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EFFECTS OF ELECTRICAL HIPPOCAMPAL STIMULATION ON ACQUISITION AND PERFORMANCE OF UNIDIRECTIONAL ACTIVE AVOIDANCE RESPONSE IN CATS

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Abstract. The influence of electrical stimulation of the posterior part of the hippocampus on acquisition and performance of the conditioned, unidirectional avoidance response was investigated in 26 cats. No significant differences in the speed of learning between stimulated and control animals were observed. However, electrical hippocampal stimulation with parameters 7/s, 100 μA, 7/s, 200 μA and 50/s, 100 μA (1 ms square pulses of negative polarity) facilitated the performance of the avoidance response. Strong hippocampal stimulation (50/s, 200 μA) caused inhibitory effects. Application of hippocampal stimulation before experimental sessions increased the performance level in comparison to sessions not preceded by hippocampal stimulation.

INTRODUCTION

Some authors pointed out that electrical stimulation of rewarding sites in the brain can facilitate the performance of conditioned avoidance response in shuttle-box. In 1965 Stein discovered that similar facilitatory influence can be observed in rats during stimulation of some points in the hippocampus which do not show rewarding properties in self-stimulation test (19). The results obtained in our Laboratory seem to fit well with Stein's observations mentioned above. We found that electrical stimulation of the posterior part of the hippocampus applied simultaneously with a conditioned stimulus can bring about faster acquisition of shuttle-avoidance response in cats (**4**).

According to the inhibitory hypothesis of hippocampal function po-

stulated by some authors, the facilitatory influence of hippocampal stimulation observed in the above experiments is unexpected. One should rather expect an inhibition of response. However, the inconsistency may be merely apparent. Shuttle-box is a very specific experimental situation due to a conflict which is inherent in it (11, 20). From a theoretical point of view it is reasonable to assume that interventions which diminish behavioral inhibition will cause facilitation of avoidance behavior in this situation (14). Such effects were frequently observed after lesions of the hippocampus in the rat (1, 7, 10, 17, 23). The similarity of results caused by lesions and by the stimulation of the hippocampus might suggest that both can cause inactivation of this structure. Nevertheless, it remains possible that the two kinds of intervention act in different ways, though the final result is similar. To avoid such ambiguity, unidirectional avoidance situation (safe island) was used in the experiments described below. Some authors have found that lesions of the hippocampus can exert a negative influence on avoidance behavior in this situation (3, 12, 13, 21).

It seemed interesting to study the effects of electrical stimulation of this structure on acquisition and performance of active avoidance response (AAR) in this experimental situation and to compare them with those obtained in shuttle-box.

MATERIAL AND METHODS

The experiments were performed on 26 male cats, weighing about 3 kg. The cats were divided into three experimental groups. All animals of groups I and II had been operated on before the experiment. Group III served as unoperated control.

Surgery. The surgery was performed under hexobarbital anesthesia (80 mg/kg, i.p.) in semisterile conditions with the aid of a stereotaxic instrument. Unipolar teflon coated stainless steel electrodes 120 μm in diameter and of 0.5 mm bare tip length were implanted bilaterally (one into each hemisphere) according to the following coordinates of the Jasper and Ajmone–Marsan stereotaxic atlas (8): A2.0, L10.0, H+4.0. Silver wire with one end coiled so as to form a plate ca 7 mm in diameter and placed on the occipital bone served as an indifferent electrode. The upper ends of all electrodes were soldered to a miniature socket and the whole assembly was fixed to the skull with the aid of acrylic cement. The experiments were started after a period of convalescence which lasted 15 days.

