INFLUENCE OF HIPPOCAMPAL KINDLING ON AVOIDANCE LEARNING IN CATS

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Abstract. The formation of avoidance response and differentiation was studied in 13 control and 7 kindled cats with developed hippocampal epileptic focus and complex partial or secondary generalized tonic-clonic seizures. Conditioning sessions lasted from 15 to 20 min in a period following afterdischarges and seizures resulting from electrical stimulation of the hippocampal formation. In two out of seven kindled cats the conditioned reflex was established within time limits (9 and 20 days) comparable to the control cats. These two animals had relatively short-lasting afterdischarges (mean values: 17 and 19 s). In cats with longer-lasting afterdischarges and complex partial seizures (mean duration: 32 to 65 s, in different animals) criterion was not reached during parallel kindling-conditioning stage of 10 to 30 days. The level of correct responses was low and variable (0-70%), despite typical reactions to the unconditioned stimuli and clear orienting responses to the conditioning stimuli on more advanced stages of training. Four kindled animals entered the stage of secondary generalization of seizures after hippocampal stimulation. Reflex performance after tonic-clonic seizures was practically abolished. The termination of kindling resulted in acquisition of the conditioned reflex in all the animals. The results indicate, that in animals with long-lasting complex partial seizures the learning of the avoidance response is impaired. In contrast to the effects exerted by afterdischarges and complex partial seizures, intensive interictal hippocampal spiking did not seem to have a direct negative influence on this particular type of learning.
INTRODUCTION

The relation between cognitive functions and epileptic discharges or seizures has always been an important question, since an impairment of learning and memory may occur in a number of persons with epilepsy. Such impairment may be due to certain types of discharges and/or seizures (1, 3, 5, 9), to antiepileptic drugs (2, 16, 26, 29), to underlying brain pathology (13, 17, 21) which is responsible for epilepsy, or to a combination of these variables (15). In clinical research it is very difficult to separate one variable from the other; therefore animal models of epilepsy should be far more utilized than presently in studies of the effects of seizures and/or drugs on the cognitive functions.

In previous studies of the AVR formation in cats, using alumina-cream neocortical model of epilepsy (18, 28), learning was delayed but not abolished. In another study (19), using better controlled kindling model of epilepsy, we found that permanent reorganization of the limbic functions due to hippocampal kindling did not interfere with memory retrieval and performance of the AVR established before kindling. The purpose of this investigation was to evaluate effects of hippocampal kindling in the dynamic process of the AVR formation.

MATERIAL AND METHODS

The study includes results obtained on 20 male and female, adult cats. The AVR was established without kindling in a control group of 13 animals. The experimental subgroup of seven cats was subjected to the following procedure.

Implantation of electrodes. A detailed description of the procedure is presented elsewhere (19). Briefly, each cat had 9 bipolar electrodes for stimulation and recording, implanted into cortical and subcortical structures. The implantation was performed under Nembutal anesthesia, 40 mg/kg body weight, i.p. About one month after implantation, kindling was started.

Kindling. Hippocampal kindling was performed by daily (6 days in a week) electrical stimulation of the right ventral or posterior hippocampal formation. One second trains of 50/s rectangular pulses, mono or biphasic (in different animals) of 1 ms duration were used. Current intensities varied from 0.2 to 0.8 mA. Pulses were delivered by the constant current stimulus isolation unit, optically coupled to the square wave generator. Conditioning started after the development of bioelectric epileptic hippocampal focus and prolonged afterdischargers and complex partial seizures.

