EFFECTS OF HIPPOCAMPAL KINDLED AFTERDISCHARGES AND COMPLEX PARTIAL SEIZURES ON PREVIOUSLY ESTABLISHED AVOIDANCE RESPONSE IN CATS

J. MAJKOWSKI and A. SOBIESZEK
Laboratory of Experimental Neurology CSK
Lindleya 4, 02-005 Warsaw, Poland

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Abstract. The performance of well established avoidance response was tested in cats with developed, kindled, bioelectric hippocampal epileptic focus and complex partial and secondarily generalized seizures. The animals were trained before kindling to perform foreleg movement in response to clicks (conditioning stimulus) in order to avoid an unconditioned stimulus — electric shocks, applied to the same forepaw. In addition, two animals were trained to differentiate between different frequencies of clicks. There was no deficit in reflex performance and differentiation if the conditioning stimulus was presented during intensive uni- or bilateral spontaneous interictal limbic spiking, occurring before or after hippocampal afterdischarges and complex partial seizures. After repeated secondary generalized tonic-clonic seizures the performance was, however, completely abolished. Presentation of the conditioning stimulus during afterdischarges and partial seizures resulted in 75% correct responses during afterdischarges shorter than 10 s and 25% correct responses during afterdischarges longer than 10 s. Correct responses were observed in cases when conditioning stimulus was applied not later than 6 s after the termination of hippocampal electrical stimulation. The results suggest that the functional integrity of the hippocampal formation is of no critical importance for the retrieval and/or performance of the previously established avoidance response and differen-
A deficit in memory retrieval and/or performance of the avoidance response during afterdischarges and partial seizures may be related to indirect effects caused by the afterdischarges in remote, other than limbic structures.

INTRODUCTION

In this paper we are basically concerned with (i) the effects of kindled, epileptic spontaneous interictal spiking (IS), (ii) epileptic afterdischarges (ADs), and (iii) epileptic seizures on memory retrieval and performance of the previously established avoidance response (AVR) in cats. To study this relation, the hippocampal formation was selected for two main reasons: (A) it has been implicated as an anatomical substrate for encoding (5), consolidation of long-term memory (23), memory retrieval (32, 34) or all of these, if related to absolute spatial and long-term temporal attributes of specific episodes (16), and B) it plays an important role in the partial seizures of temporal lobe epilepsy, which in humans are mostly associated with disturbances of cognitive processes.

An impairment of cognitive processes in epilepsy has become one of the main research problems in current epileptology. In particular, the relation between learning and memory on one side, and seizures (8, 12, 24) and drug therapy on the other (4, 30) has been subjected to many studies. These relations present a complex problem and were recently discussed from different points of view (18, 19).

Kindling with its permanent reorganization of neuronal function as manifested by the presence of IS, AD and seizures (10), by modifications of cortical evoked potentials (17, 21) and by long-lasting alterations in behavior, e.g. conditioned emotional response or passive avoidance (1, 9), provides means for the study of possible interference with memory retrieval. Our preliminary results indicated a complex relation between the functional integrity of the hippocampal formation, memory retrieval and/or performance of the AVR (22, 27).

MATERIAL AND METHODS

The study was performed on 8 adult, male and female cats. The cats were subjected to experimental procedures in the following sequence: (i) avoidance conditioning, (ii) implantation of electrodes, (iii) hippocampal kindling, (iv) kindling conducted parallel to conditioning, (v) differentiation, (vi) parallel kindling and differentiation, and (vii) histolo-
gical examination of the brains. Procedures v and vi were performed in two animals.