Experimental situation. The training apparatus used in this experiment consisted of a 100 × 100 × 100 cm wooden box, painted gray in-

side. The front wall of the box was made of transparent glass to make possible the observation of the animal's behavior during the experiment. The floor was made of metal bars 5 mm in diameter and spaced 1 cm apart from center to center, connected with the source of electric current through an autotransformer and, in addition, a 1:1 isolating transformer. An electric shock applied to the animal's paws through the bars served as unconditioned stimulus (US). A sine-wave generator connected with a loudspeaker located in the center of the ceiling of the training apparatus served as a source of conditioned stimulus (CS). A wooden shelf (shaped in a quarter circle of 25 cm radius) located in the right rear corner 25 cm above the floor level formed the "safe island". A special electrical device allowed an automatic removal of the shelf from the training apparatus through a narrow cleft cut in the right-side wall. An electrical stimulation of the brain was performed with the use of a rectangular-pulse generator (PGP-3 type, ZOPAN, Warsaw). The parameters of stimulation were controlled with the aid of a dual-beam oscilloscope (OS-102 type, UNITRA, Warsaw). The details of stimulation technique have been described by us in another paper (5).

Training procedure. One day before the training, started, each cat was placed in the training apparatus and allowed 10 min free exploration of its inside. No stimulus was applied during this time. From the next day on the animals were taught to jump onto the shelf after presentation of CS (500 Hz tone). If the cat did not respond in 10 s from the beginning of CS, short-lasting electric shocks were applied to the animal's paws until it jumped onto the shelf. Then CS action was terminated and the cat was allowed to stay on the shelf for 10 s. After this time the shelf was removed outside, forcing the cat to jump down, and then replaced immediately. This was marked as the end of the trial. When the intertrial response (ITR) occurred, the shelf was removed immediately. The animals were trained 6 days a week. Each experimental session consisted of 10 trials with 20–40 s intertrial intervals. All animals were trained until they had attained the criterion i.e., of at least 90% performance level in 10 succesive experimental sessions.

The cats of group I ($n=9$) were trained with the use of bilateral electrical stimulation of the hippocampus. The stimulation was started 0.5 s after beginning of CS. Negative rectangular pulses of 1 ms duration and 100 μA intensity (measured for each electrode separately) and of 7/s frequency were used. CS and stimulation of the brain were terminated immediately after the conditioned or unconditioned response had been performed.

The cats of group II ($n=7$) and group III ($n=10$) were not stimulated during training. After attaining the criterion, the AAR performance

was checked in cats of group I and III. The aim of that part of the experiment was to find out whether the electrical stimulation of the hippocampus applied simultaneously with CS acquired the attributes of a conditioned stimulus. The checking was performed in the following way: each experimental session was consisted of two sets of 10 trials. On the first day the first 10 trials were conducted with the use of hippocampal stimulation only, applied in an identical manner as CS. The next 10 trials were performed with the use of CS but without stimulation of the hippocampus. On the next day the procedure was reversed. Six experimental sessions have been performed in this way. US was not used in these two sets of trials. If the animal did not jump onto the shelf within 10 s the action of CS or hippocampal stimulation was terminated. However, as to avoid the extinction of conditioned response, after the first 10 trials two additional trials with the use of CS and stimulation of the hippocampus acting together were performed. If the animal did not respond in 5 s US was given.

The cats of group III (unoperated controls) were treated in a similar way. The pressing of the button triggering the stimulation of the brain in operated animals simulated stimulation for 10 s and all jumps onto the shelf which occurred during the pressing were counted as conditioned responses. This procedure enabled us, at least partially, to find the influence of other factors on response when the stimulation was acting alone.

When the animals of group II (operated control) attained the criterion, the influence of hippocampal stimulation on the performance of the conditioned response acquired during training was examined. At the beginning of each experimental session two trials with the use of CS only were made. If the cat did not perform in 5 s, US was applied. After that followed 10 trials with the use of hippocampal stimulation acting together with CS. The action of CS and hippocampal stimulation was terminated immediately after AAR had occurred or after the lapse of 10 s. US was not used in these trials. The influence of the hippocampal stimulation with frequency of 7/s and 50/s and intensity 100 μA and 200 μA was checked in that stage of the experiment.

Two succesive experimental sessions were performed with the application of each combination of current parameters. The sessions performed with the use of hippocampal stimulation were separated by two control sessions performed without stimulation.

Upon the termination of the experiment all operated cats were killed, their brains were fixed in formalin and embedded in paraffin. The 10 μm thick sections from the area of electrode penetration were stained with cresyl violet (Nissl method). For the purpose of statistical analysis

all results expressed orginally in percents (P) were transformed to arc $\sin \sqrt{P}$. Statistical significances were evaluated with the use of different analyses of variances.