Conditioning and differentiation. Clicks were used as the conditioning
(CS) and differential stimuli (DS). During conditioning the animals were trained to perform left forepaw flexion during 5 s period of CS presentation in order to disconnect an electric circuit and avoid electric shocks applied to the same forepaw (unconditioned stimulus US). A detailed description of the learning procedures is presented in another paper (19). During one session of conditioning 20 trials were given. Each session lasted about 15-20 min. It was started about 30 s after the end of ADs and seizures. The criterion for the AVR was at least 85% correct responses during 3 successive sessions. There were six kindling-conditioning sessions performed in a week. Learning continued as long as seizures were of the complex partial type, since after generalized seizures the performance of the AVR was practically abolished. For that reason, in an attempt to prolong the stage of kindling characterized by complex partial seizures, and to avoid secondary generalization, stimulating current was adjusted to its lowest effective values. In 4 animals in which generalized seizures (GS) developed after 10 to 16 days of kindling stimulation was stopped for a period of 5 to 50 days. Kindling was resumed with current intensities used during the last kindling session. In all the four animals it resulted in generalized seizures. A series of 5 to 7 stimulations associated with generalized seizures were then applied to these four cats. Kindling was alternated with conditioning sessions performed on different days in order to avoid direct effects of generalized convulsive seizures on learning. Subsequently, conditioning was continued without kindling in order to evaluate a possible delayed disturbing effect of kindling on the learning process.

During differentiation, in addition to trains of 4/s clicks (CS), trains of 8/s clicks presented during 7 s were used as differential stimuli (DS), without reinforcement. Intervals between trials lasted 35-70 s. There were 20 presentations of the DS added in semi-random order to 10 CS presentations in each session. If the animal did not reach the criterion after 2 weeks of training, this 2:1 DS to CS ratio could be changed, according to the type of behavior of the animal. If the cat was hyper-reactive and had difficulties in the formation of differential inhibition, the ratio of DS to CS was increased to 3:1, that is 30 DS and 10 CS presentations. In contrast, if the animal's behavior was of the inhibitory type, the ratio could be 1:1. The criterion for differentiation was at least 90% correct responses to the DS and CS during three successive sessions. The sequence of CS and DS presentations was arbitrary and based on previous experience. The number of stimuli of the same type (conditioning or differential) given in succession ranged from 4 to 9 during early stages of learning, and 1 to 5 in trained animals. All the animals went through the same differentiation schedule.
Histological verification. After the experiments were finished, the animals were sacrificed by an overdose of barbiturate. Brains were stained according to Klüver-Barrera method and inspected for electrode localizations. Stimulating electrodes were located in the ventral hippocampal formation: hilus of the gyrus dentatus and adjacent CA3 cortex (C3, C5, C8); gyrus dentatus and adjacent subiculum (C7), region of the angular bundle (C6), and in the CA3 region of the posterior hippocampus (C1, C2).

Data analysis. The following parameters were analysed during kindling: (i) AD duration, (ii) type and duration of epileptic seizures, (iii) number of spontaneous interictal spikes (IS) occurring during conditioning sessions, and (ii) percentages of correct responses to the CS and DS. The data were collected from (i) the control-conditioning group of animals, (ii) from experimental, kindling-conditioning group of cats, and (iii) from the experimental group of animals during conditioning without kindling. Analysis of variance was used for statistical evaluation of the learning process in the control and kindled subgroups of animals.

RESULTS

Conditioning in control cats. Control results obtained from 13 animals are part of the normative study. The criterion of AVR was reached by these animals during 8-55 days (sessions) of conditioning (median = 15 days). While one control cat required 55 days to criterion, other slowly learning control cats required no more than 20-30 days of conditioning.

Learning in kindled cats. Two subgroups could be distinguished in the experimental group of 7 animals, on the basis of behavioral and electroclinical criteria. The data illustrating the relation between EEG findings, clinical epileptic phenomena and the formation of AVR and differentiation are presented in Table I. The first subgroup consisting of two cats (C-3 and C-8) was characterized by relatively short-lasting ADs (mean: 19 and 17 s) and simple and complex partial seizures. The criterion of AVR was obtained after 10 (cat C-3) and 20 sessions (C-8). Differentiation was established during 9 sessions in cat C-3. In cat C-8 it was not tested. Acquisition of AVR differentiation in cat C-3 is presented on a graph in Fig. 1.