**Conditioning.** AVR was formed to 5 trains of 4/s clicks used as conditioning stimuli (CS). The animals were trained to pull a microswitch connected by tape to the left forepaw, in order to switch off the CS and to avoid the unconditioned stimulus (US), electric shocks applied to the same forepaw. In the absence of forepaw flexion during this period, the animal received electric shocks, isorhythmic and paired with the clicks in a period of additional 2s, regardless of the animal's behavior. The animal, or the experimenter, could terminate the CS during its 5 s duration, but could not influence the duration of the US, which was automatically regulated. The intensity of the electric current used as the US was individually adjusted for each animal, to a level resulting in a brisk forepaw withdrawal (flexion), but without generalized escape reaction. The animals during conditioning were partially restrained in a hammock which allowed them to move freely within a limited area. Conditioning was performed for six days in a week. During one session 20 trials were given. Intervals between trials ranged from 35 to 70 s. After the AVR criterion was reached (85% correct responses during 3 successive sessions), the training was discontinued and electrodes were implanted.

**Implantation of electrodes.** Each cat had 9 bipolar, permanently implanted electrodes for EEG recording and electrical stimulation. The electrodes were made of stainless steel wires, 0.3 or 0.4 mm in diam. They were glued together, with the distance between uninsulated tips of about 1.5 mm. The electrodes were implanted in the left and right ventral hippocampi, in the dorsal or posterior hippocampus, amygdala, and in the somato-sensorimotor cortex. In some animals electrodes were also placed in the right cerebellar, visual and auditory cortical areas and mesencephalic reticular formation. The localization of the hippocampal electrodes was verified during implantation by an appearance on the scope of spike discharges during the penetration of this structures by the electrode. The electrode implantation was performed under Nembutal anesthesia, 40 mg/kg body weight, given i.p. About 1 month after the implantation, reconditioning started to reestablish the AVR criterion. No defect in the AVR level due to implantation and pause in training was observed, and hippocampal kindling was started.

**Hippocampal kindling** at the threshold of afterdischarges was performed by daily (6 days/week) electrical stimulation of the right ventral hippocampus, using 1 s train of 50/s rectangular, mono or biphasic (in different animals) pulses of 1 ms duration. Current intensities ranged
from 0.3 to 1.2 mA. In the animals KW-1, KW-4, KW-5, KW-7 and KW-39 the intensity of stimulating current had to be adjusted to the level of bioelectrical response in the course of kindling (within 20% around AD threshold value). In the animals KW-2, KW-3 and KW-6 stimulating currents were kept constant during the kindling-conditioning period.

Parallel kindling and conditioning. In all cats the effect of kindling on AVR performance was analyzed in conditions of well-established bioelectric epileptic focus and partial complex seizures. In addition, in cat KW-2 and KW-3 the performance was also tested during early stages of kindling. In all cats except KW-3 the conditioning session was split into two blocks: 10 trials were given before, and another block of 10 trials followed the electrical stimulation of the hippocampus. In cat KW-3 all 20 trials were tested after the hippocampal stimulation.

In addition to 20 trials of conditioning, the CS was presented 1 to 3 times during the AD and within 20 s after its termination. These trials were analyzed separately, and were not included in the final score of responses on that day.

Fig. 1. Representative histological localization of tips of the stimulating electrodes in ventral hippocampal formation at the frontal sections A6 and A6.5 (26).
Differentiation. Two animals (KW-2 and KW-3) were trained to discriminate between two frequencies of clicks (4 and 8/s) after establishing the effects of seizures and ISs on the performance of the AVR. 4/s clicks (CS) were reinforced, while 8/s clicks presented during 7 s were used as a differential stimulus (DS) without reinforcement. The animals were trained to suppress forepaw movement in response to the DS (differential inhibition). There were 20 presentations of the DS added in semi-random order to the 10 CS presentations in each session. The criterion of differentiation was at least 90% of correct responses (to the DS and CS respectively) obtained during 3 consecutive sessions.

Histological verification. After the experiments were finished, the animals were sacrificed by an overdose of barbiturate. In 5 animals the brains were examined for localization of electrodes. Fifty μm brain sections were stained according to Klüver-Barrera method. The localization of stimulating electrodes is presented in Fig. 1.

RESULTS

The criterion of the AVR was obtained in all cats after 8 to 21 days of conditioning. Differentiation was established in two animals within a period of 10 days of learning.