RESULTS

The results of histological anylysis are presented in Fig. 1. There were slight differences in electrode placement between cats. However, the symptoms observed during stimulation were the same in all animals. Therefore all the results that have been obtained in different groups of cats were evaluated together.

The comparison of the rate of learning, based on the number of training sessions, to criterion revealed statistically significant differences between groups barely above 0.05 level (Lindquist one-dimensional analysis of variances, $F_{2,23} = 3.446$, $F_{2,23;0,05} = 3.42$. Additionally applied Duncan's test revealed that differences appeared between control groups. All groups did not differ significantly with respect to the number of ITR's and AAR latency. The analysis of results obtained during checking period (mixed-design analysis of variances, type I and II of Lindquist, variables: A, succesive experimental sessions, B, the order of CS and hippocampal stimulation application, C, experimental groups (showed a significant influence of A) ($P < 0.001$, $F_{2,34} = 49{,}751$, $F_{2,30;0,001} = 8.71$) and B ($P < 0.05$ $F_{1,17} = 5.917$, $F_{1,17;0,05} = 4.45$) on AAR performance. It was expressed by gradual extinction of AAR and an obvious influence of trials with the use of stimulation or simulated stimulation on succesive responding to the tone. A highly significant interaction between B and C variables have been also found ($P < 0.001$, $F_{1,17} = 644{,}904$, $F_{1,17;0,001} = 15.72$). The interaction suggested the use of Lindquist type I analysis of variances. Afterward Duncan's test was applied (experimental groups and successive experimental sessions were regarded as variables). That analysis again confirmed the significance of extinction and showed that, at the end of checking, this process was more pronounced in stimulated animals ($P < 0.05$). It was also shown that stimulated cats responded better to the tone in the sets of trials preceded by trials with the use of hippocampal stimulation ($P < 0.01$), whereas controls performed better in the first set of trials ($P < 0.01$). Stimulated cats failed to respond to the hippocampal stimulation applied in the same manner as CS, regardles of whether the trials were performed before or after the trials with the use of the tone. This means that the stimulation did not acquire the attributes of CS.

The analysis of effects evoked by hippocampal stimulation with the use of different stimulus parameters in group II showed a highly sig-

