EEG AFTERDISCHARGE PATTERNS AND PERFORMANCE OF THE AVOIDANCE RESPONSE IN HIPPOCAMPALLY KINDLED CATS

J. MAJKOWSKI, A. SOBIESZEK and E. DŁAWICHOWSKA

Department of Neurology and Epileptology, Medical Center for Postgraduate Education
231 Czerniakowska Str., 00-416 Warsaw, Poland

Key words: kindling, afterdischarge, EEG pattern, avoidance response, performance

Abstract. The relation between EEG limbic afterdischarge patterns and the performance of avoidance response was studied in eight hippocampally kindled cats. Five basic types of EEG afterdischarge patterns were identified in the hippocampal formation: (i) continuous, 2-4/s high amplitude spikes or spike and wave complexes; (ii) continuous, 4.5-12/s high amplitude spikes or spike and wave complexes; (iii) dysrhythmic pattern: disorganized irregular spiking, mixed with slow waves at 3-20/s frequency; (iv) continuous, 20-30/s comb-like shaped spiking of moderate amplitude, and (v) trains of high amplitude and frequency irregular spikes, lasting for 0.2-1 s. followed by EEG depression lasting 0.3-1 s. The performance of conditioned response was tested during first 10 s, of afterdischarge development after kindling electrical stimulation of the hippocampus. A statistically significant relation was observed between the EEG afterdischarge patterns seen at the beginning of afterdischarge formation (basically types I, II and III) and the performance of avoidance response, with greatest probability of the response failure with type III of afterdischarge pattern. Latencies of the avoidance responses were prolonged during afterdischarges, in comparison to latencies observed before and after kindling hippocampal stimulation.
INTRODUCTION

The increase of reaction time and the lack of responsiveness are common facts which may be observed during 3/s spike and wave EEG generalized discharges associated with the absence type of primary generalized nonconvulsive epilepsy in humans (2-4, 7, 12). Also hippocampal stimulation, below or at the afterdischarge (AD) threshold may result in an impairment of human performance on sensitive tests (6, 8). These clinical observations have been recently confirmed using animal models of epilepsy. In the feline penicillin model of absence type seizures associated with generalized spike and wave discharges, the performance of the operant reflex was selectively impaired (5, 14). In the hippocampal animal models of partial epilepsy indications of the long term memory formation deficit was reported after intra-hippocampal tetanus toxine injection (1, 11) or as a result of hippocampal kindling (13). In all of these studies the duration of epileptic discharges, spontaneous or provoked, more or less generalized, was the main feature taken into consideration. No effort was undertaken to correlate the types of EEG patterns with the performance of learned responses.

In our previous comparative studies of kindling in the hippocampus, amygdala and neo-cortical structures of guinea pigs, four different types of EEG afterdischarge patterns have been identified, occurring during ADS in the hippocampal formation only (9). However, in these studies the relation between EEG patterns and learned responses was not studied. Later on, an additional fifth pattern called dysrhythmia was identified.

The purpose of this study was to look for a possible relation between a) the types of the EEG patterns occurring during ADS in hippocampal kindling and the performance of the previously established conditioned response to the conditioning stimulus presented during different EEG patterns of the ADS, and b) the duration of hippocampal ADS and the latency of the avoidance response.

MATERIAL AND METHODS

The experiments were performed on 8 adult cats, male and female. The animals were subjected to the following experimental procedures described in more detail in the previous papers (10, 13): i) formation of the avoidance response (AVR), (ii) implantation of wire cortical and depth electrodes, (iii) hippocampal kindling, (iv) memory retrieval of the AVR in kindled animals.

Conditioning. The AVR was formed to trains of 4/s clicks, lasting 5 s. They served as conditioning stimuli (CS). As unconditioned stimuli
(US) 2 s trains of electric impulses, isorhythmic with the 4/s clicks were applied to the left forepaw after 5 s CS presentation. Current intensity of the US was individually adjusted to produce vigorous paw withdrawal without generalized escape reaction. The cats were trained to pull a microswitch connected by tape to the left forepaw, and to avoid electric shocks. However, when there was no proper reaction within the 5 s of CS presentation, and the US was on, the shocks could not be stopped by pulling. Thus, the US was avoidable but not escapable.

The animals during conditioning were placed in a hammock which allowed them to move freely within a limited area. During one session, which lasted for about 30 min, 20 trials were performed. Six daily sessions in a week were performed. The AVR criterion was 85% of correct responses to the CS during 3 successive sessions.