The second subgroup of 5 animals (C-1, C-2, C-5, C-6 and C-7) was characterized by longer duration of ADs and complex partial seizures (mean values varying from 32 to 65 s in different animals; Table I). Symptomatology of partial seizures is described in another paper (in
Summary of the results on conditioning during and after hippocampal kindling. Number of sessions refers to period of conditioning during kindling characterized by long-lasting ADs and complex partial seizures. IS, interictal spiking

<table>
<thead>
<tr>
<th>Cat No.</th>
<th>Number of sessions (days)</th>
<th>AVR criterion</th>
<th>AD duration range and mean</th>
<th>Number of IS/min. range and mean</th>
<th>Differentiation No. of sessions for criterion</th>
<th>No. of secondary tonic-clonic seizures</th>
<th>AVR No. of sessions for criterion</th>
<th>Differentiation No. of sessions for criterion</th>
</tr>
</thead>
<tbody>
<tr>
<td>C-3</td>
<td>10</td>
<td>+</td>
<td>15–26; $\bar{x} = 19$</td>
<td>HVR 0–55;</td>
<td>18</td>
<td>9</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>C-8</td>
<td>20</td>
<td>+</td>
<td>7–29; $\bar{x} = 17$</td>
<td>HVR 0–14;</td>
<td>2</td>
<td>No. criterion during 20 sessions</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>C-2</td>
<td>30</td>
<td>-</td>
<td>7–105; $\bar{x} = 54$</td>
<td>HVR 0–24;</td>
<td>4</td>
<td>-</td>
<td>7</td>
<td>not tested</td>
</tr>
<tr>
<td>C-1</td>
<td>15</td>
<td>-</td>
<td>25–61; $\bar{x} = 32$</td>
<td>HVR 0–24;</td>
<td>5</td>
<td>5</td>
<td>24</td>
<td>51</td>
</tr>
<tr>
<td>C-5</td>
<td>15</td>
<td>-</td>
<td>32–57; $\bar{x} = 46$</td>
<td>HVR 0–26;</td>
<td>6</td>
<td>7</td>
<td>42</td>
<td>23</td>
</tr>
<tr>
<td>C-6</td>
<td>10</td>
<td>-</td>
<td>52–142; $\bar{x} = 65$</td>
<td>HVR 0–14;</td>
<td>2</td>
<td>5</td>
<td>25</td>
<td>22</td>
</tr>
<tr>
<td>C-7</td>
<td>16</td>
<td>-</td>
<td>23–94; $\bar{x} = 55$</td>
<td>HVR 0–6;</td>
<td>0.2</td>
<td>5</td>
<td>4</td>
<td>7</td>
</tr>
</tbody>
</table>
preparation). In this subgroup the criterion of AVR was not obtained. The animals displayed however clear responses to the US and orienting reaction to the CS in advanced stages of conditioning. Also EEG activity after CS application showed changes of activation type. The percentages of AVR throughout the whole stage of kindling-conditioning were low and variable; from 0-35% in cats C-6 and C-1, or showed great variability: 0-75% in cats C-2, C-5 and C-7. Figure 2 presents acquisition curve of cat C-2. Learning of cat C-5 is illustrated in Fig. 3.

The statistical evaluation of learning processes in the control-conditioning and kindling-conditioning groups of animals was performed by

Fig. 1. Acquisition curve of the AVR (continuous line) and differentiation (broken line) in cat C-3. CR, conditioned responses; DR, differential responses.

Fig. 2. Acquisition curve of cat C-2 with prolonged afterdischarges and complex partial seizures during stages of kindling-conditioning and conditioning without kindling. Arrow indicates criterion of correct responses.
analysis of variance, type I, according to Lindquist and Duncan. The subgroup of cats characterized by prolonged ADs (n = 5) differed significantly from the control group (n = 13) in the level of correct responses \( (F_{1,16} = 5.16, \ P = 0.035) \) and interaction \( (F_{14; 224} = 1.84, \ P = 0.035) \). The group of animals with prolonged seizures had lower scores of correct responses than control cats during the same period of conditioning. Besides, there was no clear trend toward higher scores of correct responses with the progress of conditioning in animals with seizures.