In all cats hippocampal kindling resulted (i) in an increase of AD duration, (ii) in AD generalization to the contralateral hippocampus and to the amygdala on both sides, (iii) in the appearance of spontaneous focal spiking (at first after, and later on before the hippocampal stimulation) and (iv) in the occurrence of simple and complex partial seizures, which at later stages of kindling generalized to tonic-clonic seizures. Hippocampal stimulation never produced left or right forepaw movements.

A summary of experiments on AVR performance during different states of epileptogenicity is presented in Table I. The total duration of selected EEG samples recorded in the postseizure periods lasted in different animals from 10 to 124 min (mean = 56.4 min). The samples were collected during 3 to 9 sessions (mean = 5.7) of parallel kindling and conditioning. AD duration and seizures varied from 15 to 128 s (mean = 55.4 s).

The number of spontaneous spikes was determined during 10-15 min periods, starting 30 s after the termination of ADs and complex partial seizures.

In all animals the number of ISs after hippocampal stimulation was much greater than before (an example is presented in Fig. 2).
Performance of the avoidance response (AVR) and hippocampal interictal spiking before and after kindled complex partial seizures: summary of results. HVR and HVL, right and left ventral hippocampal formation. In the cat KW-3 AVR performance was tested after seizures only.

<table>
<thead>
<tr>
<th>Cat No.</th>
<th>Number of seizures selected for analysis</th>
<th>Duration of afterdischarges: range and mean (seconds)</th>
<th>Intensity of interictal spiking after seizures, at the time of AVR testing</th>
<th>AVR performance level % during spontaneous spiking</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Total duration of EEG samples (minutes)</td>
<td>Number of hippocampal spikes following afterdischarges (range and median/min)</td>
</tr>
<tr>
<td>KW-1</td>
<td>7</td>
<td>73–128; $\bar{x} = 87$</td>
<td>45</td>
<td>Transient EEG depression</td>
</tr>
<tr>
<td>KW-2</td>
<td>5</td>
<td>22–58; $\bar{x} = 37$</td>
<td>49</td>
<td>HVR 1–21;</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>HVL 0–16;</td>
</tr>
<tr>
<td>KW-3</td>
<td>7</td>
<td>15–25; $\bar{x} = 18$</td>
<td>124</td>
<td>HVR 9–55;</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>HVL 4–32;</td>
</tr>
<tr>
<td>KW-4</td>
<td>3</td>
<td>101–104; $\bar{x} = 103$</td>
<td>10</td>
<td>HVR 3–39;</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>HVL 2–13;</td>
</tr>
<tr>
<td>KW-5</td>
<td>2</td>
<td>48 and 87 not counted</td>
<td>not counted</td>
<td>not counted</td>
</tr>
<tr>
<td>KW-6</td>
<td>9</td>
<td>26–88; $\bar{x} = 44$</td>
<td>62</td>
<td>HVR 0–22;</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>HVL 0–18;</td>
</tr>
<tr>
<td>KW-7</td>
<td>7</td>
<td>33–72; $\bar{x} = 51$</td>
<td>35</td>
<td>HVR 0–11;</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>HVL 0–14;</td>
</tr>
<tr>
<td>KW-39</td>
<td>8</td>
<td>35–51; $\bar{x} = 38$</td>
<td>35</td>
<td>HVR 0–23;</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>HVL 3–22;</td>
</tr>
</tbody>
</table>
AVR performance during postseizure spontaneous hippocampal spiking. As can be seen in Table I, there was no difference in the percentage of correct AVR before and after hippocampal stimulation in spite of great differences in spiking levels. The percentage of correct responses after stimulation varied from 76% to 100% with respect to 76% and 100% before hippocampal stimulation. The difference is not statistically significant (Wilcoxon test).

The absence of clear relation between an increased number of ictal spikes during postseizure period and AVR performance illustrates Fig. 2.

![Fig. 2. The number of spontaneous spikes (ID: ictal discharges) and percentage of the AVR correct responses (CR), before and after electrical stimulation (S) of the hippocampus. The number of hippocampal spikes (n) was determined in 10 min EEG samples. The values are means and standard deviations of the data obtained during 6 kindling-conditioning sessions (N) in the cat KW-39.](image)

The same can be said about click differentiation in cats KW-2 and KW-3. Figure 3 shows correct responses to the CS and DS presented during hippocampal EEG depression and spiking after prolonged bioelectric and behavioral complex partial seizures in cat KW-2.

