EFFECTS OF HIPPOCAMPAL STIMULATION ON RETENTION AND EXTINCTION OF ONE WAY ACTIVE AVOIDANCE RESPONSE IN CATS

Krystyna GRALEWICZ and Sławomir GRALEWICZ

Department of Animal Physiology, Institute of Physiology and Cytology, Łódź University
90-222 Łódź, Rewolucji 1905 r. str. 66, Poland

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Abstract. We found previously that hippocampal stimulation (HiSt) at 20 cps, 100 μA, applied jointly with a tone (500 Hz) CS in the course of retention test, improved the performance and retarded the extinction of one-way active avoidance response (AAR) in cats. During this test failures to perform the AAR were not punished in all but two trials at the beginning of each session. The first experiment of the present studies demonstrated that (i) the AAR facilitating the effect of HiSt might be prevented by small electrolytic lesions made around the tips of the stimulating electrodes, (ii) large lesions of the hippocampus exerted little effect on the AAR acquisition, but the response was extinguished faster during the retention test. In the second experiment two response prevention trials (non-reinforced presentations of the CS with no possibility to make the AAR) were run at the beginning of each session after the end of training. In these conditions the HiSt resulted in a faster extinction of the AAR as compared with implanted unstimulated animals. Large lesions of the hippocampus had no effect on the extinction rate. We conclude that the facilitation of retrieval from memory may be responsible for the effects of HiSt on conditioned behavior.
INTRODUCTION

There are several reports in the literature which show that the influence of electrical stimulation of the hippocampus (HiSt) on conditioned behavior is not exclusively inhibitory as some authors suggested (7, 15). A low frequency and low intensity HiSt applied jointly with CS does not inhibit the performance of instrumental responses (6, 12, 19), and it may even facilitate the development of conditioned behavior (12, 21). Inhibitory effects, on the other hand, appear when the strength of HiSt is raised (11, 13, 19). In our previous experiments on cats we found that a weak stimulation of the posterior part of the hippocampus facilitated the performance of one-way active avoidance response (AAR) whereas a strong stimulation exerted an inhibitory influence (11). In further studies we observed that when a weak HiSt was applied during a retention test after training, it resulted in a much longer maintenance of a high level of AAR performance as compared with unstimulated animals. Interestingly, the stimulated cats had no troubles with withholding the conditioned response; after one or two unpunished failures to perform the AAR they reduced the rate of responding as efficiently as controls. Moreover, when HiSt was added to CS after the response was extinguished, it did not result in a reappearance of AAR in the majority of animals (14). The purpose of the present work was to clarify further the effect of a weak HiSt on one-way AAR in cats. It has been argued that owing to the use of the monopolar stimulation technique the facilitatory effects observed in our experiments might be due to stimulation of some extrahippocampal structures by current loops (E. Grastyan, personal communication). There is also a possibility that the long maintenance of a high level of AAR performance in stimulated animals might be analogous to the retardation of extinction commonly observed in rats with a lesioned hippocampus (2). These assumptions were tested in the Experiment I of the present study. The fast reduction of the rate of responding to the CS seen in the stimulated animals after several unpunished failures to perform the AAR suggested still another possibility, namely that the effect of HiSt was dependent on the animal's recent experience with the situation. The validity of this suggestion was checked in the Experiment II.

EXPERIMENT I

This experiment is in fact the second part of the one mentioned in Introduction (ref. 14). The necessity to publish the two parts separately was justified by the variety of problems and the large number of
groups. Here we tried to find out whether the facilitatory effect of HiSt would be present if small electrolytic lesions were made before training around the tips of stimulating electrodes. It was expected, on the basis of some literature data (8), that small lesions of the hippocampus, contrary to large ones, should have no effect on the rate of AAR acquisition and on the subsequent extinction of the response. They should, however, eliminate the hippocampal neural elements which might be activated directly by the HiSt. The next purpose was to find out whether large lesions of the hippocampus would result in a retardation of AAR spontaneous extinction after training.