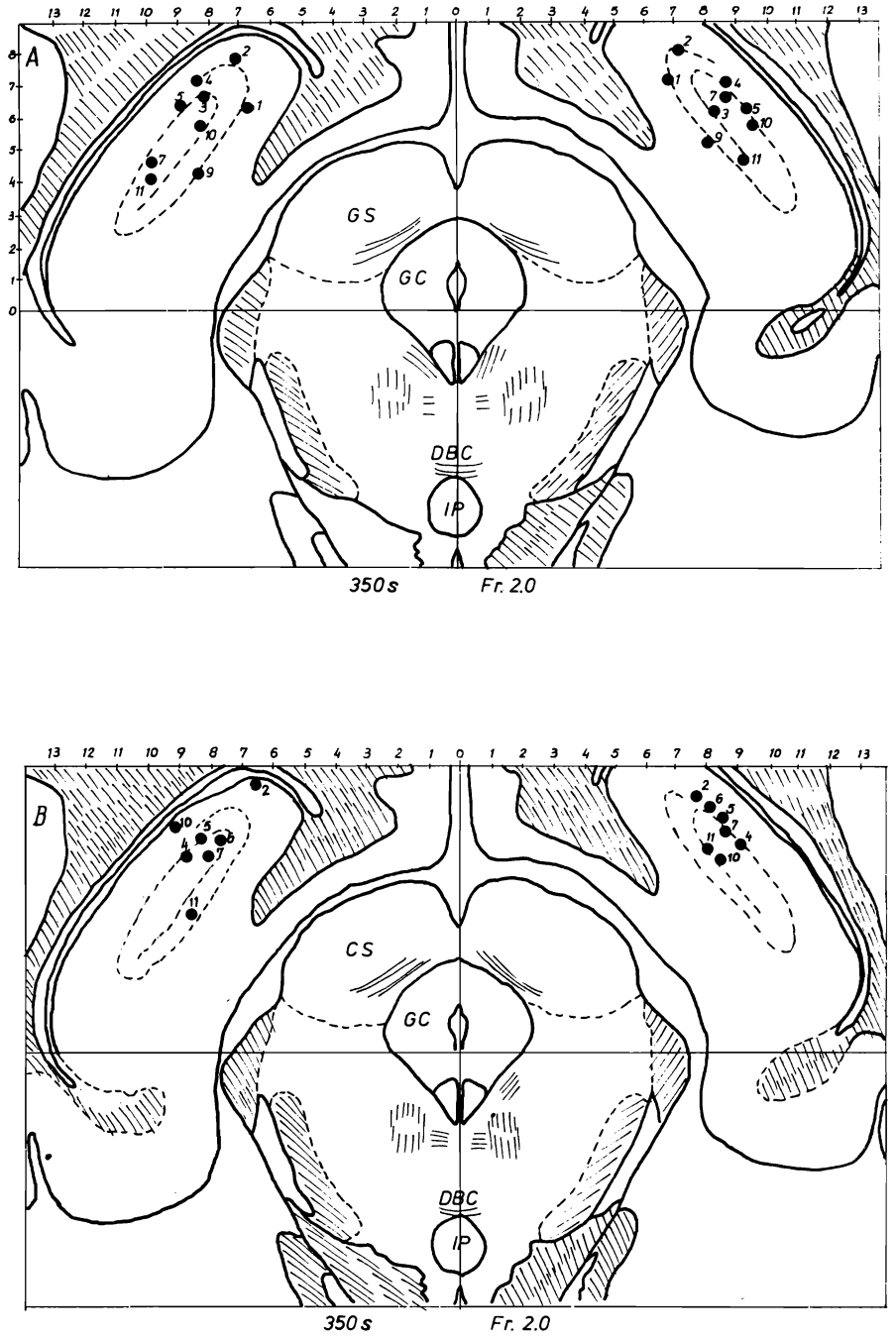

Fig. 1. The distribution of electrode placements in cats of group I (*A*) and group II (*B*). Black circles, the place of electrode placements; digits, the cat numbers.

nificant influence of the stimulation on AAR performance ($P < 0.001$, $F_{3,15} = 120.667$, $F_{3,15;0,001} = 9,34$ with regard to stimulus parameters, and $P < 0.001$, $F_{1,5} = 49.458$, $F_{1,5;.001} = 47.04$ in comparison with control sessions). That influence appeared exclusively in the case of 50/s, 200 μA stimulation which caused a strong inhibition of AAR performance and an increase of ITR number. This increase was also highly significant ($P < 0.01$, $F_{3,15} = 7,618$, $F_{3,15;0,01} = 5.42$ with regard to stimulus parameters and $P < 0.05$, $F_{1,5} = 10,163$, $F_{1,5;0,05} = 6.61$ in comparison with control sessions). Application of the remaining combinations of stimulus parameters did not influence neither the level of AAR performance nor the ITR number, but it shortened the latency of AAR significantly ($P < 0.05$, $F_{1,6} = 8.886$, $F_{1,6;0,05} = 5.99$) in comparison with control sessions. It suggests that the stimulation exerted a positive, facilitatory effect on AAR performance.