Implantation. After the AVR criterion was established, wire electrodes were implanted for bipolar EEG recording in the left and right cortical areas (somato-sensory, visual or auditory) and stereotaxically into the right and left ventral and dorsal hippocampi, amygdalae, and mesencephalic reticular formation. The depth electrode in the right hippocampus was used also for kindling stimulation. The implantation was performed under Nembutal anesthesia, 40 mg/kg body weight, i.p. About 1 month after implantation, kindling was started.

Hippocampal kindling at the threshold for ADs was performed by daily electrical stimulation of the right ventral hippocampal formation. For electrical stimulation 1 s train of 50/s rectangular mono or biphasic pulses of 1 ms duration were used. Current intensities were adjusted individually to produce local AD of a few second duration, and varied from 0.16 to about 1 mA.

Once the epileptic focus in the right hippocampus was formed, as judged by spontaneous spiking, the CSs were presented before, during and after the ADs. The presentation of CS during AD was aimed at different EEG patterns. The data presented in this paper include the results of tests performed during the first 10 s of AD duration. The reason for this was that the onset of the CS was delayed 2-3 s with respect to the termination of the 1 s train of kindling electric pulses applied to the hippocampus.

For the analysis of AVR latencies in relation to AD durations, comparisons were made between trials immediately preceding and following the kindling stimulation.

A statistical analysis of reflex performance and EEG data during different EEG afterdischarge patterns was performed using analysis of variance, chi square and Kolmogorov-Smirnov tests.
RESULTS

Types of EEG afterdischarges. Five types of the EEG patterns of hippocampal ADs were identified and analyzed in relation to the responsiveness to conditioning stimuli (Fig. 1A and B):

I: continuous high amplitude spikes or spike and wave complexes at 2-4/s frequency;
II: continuous high amplitude spikes or spike and wave complexes at 4.5-12/s;
III: disorganized irregular spiking activity mixed with slow waves at frequencies 3-20/s (dysrhythmic pattern);
IV: continuous high frequency, regular, comb-like shaped spiking at 20-30/s and of moderate amplitude;
V: trains of high amplitude and frequency irregular spikes lasting for 0.2-1 s and followed by EEG depression lasting 0.3-1 s. This intermittent type of activity looks like an alternating pattern.

The EEG patterns were related to the duration of ADs, which usually increased with kindling. At the beginning of the hippocampal kindling usually the patterns of type I and II were observed. As the kindling proceeded, type III, IV and finally V appeared. Type V occurred at the end of ADs.

The occurrence and duration of a given type of the EEG pattern varied. Moreover, during one AD each type could reappear 2-3 times in variable sequence. This observation could be applied to types II-V. For identification of a given type of the EEG pattern at least 1 s of its activity was required. These types of the EEG afterdischarge patterns could be, to some extent, seen in other than hippocampal brain regions, however greatly modified.

Figure 2 presents the distribution of percent values of each type of the AD EEG patterns in the overall AD composition in the two groups of afterdischarges: shorter and longer than 10 s. The two distributions differ significantly (analysis of variance, $F (df 4) = 14.2, P < 0.001$).

Effects of afterdischarges on AVR performance. From 8 cats 537 ADs were analyzed in relation to the animal’s behavior and responsiveness to the CS presentation. The ADs were analyzed according to their duration: (i) shorter or equal to 10 s ($n = 228$ ADs) and (ii) longer than 10 s ($n = 309$ ADs). The total duration of the analyzed ADs was 7455 s.

During short lasting ADs (group 1) the animals remained motionless or sometimes displayed a head turn to the side opposite to the stimulated hemisphere. With increasing AD duration additional signs developed, including: twitches of the facial muscles (at first contralateral), dilatation of pupils, salivation, jaw movements, swallowing, gradually more and
Fig. 1. Examples of EEG afterdischarge patterns I, II, III (Fig. 1A) and IV, V (Fig. 1B) in a hippocampally kindled cat. Abbreviations: SR, right somato-motor cortex; EnR, right entorhinal cortex; HVR, HVL, right and left ventral hippocampus; HPR, right posterior hippocampus; AmR, right amygdala.
more evident body jerks, urination and defecation. Vocalization and violent cleaning reaction were observed after the termination of seizures. Massive body jerks (secondary generalized clonic seizures), urination and defecation usually were observed during longer lasting partial seizures.

The distribution of the correct conditioned responses and failures to the CS presentation in relation to the EEG patterns is shown in Table I. AD types indicated in Table I are those seen at the beginning of AD.

![AD Patterns](image)

Fig. 2. Distributions of the hippocampal EEG afterdischarge patterns (percent values) in two groups of afterdischarges: shorter and longer than 10 s duration.