Material and methods

Sixteen male cats were used. Eleven animals (IL group) had electrodes implanted bilaterally in the hippocampus. Around the uninsulated tips of these electrodes small electrolytic lesions were made. In five cats (L group) a large part of the posterior hippocampus was lesioned bilaterally.

Surgery. In cats of the IL group two monopolar, stainless steel, teflon coated electrodes of 120 μm in dia. and 0.5 mm bare tip were implanted bilaterally into the posterior part of the hippocampus (A = 2.0, L = 10.0, H = +4.0, according to Snider and Niemer's stereotaxic atlas (25)). At the end of the surgery a 3.0 mA current was passed during 15 s between each electrode (cathodes) and a surgical clamp (anode) attached to the edge of the scalp wound. For making large lesions in cats of the L group a high frequency (200 KHz) current was passed through a glass coated 0.2 mm in dia. platinum-iridium electrode with uninsulated tip of 1.0 mm. The intensity of the current was adjusted before the surgery so as to obtain a lesion of 1.5–2.0 mm in diameter the raw flesh during 15 s of coagulation. The stereotaxic coordinates of the 3 damaged loci were follows: 1. A = 2.0, L = 10.0, H = +4.0, 2. A = 2.0, L = 8.0, H = +5.0, 3. A = 2.0, L = 7.0, H = +6.0. The experiments began 15 days after the surgery.

Stimulation. The stimulation equipment and the stimulation parameters were the same as used previously (14). The electrodes, each in series with a 10 kOhm resistor, were connected in parallel to the output of the stimulator. The hippocampus was stimulated bilaterally. Negative, 1.0 ms square pulses, at 20 Hz frequency were used. The current passing through each electrode was 100 μA.

Apparatus and procedure. The training box and the procedure were identical as before (14). Shortly, the cats were taught to jump onto
a wooden shelf placed 25 cm above the grid floor within 10 s presentation of a CS (500 Hz pure tone) in order to avoid an electric footshock (50 Hz Ac of about 0.2 s duration, applied at a frequency of about 1.0 Hz). The intensity of the footshock (US) was 0.5 mA above the threshold established individually for each animal. It rarely exceeded 1.0 mA. The jump response terminated the CS immediately. Each daily session consisted of ten trials spaced by 20–30 s intertrial interval. The training continued until an acquisition criterion (at least 90% of AAR in each session during ten successive sessions) was attained. On the next day a retention test began. Each daily session during this phase was composed of twelve trials. During the first two trials, denoted as antiextinction trials, the maximum CS duration was 5 s and if the cat failed to perform during this time US was applied. Afterwards, ten trials were run with maximum CS duration of 10 s, as usually, and no US. The procedure was continued until the level of AAR performance dropped to 10% as maximum (disregarding the antiextinction trials) during four consecutive sessions (extinction criterion). In six cats of the IL group (denoted as ILs subgroup) during the retention test the HiSt was applied jointly with the CS in all but the two antiextinction trials. The remaining five cats of this group (denoted as ILns subgroup) were not stimulated.

After completion of the Experiment the animals were killed by an overdose of hexobarbital and their brains were subjected to a standard histological procedure.

Nonparametric tests were used for statistical evaluation of the data.

Results and discussion

Histological investigations. The lesions made around the bare tips of the electrodes in the IL group were of oblong, irregular shape. Their short dimension varied from 1.5 to 2.5 mm. There were some differences in the location and size of the lesions in both hemispheres. In the majority of cases the lesions encompassed a part of the dentate gyrus and overlying CA fields. In some animals the lesions were more superficial and comprised mainly the CA-1 — subiculum area. The large lesions in the L group were irregular and encompassed mainly the postero-dorsal part of the hippocampus. In all cases in this group the white matter over the hippocampus was also damaged. The extent of the hippocampal lesions is shown in Fig. 1.