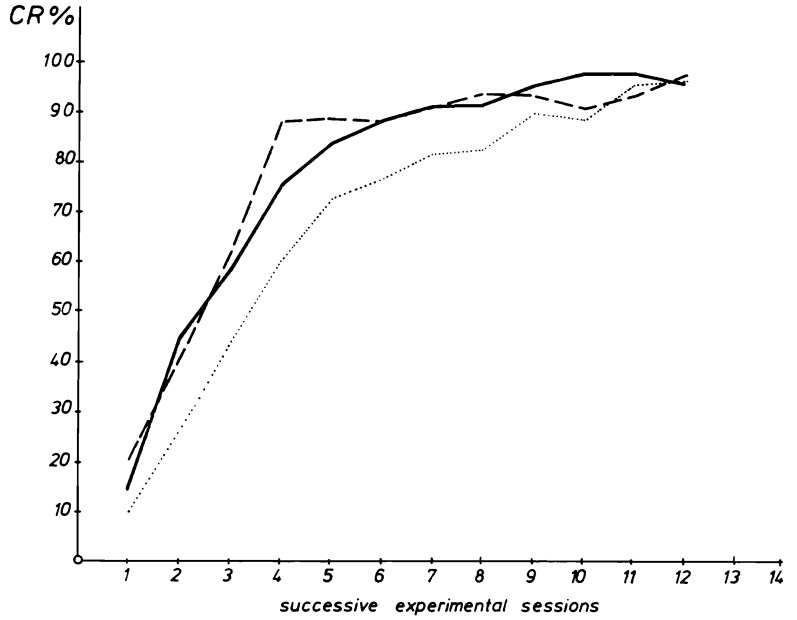

Fig. 2. Mean percentage avoidance responses during acquisition training in cats of group I (continuous line), group II (dashed line) and group III (dotted line).

The inhibition of AAR performance by 50/s, 200 μA hippocampal stimulation was correlated with the changes in animal behavior observed during stimulation. A few seconds after starting the stimulation, the inhibition of spontaneous locomotion occurred followed by pupil dilation, clonic contractions of lip movements, piloerection and salivation. These changes outlasted the time of stimulation by tenths of second. Loud miaowing in series marked the end of motor inhibition, and afterwards

the motor activity was usually much higher than before stimulation. These symptoms were most pronounced in the first trial. In further trials they were much weaker. As a result of increased motor activity that occurred after stimulation, the number of intertrial responses was higher than in control sessions.

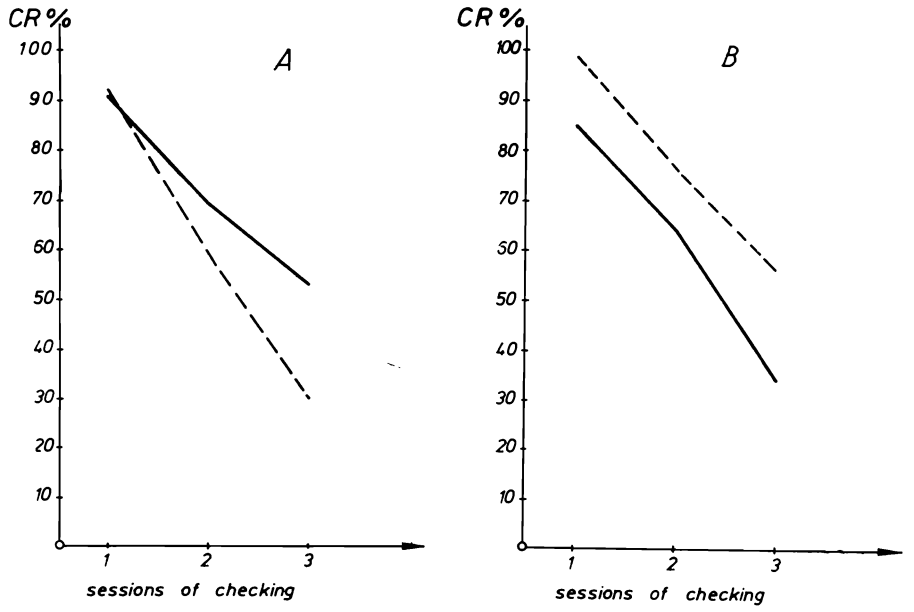

Fig. 3. Mean percentage of avoidance responses during checking in response to the tone in stimulated (*A*) and unstimulated (*B*) cats. The dashed line, the first set of 10 trials (before the trials with the use of stimulation or simulated stimulation); the continuous line, the second set of 10 trials (after the trials with the use of stimulation or simulated stimulation).

