs, indicating that the IISs and evoked potentials may have resulted in part from the activation of different transmission pathways.

It is also interesting to note that the IISs recorded from the amygdala were in all animals delayed with respect to hippocampal discharges of spikes.

Figure 7 illustrates yet another finding, namely, that the direct electrical stimulation of kindled hippocampal region did not produce waveforms resembling discharges of real interictal spikes. On the contrary, paroxysmal responses could be obtained by stimulation of brain regions projecting to the kindled hippocampal region. An illustration of this finding is Fig. 8. Double pulse electrical stimulation of the left (contralateral) hippocampal gyrus in cat C12 (45 ms interstimulus interval) evoked giant population spikes and fast (about 30/s) oscillations of waves recorded simultaneously from the hippocampal and dental gyri of the ventral and posterior hippocampal regions on the right (kindled) side.
DISCUSSION

Morphological as well as electrophysiological evidence clearly shows that the hippocampal formation is, basically, designed as an ensemble of transverse neuronal circuits composed of granular cells in the gyrus dentatus, hippocampal pyramidal cells and subicular neurons (lamellar system) arranged along the septo-temporal hippocampal axis (Andersen et al. 1971, Andersen 1975). These transverse circuits are interconnected by the intrahippocampal longitudinal association fibres, originating in the hilar and CA3 hippocampal regions (Berger et al. 1980, Laurberg and Sorensen 1981, Swanson et al. 1978, 1980). The transverse systems receive topographically organized afferents from different regions of the entorhinal cortex (Andersen 1971, Lopes da Silva et al. 1985, Room and Groenewegen 1986). Consequently, local as well as widespread electrophysiological effects may be observed after electrical stimulation of the hippocampus. The effects may be immediate or delayed: long-term potentiation of synaptic transmission, long-term depression (Bliss and Lynch 1988, Racine et al. 1983, Racine and Milgram 1983, Racine and de Jonge 1988) and the kindling effect (Goddard 1969).

Fig. 7. IIS, superimposed waveforms of the spontaneous interictal spikes recorded in cat C12 after 160 days of kindling. AEP, average evoked field potentials (n=16) evoked by 3 times threshold pseudorandom stimulation of the posterior hippocampus.
Kindling–related modifications of the hippocampal field potentials evoked by entorhinal stimulation

A complex evoked potential waveform of 40–50 ms duration may be recorded by a macroelectrode located in the CA3/CA4, dentate hippocampal regions, during entorhinal stimulation. The late components are especially well developed after repetitive stimulation. In may be assumed that this waveform reflects the activation of the polysynaptic neuronal chain including granular cells and CA4, CA3 and possibly CA1 pyramidal cells. The phenomena of frequency and post–tetanic potentiation of synaptic transmission along this pathway are very well known (Andersen 1975, Bliss and Lynch 1988, Racine and Milgran 1983, Racine et al. 1983). The early component apparently included population excitatory postsynaptic potentials elicited in granular cells by perforant path discharge. However, the recording technique used in this study did not allow a precise determination of the origin of late components. Besides, the transmission time of 15–20 ms along the whole hippocampal three or four synaptic pathways was found in rats, rabbits and guinea pigs. The transfer of excitation from the region...
of perforant path to the CA1 cells (trisynaptic excitation) in unanesthetized rats required about 15 ms (Herreras et al. 1987). The 20 ms latency of the CA1 response was observed in rabbits anaesthetized with a mixture of urethane and chloralose (Andersen et al. 1971). The latencies of subicular responses as long as 20 ms were found in guinea pigs anaesthetized with sodium pentobarbitone (Bartesaghi and Gessi 1986). The longer duration of the evoked hippocampal field potential in cats could be attributed to the greater dimension of the cat's brain. In that case the late spikes would reflect synchronized discharges of pyramidal cells in the hippocampal gyrus. The alternative hypothesis would be reactivation of the lamellar circuit by the discharge of CA3 neurons and subsequent excitation of entorhinal and dentate cells (Deadwyler et al. 1975, Hjorth-Simonsen 1971) or due to the activation of a more complex pathway including CA1 and subicular cells (Bartesaghi and Gessi 1986, Van Groen and Lopes da Silva 1986, Van Groen et al. 1986). Operation of a hippocampal neuronal feed–back system could offer still another possibility for the generation of late components. The existence of some kind of feed–back mechanism was suggested by Hablitz (Hablitz 1984) as an explanation of reverberation of picROTOXIN induced bursts of spikes between the CA3 and CA1 subfields in guinea pig hippocampal slices. Also post–excitation excitability changes, interhemispheric transfer of impulses or response to some unidentified afferent system should be considered as possible sources of the delayed spikes.