Effects of ADs on AVR performance. Proportions of correct forepaw movements performed on the background of ADs illustrates Fig. 4. The CS presentation during afterdischarges shorter than 10 s resulted in
about 75% of correct responses. The data ($n = 85$) were collected from 7 animals. The CS presentation during ADs longer than 10 s resulted in 25% of correct responses (number of observations, $n = 175$). These differences of performance are statistically significant ($P < 0.001$, $x^2 = 22.4$; $df = 1$). Correct responses occurred when the CS was applied

![Fig. 3](image)

**Fig. 3.** Correct responses to the CS and DS presented 16 and 30 seconds after the end of afterdischarge in cat KW2. The termination of the AD is shown on the left side of this Fig. ML and MR, left and right motor cortex; HVR and HVL, right and left ventral hippocampus; AmR, right amygdala.

![Fig. 4](image)

**Fig. 4.** The performance of the AVR (+, emission of the conditioned forepaw movement; −, no response) during initial phase of afterdischarges. The results are grouped according to the duration of ADs: shorter or longer than 10 s. CS presentation begun 2-6 s after termination of kindling electrical stimulation of the hippocampus.
within 6 s after the termination of hippocampal stimulation. They were occasionally observed even during bilateral ADs, involving both hippocampi and amygdalae, providing that the conditioned movement was accomplished within a period of about 10 s after stimulation (Fig. 5B, KW-1).

Fig. 5. A, Spontaneous interictal spiking preceding 61st kindling hippocampal electrical stimulation (kHVR) marked by one second artefact present in part B. B, Correct AVR (MV: forepaw movement marked by the arrow) performed during bilateral hippocampal and amygdalar afterdischarges. CrR and CrL, right and left cerebellar cortex respectively. Other abbreviations like in previous figures. Top line, one second time marker.

In cases of longer ADs, the CS was also presented 2-3 times later on, between 20-100 s of AD duration. In such situations there was no conditioned reflex.

The performance of the AVR to first CS presentation, depending on afterdischarge duration illustrates Fig. 6.

Effects of seizures on the AVR performance. Generally, complex partial seizures were associated with longer durations of ADs and mo-
tor and autonomic phenomena (facial and body jerks, salivation, urination, etc.). The presentation of the CS during the first 6 s after hippocampal stimulation failed to produce a correct AVR in 75% of trials. These results may be due to different patterns of AD distributions and/or different type and complexity of partial seizures. The presentation of the CS in later stages of the ADs (between 20 and 100 s) associated with complex partial seizures resulted in no response. However, the ani-

mals displayed a typical motor response to unconditioned stimuli. The presentation of the CS after the termination of ADs associated with complex partial seizures resulted, usually, in correct responses of the AVR, just indicating the recovery of the animals (Fig. 3).

In the final stages of kindling, when complex partial seizures generalized to tonic-clonic seizures, the presentation of the CSs during 20 min. after the termination of seizures resulted in a dramatic decrease of AVR percentage. If the seizures were occurring during successive days, the performance dropped to zero. The CS could produce, however, an orienting response and, occasionally, a forepaw extension instead of flexion.
This study shows that the presence of kindled hippocampal epileptic focus resulting in spontaneous interictal spiking occurring unilaterally or bilaterally in the hippocampal formation and amygdala, does not prevent memory retrieval and performance of the well established AVR. The same type of hippocampal function impairment does not interfere with learning the AVR (28).

The effects of hippocampal ADs on the memory retrieval appear to depend mainly on their duration and on the time of CS presentation during the AD. In this respect our results are consistent with observations of Delgado and Sevillano (6), who studied the performance of motor conditioned reflex in cats during after-discharges and seizures evoked by hippocampal electrical stimulation at seizure threshold level. Therefore, AD itself, its duration and spreading seems to be the main factor contributing to an impairment of memory retrieval and/or performance of the previously established behavior. A role of long-lasting synaptic changes produced by kindling would be of minor importance. Using a cortical alumina cream model of epilepsy in cats, Szwed (29) in our laboratory found that focal neocortical epileptic discharges did not have deteriorating effects on the memory of AVR.