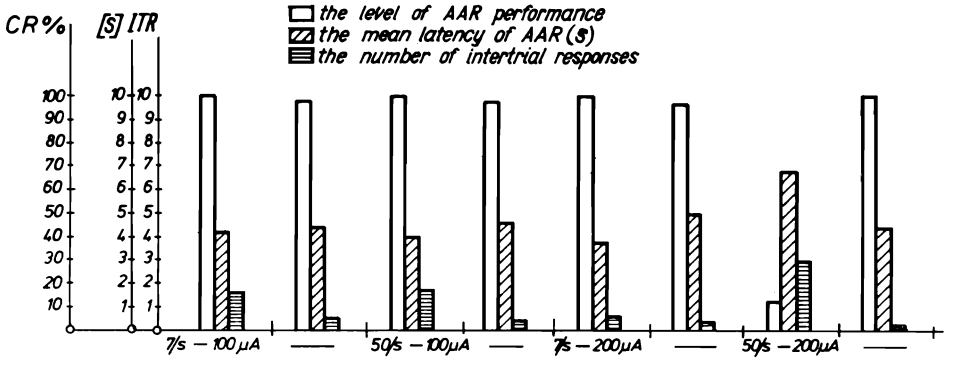

Fig. 4. Diagrams showing the influence of hippocampal stimulation on AAR performance.

DISCUSSION

The results obtained in this experiment during training are different from those obtained in the shuttle-box, where significant facilitation of learning was found (4), although the stimulated region of the hippocampus and the parameters of stimulation were the same. Some authors have pointed out that the acquisition of AAR in one-directional avoidance situation is much faster than in the shuttle-box (11, 14, 20), because animals do not hesitate to enter into the safe compartment which is always safe, or to jump onto the shelf where they are never punished. Moreover, the external spatial cues that can help to determine the direction of movement during avoidance do not change when a unidirectional avoidance apparatus is used. These factors make the experimental situation simpler and, as a consequence, faster learning can occur. It seems very likely that the simplicity of experimental situation used in the experiments described above made the facilitative effect of hippocampal stimulation on learning impossible to detect. In most cases only two or three experimental sessions were sufficient to attain 90% of AAR performance in comparison to 10 or even 20 sessions required to reach a similar level in the shuttle-box (4).

The barely significant differences between unoperated cats and operated controls are very difficult to asses. We suspect, on the basis of the analyses of ITR's and the latency of AAR, that they were caused by an accidental selection of a single animal. Although the expected influence of hippocampal stimulation was not visible during acquisition it seems clear from the results that have been obtained during the checking period that the stimulation had some facilitatory influence on AAR. It was manifested by a shorter latency of AAR in group II when stimulation was performed with the use of low parameters of electric current, and the higher level of AAR performance in response to tone when the trials were preceded by stimulation of the hippocampus. The latter result is of special interest, because it shows that a weak hippocampal stimulation can evoke a long lasting (at least a couple of minutes) proactive effect on AAR performance. This effect becomes more pronounced when compared with the decrease of performance observed in unoperated animals after simulated stimulation. The lower level of performance in unoperated cats was probably connected with a decrease of excitability in some neuronal circuits due to a long stay in the experimental apparatus without presentation of the conditioned stimulus. If this supposition is correct, the higher level of performance after stimulation would be dependent on an increase of excitability. It is known that weak stimulation of the hippocampus can cause an in-

crease of the theta rhythm — a specific bioelectrical activity of this structure (9). Moreover, it has been shown that changes in animal behavior and bioelectrical activity of the neocortex during low frequency hippocampal stimulation are similar to those occurring during the theta rhythm (15, 16). So it is possible to assume that in the experiments presented above, the weak electrical stimulation of the hippocampus could evoke an increase of theta activity and the neuronal processes related to it.

It has been hypothesised by some authors that the theta rhythm is connected with the state of attention or arousal (6), or that it is correlated to the planning and performance of voluntary movements (2, 22). If the hippocampal stimulation could cause a temporary intensification of hippocampal functions, the increase of arousal or attention as well as the facilitation of processes connected with voluntary movements could explain the facilitation of AAR performance. Further experiments with the use of electrophysiological methods seem to be necessary to check this supposition.

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