The differentiation of evoked potential components and development of late hippocampal spikes as a result of kindling suggest progressive strengthening of the mechanisms responsible for the synchronization of cellular discharges in addition to the widespread long–term enhancement of evoked responses. The kindling–related development of population spike in the CA1 pyramidal cells was described in rats. Kindling was performed by stimulation of the Schaffer collaterals (Kamphuis et al. 1988).

Activation of a polysynaptic transverse hippocampal circuit may be one of the possible mechanisms responsible for the appearance of repetitive discharges of spikes during after–discharges, or the existence of late evoked potentials described by different authors. The late hippocampal spikes were observed by Racine et al. (1983) after fornix/fimbria stimulation in kindled rats. Steriade (1964) observed late components of the hippocampal evoked potentials in cats during repetitive stimulation of the amygdala, leading to the development of after–discharges. Kindling–related augmentation of the five– component evoked potential recorded from the CA1 hippocampal region in rats after stimulation of the ipsilateral amygdala was described by Racine et al. in another paper (1975). Morphological as well as electrophysiological investigations show that entorhinal cortex and subiculum are the target structures of amygdaloid afferents (Finch et al. 1986, Price 1981).

Sequential activation of the neuronal populations within a lamellar circuit may possibly be responsible for the appearance of episodes of double or triple spikes observed during some (especially dysrhythmic) EEG types of hippocampal after–discharges (Majkowski et al. 1989).

**Modifications of the hippocampal field potentials evoked by intra hippocampal stimulation**

The development of new components or enhancement of already existing responses were observed in this research in hippocampally kindled cats after intrahippocampal stimulation. The origin of these components is unclear. They may have appeared as a result of long term potentiation of synaptic transmission in the ipsilateral associational pathways originating in the hilar and CA3 hippocampal regions (Bartesaghi et al. 1983, Berger et al. 1980, Laurberg and Sorensen 1981, Swanson et al. 1978, 1980). Reactivation of the transverse hippocampal circuits by hippocampal afferents to the entorhinal cortex (Deadwyler et al. 1975) would be another possibility.

Transient suppression of this type of response observed in one animal after prolonged after–discharges and partial complex seizures presents a potentially useful information for further studies of the relation between functional states of the hippocampal formation and learning. Impairment of learning of the conditioned avoidance response in cats after kindled prolonged after– discharges and seizures was described in another paper (Sobieszak and Majkowski 1988).

**Interictal spikes**

Three groups of components seemed to contribute to the different patterns of the spontaneous hippocampal interictal spikes. These were: (1) clusters of localized, brief asynchronous spikes (Fig. 7, HPR), (2) more or less widespread, synchronous discharges of high amplitude spikes, and (3) random components.
(1) The appearance of clusters of spikes is a common observation under certain conditions in tissue slices and may be observed in vivo in cats during hippocampal kindling (Sobieszek 1989). Results of tissue slices experiments show that hippocampal pyramidal cells are especially prone to cluster firing, predominantly in the CA3 hippocampal subfield.

(2) It may be assumed that widespread, synchronized discharges of high amplitude spikes result from direct or indirect synchronous activation of cellular populations with widespread projections. The best candidates appear to be pyramidal cells of the hippocampal gyrus, especially CA3 subfield. Increased tendency of the nerve cells to fire synchronously, amplified by the widespread long-term potentiation of synaptic transmission observed during kindling, could be responsible for the appearance of both: giant population spikes. This types of activity may well express the augmentation of the physiological mode of action of the pyramidal cells, responsible for the high content of sharp waves observed in hippocampal EEG in certain functional brain states (Buzsaki 1986, Suzuki and Smith 1987).

The results of this research show (Fig. 6) that widespread IISs recorded almost simultaneously from different regions of the hippocampal formation in one hemisphere may also result from activation from the contralateral hippocampus with an independent epileptic focus.

(3) It may be assumed that the instability of IIS waveforms may result from the interaction of many factors. The degree of regional (focal) excitatory drive, as well as responsiveness of distant cellular populations, may be state dependent in addition to differences of excitability observed in different stages of kindling. It could be expected that the possible factors influencing the IIS wave shapes are common with the factors influencing patterns of the hippocampal field potentials, discussed in the first two paragraphs.

The finding that the interictal spikes recorded from the amygdala were always delayed with respect to hippocampal spikes does not support the hypothesis about amygdaloid dominance during the formation of interictal spikes (Kairiss et al. 1984).

Altogether, the results point to the increased tendency toward synchronized cellular activity as one of the main effects of kindling.

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