For that reason only the first three EEG types are included, since, basically, types IV and especially V were observed in later stages of prolonged ADS, which were not analysed in this study. It can be seen that the number of failures evidently increases from the AD EEG type I to III. The likelihood of the presence of an avoidance response in relation to the particular EEG afterdischarge type is significant at the $P < 0.01$ level, $x^2(df 2) = 11.3$.

<table>
<thead>
<tr>
<th>AD type</th>
<th>Conditioned response present $(n)$</th>
<th>No response $(n)$</th>
<th>Ratio of response failures to correct responses</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>16</td>
<td>22</td>
<td>1.4</td>
</tr>
<tr>
<td>II</td>
<td>58</td>
<td>165</td>
<td>2.8</td>
</tr>
<tr>
<td>III</td>
<td>42</td>
<td>186</td>
<td>4.4</td>
</tr>
</tbody>
</table>

Significance level $P < 0.01; \ x^2 = 11.3; \ df = 2$
An example of the AVR performance during EEG afterdischarge pattern type II of 8 s duration is presented in Fig. 3.

**Fig. 3.** Performance of AVR during afterdischarges of type I evoked by stimulation of the right ventral hippocampus, using current intensity of 190 μA. Abbreviations as in Fig. 1, and AR, auditory cortex; HDR, right dorsal hippocampus; ThR, right anterior thalamus; FRm, right mesencephalic reticular formation; EMG, electromyogram of the left forepaw; CS, conditioned stimulus; EKG, note acceleration of the heart rate after performance of the ACR.

**Effects of afterdischarges on AVR latencies.** Figure 4 presents the distribution of AVR latencies measured in 6 animals. It includes the latencies of reflex movements performed during (D) shorter and longer ADS in comparison to response latencies seen before (B) and after (A) hippocampal stimulation. An analysis of variance showed statistically significant differences between the distributions in the group of ADS both shorter and longer than 10 s \( F(df 2) = 13.54; P < 0.01 \). The application of the Duncan test showed that regardless of the AD duration, latencies during ADS (D) were significantly longer than before (B) and after (A) kindling hippocampal stimulation.
DISCUSSION

In our previous studies of the AVR performance tested in the background of afterdischarges (10) we observed a relation between the performance and duration of the ADS and seizures: namely, about 75% correct responses during ADS shorter than 10 s and 25% correct responses during ADS longer than 10 s. However, the pattern of afterdischarges was not specified in that analysis. Variability of the AVR performance led to the conclusion that AD duration was not the only factor responsible for AVR failures in both groups of AD durations.

The results of the present investigations support an earlier observation (9) about the existence of several basic EEG patterns of the hippocampal afterdischarges in hippocampally kindled animals. The patterns of EEG afterdischarges in hippocampally kindled guinea pigs are different than AD patterns in guinea pigs during cortical or amygdalar kindling (9). The present results indicate also that the type of the AD observed during CS presentation (types I, II and III) may be related to the AVR performance (Table I). Namely, EEG dysrhythmia (type III) was associated with the greatest probability of the AVR failure. The ratio of response failures to correct responses was 4.4 for type III of the EEG afterdischarge pattern, while it was 2.8 for type II and 1.4 for type I pattern. AD type V was not observed during early phases of AD deve-
lopment after electrical stimulation of the hippocampus. It was observed at the end of prolonged afterdischarges.

These findings show that both AD patterns as well as behavioral performance are good indicators of the degree of limbic dysfunction.

AVR latencies and their prolongation during limbic ADs observed during our experiments are comparable to reaction times found by Taylor-Courval and Gloor (14) in their studies of instrumental reflexes during neocortical spike and wave bursts evoked by penicillin injections in acute animal epilepsy model. However, in their study performance depended also on the exact timing of the CS with respect to spike and wave bursts. The application of conditioned stimuli in the middle of a burst had the greatest likelihood of response failure. The application of the CS at the beginning or at the end of a burst could result in a correct response, after a longer latency, or a motor response could be completed after the spike and wave burst was over. Since in our studies time relations between electrical stimulation and CS presentation were relatively stable, the variability of reflex performance seemed to be attributed to a different degree of hippocampal dysfunction, expressed by different EEG afterdischarge patterns. This hypothesis will be verified during a subsequent study on the relation between AVR performance and different EEG AD patterns during different stages of AD duration.

This investigation was supported by Project 04.01.6.16 of the Polish Academy of Sciences.

REFERENCES


Accepted 10 August 1988