Acquisition and retention tests. For the purpose of comparison two groups of cats from the previous work (14) have been included here.
This is the implanted group in which HiSt was applied during the retention test (NS–S group) and the implanted, unstimulated group (NS–NS group). Only the animals which participated in statistical analyses were taken into account i.e., seven cats from the NS–S group and eight cats from the NS–NS group (ref. 14, Table I). In the present work, they were treated as one implanted group (I group) during the analysis of the acquisition data, and as two subgroups (I_s — implanted stimulated and I_ns — implanted nonstimulated) during the analysis of the data from the retention test.

![Fig. 1. The extent of small (A) and large (B) lesions of the hippocampus. Minimum tissue destruction, black; maximum, lined.](image)

There were no clear-cut differences during training between the groups of cats from the present work and the I group. The median number of training sessions to the acquisition criterion was 14 in the L group, 12 in the IL group and 13 in the I group. The mean median number of intertrial responses per session during the criterion was 2.7, 2.5 and 2.3, respectively. The mean median latency of the AAR during the criterion was 4.3 in the L group, 4.1 in the IL group and 3.5 in the I group. Only the differences in the response latency were statistically significant. The cats of the L and IL group were slower in responding to the CS than the animals of the I group (P < 0.01 in both cases, Smirnov test).

In all groups the level of AAR declined during the retention test. The median number of sessions to attain the extinction criterion was 7 in the L group, 8 in the IL_{ns} subgroup and 7 in the IL_s subgroup. The corresponding values in the I_s and I_{ns} subgroups were 25 and 10.5,
Fig. 2. The extinction rate of the AAR in the course of the retention test in Experiment I. $I_s$, implanted cats, stimulated during the test, $I_{ns}$, implanted unstimulated cats, $I_{LS}$, implanted cats with small lesion around the tip of the electrode, stimulated during the test, $I_{LNS}$, implanted cats with small lesions around the tips of the electrodes, unstimulated during the test, $L$, unimplanted cats with large lesions of the hippocampus, respectively (Fig. 2). The Kruskall–Wallis one-way ANOVA showed that the group differences were highly significant ($H = 24.35$, $df = 4$, $P < 0.001$). Detailed comparisons with the Mann-Whitney U test revealed that the $I_{LS}$ and $I_{LNS}$ subgroups did not differ significantly from each other nor from the $L$ group and from the $I_{ns}$ subgroup. The cats of the $L$ group, however, attained the extinction criterion significantly faster than the $I_{ns}$ subgroup ($U = 2$, $P = 0.006$, two tailed). All groups of cats from the present experiment extinguished the AAR more readily than the cats of the $I_s$ subgroup ($U = 0$, $P = 0.002$, $U = 0$, $P = 0.002$ and $U = 0$, $P = 0.002$, two tailed, for the $L$ group, $I_{LS}$ and $I_{LNS}$ subgroups, respectively). It appears from the above that the small lesions of the hippocampus around the tips of the stimulating electrodes prevent the facilitatory effect of HiSt seen previously. Therefore, it is rather unlikely that the long maintenance of a high level of AAR performance in the $I_s$ subgroup was due to stimulation of extrahippocampal areas by current loops. The slight disturbance of the AAR acquisition (longer latencies) in the lesioned

1 Statistical evaluation of the number of US applications during the antiextinction trials excluded this factor as a possible cause of the long-lasting maintenance of AAR in the $I_s$ group (14).
cats are in agreement with literature data which showed that hippocampal lesions disrupt rather than improve the one-way AAR (22). The faster extinction of the conditioned response by the L group, on the other hand, is in contrast with expectations based on literature (2). Therefore it lessens the probability that the lesion-produced disturbances in inhibitory processes (2) and the facilitatory effect of HiSt are the same phenomena.