Two aspects of our results are worth further discussion: (i) a possible role of the hippocampal formation in the storage of long-term associative memory and its retrieval, and (ii) a motor performance of conditioned reflex.

It is reasonable to assume that the AD disrupts normal ongoing neuronal activity within the vicinity of stimulating electrodes and in the neighboring areas, where the AD spreads. Therefore hippocampal AD effects on memory retrieval may be direct and local. Since such an effect does not interfere with the long-term associative memory of the AVR, the conclusion would be that structures other than the hippocampal formation and amygdala are involved in the storage and memory retrieval of the AVR. The spreading of the AD or its indirect suppressive effects in remote neuronal structures would be responsible for deficits in memory retrieval. A similar supposition is expressed by other authors. The spread of seizure activity from the hippocampus was considered to be responsible for the enduring memory deficit in hippocampal tetanus toxin induced epilepsy in rats (11).

The local epileptic discharge can be treated as a sign of temporary functional blocking of the brain structure. Blocking may be also obtained by appropriate electrical stimulation, by spreading depression (2),
or by local structural lesions. The major difference is in the time of blocking. Moreover, the size and location of lesions has different effects on the learning of different tasks and memories (13, 14). The same is true for different intensities of electrical stimulation, or epileptic discharges, which have different effects on the learned tasks (12). For example, it has been found that subthreshold stimulation, which failed to interrupt slow wave hippocampal activity, did not affect discrimination in an 8 arm maze (25). However, when the slow wave activity was interrupted, even without behavioral seizures, the rats showed substantial retrograde amnesia. In similar behavioral tests, subseizure levels of electrical stimulation of the dorsal hippocampus of rats disrupted the retention of the space oriented learning (3).

Majkowski and Jasper (20), as many others, found an impairment of the AVR performance after the removal of both ventral and dorsal hippocampi in cats with a previously established reflex. However, by methodological modifications it was possible to retrieve the memory of the AVR. Similar observations were reported by Karmos and Grastyan (14) and Karmos et al. (15). They found that extensive hippocampal lesions did not abolish simple and delayed alimentary conditioned reflex, and did not prevent the formation of a conditioned response and differentiation after lesions. Multiple-choice delayed reflex could not be, however, established in lesioned cats. Thus, lack of clear effects of bioelectric epileptic focus and complex partial epileptic seizures on the memory performance of the AVR is consistent with the results of bilateral extirpation of the hippocampi.

The second aspect of our study, namely, the failure to perform an AVR, may be related to a deficit in memory retrieval or alteration of motor performance. Such a possibility should be also taken into account, since it was found that latency of cortically evoked forepaw movement in cats increased during propagated hippocampal ADs and, correspondingly, that the magnitude of movement decreased (7). In our study we observed an abortive performance of the AVR during the postseizure period, however, after tonic-clonic seizures only. The CS presentation could result in a foreleg extension instead of its flexion. This observation indicated that motor functions were impaired. However, it does not exclude the possibility that it was due to an impairment of the memory storage of the AVR. Moreover, normal forepaw flexion in response to US observed during ADs and complex partial seizures (especially within 10 s after electrical stimulation of the hippocampus) indicates that the functional integrity of the motor cortex is in that period sufficient to subserve motor reaction to CS presentation.

Taking into consideration Kesner's theoretical framework for the
multidimensional relationships between brain structures and an attribute theory of memory representation, our results support the view that there are independent and selective impairments of different memory system (16, 31, 33). Each system has to a great extent its own functional and structural properties and localizations. Thus, on the experimental and clinical levels there are several dissociable kinds of memory. Consequently, the final results of local hippocampal dysfunction and its relation to memory storage and/or retrieval depend, mainly, on the type of task that is to be memorized and on the degree of hippocampal dysfunction.

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REFERENCES


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