In 4 animals with long-lasting ADs (C-1, C-5, C-6 and C-7), partial seizures secondarily generalized to tonic-clonic seizures after a period of 10-16 days. These animals practically ceased to respond to CS during the postseizure period.

**Learning in cats after kindling withdrawal.** In the animals with secondary generalized seizures kindling was stopped for a period of 5 to 50 days. When it was resumed after that time with the same current

![Graph](image-url)
intensities, it resulted in generalized seizures in all four animals. They were then subjected to a series of 5 to 7 kindling stimulations. Kindling was alternated with conditioning sessions (on different days). After that period kindling was stopped and conditioning was continued.

The criterion of AVR was reached by all the four animals which had generalized seizures. However, the number of sessions required varied from 4 to 42 days (Table I). Cat C-7 required only 4 days to reach the AVR criterion. A high level of AVR performance was observed in this animal already during alternating kindling-conditioning sessions.

The criterion of differential inhibition was also established in all four cats which had generalized seizures (Table I). Three types of behavior were observed during the formation of click discrimination: (i) hyperexcited (formation of differential inhibition was difficult while percentages of the AVR were high; cat C-1), (ii) inhibited (with low level of AVR performance and high percentage of correct responses to the DS; cat C-5), and (iii) with well-balanced processes of excitation and inhibition (cats C-6 and C-7). Figure 3 illustrates the time course of learning during kindling and post-kindling periods in cat C-5.

In cat C-2 secondary generalization did not occur during kindling-conditioning stage of 30 days. After termination of kindling in this cat the criterion of AVR was obtained during seven days (Fig. 2).

Effects of spontaneous interictal spiking. Table I shows numbers of spontaneous interictal spikes found in the stimulated hippocampal formation during conditioning following kindling stimulation. The numbers of spikes were determined during periods ranging from 100 min to 196 min (mean value for all cats was 159 min from 8.2 sessions). High intensity of interictal spiking during conditioning session did not seem to affect the learning process. The highest number of spikes was recorded in cat C-3 (Med. = 18/s) and in this cat the AVR was established during 10 days. In the animals which did not reach the criterion of AVR the numbers of spontaneous spikes were lower and varied from 0.02 to 6/min, thus indicating that other factors than interictal spiking play a critical role in learning impairment.

DISCUSSION

Since the relation between epileptic phenomena and learning processes is poorly understood, it seems to be worthwhile to point out at first some methodological difficulties. The limbic system, in particular the hippocampus and amygdala, have been considered to be involved in learning and memory processes for a long time. However, despite
a great number of publications, the obtained results vary and are far from conclusive (12). The reasons seem to be partly due to methodological differences related to the three groups of variables: (i) the method and degree of production of hippocampal dysfunction: e.g. short-lasting electrical stimulation, kindling, alumina cream induced epileptic discharges with varying durations, localization and extent of spreading; (ii) type of tasks to be learned or memorized during hippocampal dysfunction; (iii) temporal relation between the occurrence of hippocampal epileptic activity and associative learning: specifically, before, during or after pairing the CS and US. The combination of these variables, which sometimes are poorly controlled by the experimenter, e.g. spontaneous epileptic spiking or discharges in the alumina-cream induced epilepsy model, contributes to the variability of obtained results and conclusions. As an example: in a model of a conditioned reflex with short delay (4) learning was facilitated after low current stimulation of the amygdala (without EEG changes) if applied before or after CS-US pairings. In another study, amygdalar stimulation applied after CS-US pairing impaired retention (6). Low current intensity posttrial stimulation of the amygdala can interfere with, or can enhance retention of the avoidance reflex depending on current intensity of the US (7). Kesner and Doty (11) observed amnesia for an aversive experience following afterdischarges without overt convulsions, evoked by electrical stimulation of the dorsal hippocampus and amygdala. This effect was not observed if initiation of the ADs was from the septum, fornix or ventral hippocampus. Amnesia was also obtained after bilateral low intensity stimulation of the dorsal hippocampus (22).