EXPERIMENT II

In the previous study (14) and in Experiment I the moment when the animal could notice that the reinforcement contingencies had been changed was not controlled by the experimenter during the retention test (in fact, this moment was delayed by the use of the two antiextinction trials). In the Experiment II, after training, the animals were "informed" at the beginging of each session that CS presentation was not followed by US. In order to do this a response prevention procedure was employed. Such a procedure is known to diminish the aversiveness of the CS and to facilitate extinction (5).

Material and methods

The experiment was performed on two groups of cats. Twelve animals (I group) had electrodes implanted in the hippocampus. Six cats (L group) had the posterior part of the hippocampus lesioned extensively by thermocoagulation. After training the I group was divided into two subgroups of six animals each. One of the subgroups (I_s) was stimulated during testing and the second (I_nS) served as unstimulated control. The surgery, the stimulation equipment and the stimulation parameters were the same as in Experiment I.

Procedure. The experiment was composed of two phases: acquisition and extinction. The acquisition procedure was the same as in Experiment I. During the extinction phase two response prevention trials were run at the beginning of each session. The shelf was outside during these trials, which made the AAR performance impossible, and the CS was on for 10 s. It was not followed by US. The following ten trials were run in the same manner as during the retention test in Experiment I. The procedure was continued until the extinction criterion was attained.

Results and discussion

Histological investigations. There were some differences in the placement of the left and right electrode in the implanted cats. In one
animal of the $I_s$ subgroup both electrodes were located symmetrically in the hilus of the fascia dentata and in three both were in the CA-1 field at different depth. In two animals of this subgroup one electrode was in the hilus and the other in the stratum radiatum of the CA-3 field. The lesions in the L group varied in size. In two cats a great part of the dorsal and posterior hippocampus from $A = 0.0$ to $A = 5.0$ was destroyed bilaterally. In the remaining animals the lesions comprised mainly the posterodorsal part from $A = 0.0$ to $A = 3.0$ plane. In all animals of this group there was some damage to the white matter over the hippocampus.

Acquisition and extinction. The two cats of the L group with the largest lesions have not learned the AAR and had to be rejected. The remaining animals of this group acquired the response at a similar rate as the cats of the I group. The only difference between the lesioned cats and the implanted one was a longer AAR latency in the L group. The mean median latency was 4.5 and 3.4 in the L and I group, respectively ($P < 0.01$, Smirnov test). The application of the two response prevention trials after training resulted in a very rapid decline of the level of AAR performance (Fig. 3). Therefore, statistical evaluations were performed on the number of AAR-s made instead on the number of sessions to the extinction criterion. The highest number of AAR-s was made by the $I_{ns}$ subgroup (median 15), the next were the cats of

Fig. 3. The extinction rate of the AAR during the extinction phase of Experiment II. $I_s$, implanted cats, stimulated during the extinction phase. $I_{ns}$, implanted unstimulated cats. $L$, unimplanted animals with large lesions of the hippocampus.
the L group (median 13). The smallest number of AAR-s was made by the cats of the I_s subgroup (median 2). The group differences were highly significant (Kruskall–Wallis ANOVA: H = 348.9, df = 2, P < 0.001). Detailed comparisons with the Mann–Whitney U test showed that the I_s subgroup differed significantly from the L group (U = 1, (4, 6), P = 0.02, two tailed), and from the I ns subgroup (U = 0, (6, 6), P = 0.002, two tailed). The L group and the I ns subgroup did not differ from each other.

As it appears from the above data, the replacement of two antiextinction trials by two response prevention trials after training changed radically the pattern of results obtained on the stimulated and on the lesioned animals. The almost instantaneous extinction of AAR in the I_s subgroup suggests that in the present conditions HiSt facilitated this process. Lesions of the hippocampus, on the other hand, seemingly had no effect on the extinction rate, although they resulted in a faster extinction of AAR in Experiment I.

GENERAL DISCUSSION

The main result of the present study is that the same HiSt, which has been described previously (14) as facilitating an instrumental avoidance response, may also result in a faster extinction of the same conditioned behavior. The present data, as well as those obtained earlier, support the assumption that the type of HiSt influence is determined by what the animal has learned about the experimental contingencies. It seems to strengthen the effect of the recent experience on behavior. As we have seen before (14), when no noticeable (for the animal) change was introduced to the experimental procedure after training, the HiSt facilitated the AAR, or rather, the behavior of the stimulated cats was more efficiently controlled by what they had just learned. In the lesioned cats this control might be weakened, which was suggested by the faster extinction of the AAR (Experiment I). When after training two response prevention trials were run at the beginning of each session, the animals might learn that the CS was no longer followed by US. The behavioral effect was a fast decline of the level of AAR performance and, again, it was stronger in the stimulated animals. The fact that there were no differences in the extinction rate between the I ns and L group (in spite of the diminished responsiveness of the lesioned cats, which should lead to a faster extinction) might suggest a reduction of the extinction facilitating effect of the response prevention procedure in the L group (Experiment II). Thus, in both situations the effect of HiSt and that of the lesions of the hippocampus seems to be opposite. This conclusion, however, should be confirmed in further experiments.
The assumption that the recent experience determines the effect of HiSt may also account for the lack of changes in behavior when HiSt was introduced to the experimental procedure after the AAR was extinguished (14). It is also consonant with the ability of HiSt to induce a learned avoidance response in the absence of CS (13). A facilitation of retrieval of stored information may be the most straightforward explanation of all the above changes in the conditioned behavior during HiSt. Accordingly, the effects of hippocampal lesions may be interpreted in terms of a deficient retention and/or retrieval. As far as the lesion data are concerned, a similar conclusion has also been drawn by other authors. The deficits in retention observed in some experimental situations in animals lesioned before training (24, 27) as well as in those operated after training (3, 23) were interpreted as a disfunction of a retrieval/comparator mechanisms (3) or as an inability to use background stimuli to facilitate retrieval (24, 27). Stimulation studies, on the other hand, suggested an involvement of the hippocampus in the process of consolidation rather than retrieval. It has been shown that a posttrial HiSt results in amnesia 24 h later, although the memory may be normal immediately after HiSt (4, 17). There are some reasons to assume, however, that the amnestic effect may be in fact a retrieval deficit (20).

If at least one of the hippocampal functions is the participation in retrieval and if HiSt produces results suggesting a facilitation of this process, one may be tempted to conclude that HiSt facilitates the physiological function of the hippocampus. Such conclusion, however, may be unacceptable for some authors who regard HiSt as a disturbing factor (7, 17). Nevertheless there is a growing body of evidence that apart from the amnestic and other negative effects a facilitation of learning and retention may be also produced by HiSt. Such results were obtained when the hippocampus was stimulated during trials (12, 21), between trials (10) or after training (9, 18, 26). It does not seem likely that the above effects were due to only quantitatively different disfunction of the hippocampus. An intriguing fact is the lack of differentiation of the effects of HiSt depending on the location of the stimulating electrodes. However, considering the existence of the four membered neuronal loop (perforant pathway — mossy fibers — Shaffer collaterals — CA-1 axons) within the hippocampal formation (1), such a situation is conceivable. Stimulation at any point of this loop may lead to an increased output from the CA-1 area. This may be directly responsible for the behavioral effect.

Although the data obtained in the present study (Experiment I) have shown that activation of the neuronal elements around the tips of the stimulating electrodes is necessary to obtain the effect on behavior,
they do not preclude the possibility that similar effects may be obtained from other brain areas. It has been shown, for example, that contrasting motivational states (fear or sleep) may be produced by stimulation of the same diencephalic loci depending on what the animal has learned about the situation in which it was stimulated (16 and ref. 14). This points to the necessity of broadening the area of search and looking for a system of structures involved in the same phenomena.


REFERENCES


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