Epileptic bilateral discharges from the inferotemporal cortex, fusiform and hippocampal gyri in monkeys in an alumina cream model of epilepsy resulted in delayed learning of visual pattern discrimination (27). The EEG discharges in this study were of the spontaneous spiking type, sometimes predominantly unilateral. However, in the alumina-cream model, in addition to epileptic spiking there is yet another variable, local or diffuse encephalopathy (20) which is absent during kindling. Bilateral electrical stimulation of the inferotemporal cortex or amygdalae, resulting in partial seizures immediately following CS-US pairing, markedly impaired or blocked the development of paw avoidance conditioned reflex (14). This effect was modality specific and consistent when visual stimuli served as the CS, but there was little or no effect with auditory stimuli. The results suggest that seizures have some amnestic effect on the visual association process, since the inferotemporal cortex plays an important role in visual perception.

Results of present investigations may be interpreted in terms of im-
pairment of avoidance learning after prolonged afterdischarges associated with complex partial seizures. Intensive spontaneous epileptic spiking after relatively short lasting bioelectric seizures (ADs shorter than 20 s) did not impair this particular type of learning process.

This interpretation has several aspects open to criticism. First of all: a relatively short duration of the kindling stage characterized by the presence of prolonged afterdischarges and complex partial seizures, but before secondary generalization of seizures. In four animals it lasted between 10 and 16 days. None of the animals has reached the AVR criterion within that time. Within comparable period of time (8-15 days) more than 50% of the control group of animals (good achievers) reached the criterion. This could suggest that none of the kindled cats would have belonged to the control group of good achievers, unless the slow progress of learning was a result of prolonged afterdischarges and seizures. Indeed, two animals: C-2 (30 days of kindling-conditioning with no criterion) and C-7 reached the criterion within 7 and 4 days respectively, after they had been trained without kindling. An impairment of learning process was also observed in three other animals, since all the cats served as controls for themselves, during the stage of conditioning and differentiation without kindling. Taking as a whole duration of the kindling-conditioning stage and conditioning without kindling, the criterion of correct responses was obtained in these animals after 40, 44 and 64 days, indicating disturbed learning. These effects, together with low and unstable behavioral responses to conditioned stimuli in comparison to control animals, speak in favor of learning impairment in cats with prolonged ADs and seizures in spite of a relative simplicity of the avoidance task.

The failure to respond reliably to conditioned stimuli after prolonged ADs and complex partial seizures of hippocampal origin could be associated with indirect effects exerted by the ADs on distant structures. The failure of the motor performance in the strict sense could be excluded, since the animals showed typical responses to unconditioned stimuli. Besides, very well coordinated escape behavior was occasionally observed in the animals even during fully developed partial seizures.

Three possibilities arise with respect to a possible involvement of the hippocampal formation in the learning task used in this study. (i) Hippocampal formation is not important for that particular type of learning process. (ii) Hippocampal dysfunction is expressed by an indirect effect caused by a spread of ADs along hippocampal projection pathways (or elsewhere). (iii) Hippocampal formation is important during the process of consolidation of a new pattern of motor behavior. In the last case, a selective involvement of neurones in short-lasting ADs and ISs would
not interfere with the learning process. However, an increased neuronal recruitment during prolonged ADs and complex partial seizures would interfere with the information processing. Since avoidance learning paradigm used in this study did not require spatial differentiation, or delayed retention, usually used for the demonstration of hippocampal participation in cognitive processes and memory functions (8, 10, 25, 30), we cannot exclude the first possibility. From the epileptological point of view however, as far as the effects of complex partial seizures are concerned, our results indicate a possible hippocampal involvement (direct or indirect) in working memory rather (24, 25) than, specifically, in cognitive mapping functions (23).

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REFERENCES


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