FACILITATORY EFFECTS OF ELECTRICAL STIMULATION OF THE HIPPOCAMPUS ON A ONE WAY ACTIVE AVOIDANCE RESPONSE IN CATS

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Abstract. Effects of a low intensity electrical stimulation of the hippocampus (HiSt) on acquisition, performance and retention of a one-way active avoidance response (AAR) were studied in four groups of cats. The animals were first trained in avoiding an electric foot-shock signalled by a CS (500 Hz tone) presentation and then they were tested for retention until the response extinguished. The HiSt was applied simultaneously with the CS presentation and terminated immediately after the performance of the AAR. One group of cats was stimulated during training, one during the retention test, one during training and during the retention test and one served as unstimulated control group. It was found that application of the HiSt during training did not influence the acquisition rate, but the stimulation became indispensable for a normal performance and for retention of the response. Application of the HiSt after training facilitated performance and improved retention, but only in cats unstimulated during training. Additional tests revealed that the facilitatory effects of the HiSt could not be attributed to disinhibitory or cue properties of the HiSt.

INTRODUCTION

Inhibition of conditioned behavior by hippocampus stimulation (HiSt) applied during trials is a well documented phenomenon (3, 5, 7, 12, 14). Some authors regarded this effect as a proof of an inhibitory function
of the hippocampus (1, 12). In some cases, however, inhibitory effects were not observed unless seizure activity was evoked (2, 4, 17). There are also reports describing a facilitated response performance when a brief HiSt was applied at the beginning of each trial (24), and a faster acquisition of a conditioned EEG (theta rhythm) and fear response when a low intensity and low frequency HiSt was applied during CS-US pairings (19). In our previous studies, devoted mainly to establishing the significance of the HiSt parameters, we found that a low intensity HiSt, even of a relatively high frequency, might lead to a faster acquisition of a two way AAR (9, 10) and to a marked improvement of performance of a one-way AAR (8). Some observations suggested that such stimulation might also improve the retention of responses. High intensity stimulation produced inhibitory effects. The aim of the present studies was to gain more information concerning the mechanism of the facilitatory effects. It is conceivable that the faster acquisition and the better performance of AAR-s produced by application of HiSt during trials were forms of disinhibition due to a disruption of a response inhibiting mechanism (lesion-like effect (1)). Another possibility is that the HiSt possessed cue properties and became a part of a compound CS with the external stimulus. If such was the case, the HiSt produced facilitation might be interpreted simply as a summation effect. In the present experiments we tried first to distinguish more precisely the effects of a low intensity HiSt applied during trials on acquisition from the effects on performance and retention of a one way AAR. This was done in order to find out which of the processes was primarily influenced by HiSt. Then, in two separate tests we checked the disinhibition and summation hypotheses of HiSt action mentioned above.

MATERIAL AND METHODS

The experiments were performed on 38 adult male cats with electrodes implanted into the posterior part of the hippocampus. The implantation technique was described previously (9). Monopolar electrodes made of a stainless steel, teflon coated wire of 120 \( \mu \)m diam. were implanted bilaterally (one electrode into each hemisphere). The tips of these electrodes were bared at 0.5 mm length. The stereotaxic coordinates were as follows: \( A = 2.0, L = 10.0, H = +4.0 \) according to Snider and Niemer's stereotaxic atlas (23).

Stimulation. Bilateral brain stimulation was performed with the use of a square pulse stimulator. The stimulation always paralleled the CS. Negative pulses of 1.0 ms duration and 100 \( \mu \)A intensity (measured for each electrode separately) were used in all cases. The stimulation frequency was 20 cps during the main part of the experi-
ment. Exceptions are noted in the text. Pilot investigations showed that HiSt at these parameters had a facilitatory effect on the AAR and might be applied during many successive sessions without the danger of seizures. The details of the stimulation technique and stimulation equipment were described previously (11).

**Apparatus.** The experimental situation was the same as used previously (8). In short, it was a cubic 100×100×100 cm box with a grid floor and a shelf placed in the left, rear corner, 25 cm above the floor level. The CS was a pure (500 Hz) tone and the US was an electric footshock (AC) applied from a ground isolated source. The US intensity was 0.5 mA above the threshold established individually during the first training session.

**Procedure**

(a) *Effects of HiSt on acquisition, performance and retention of the AAR.* The first part of the experiment was composed of two phases: an acquisition and a retention test. During acquisition the cats were taught to jump onto the shelf within 10 s after the CS onset in order to avoid the US. After the jump the CS was switched off and the animal was allowed to stay for 10 s on the shelf. Each daily session was composed of ten trials with 20–40 s (mean 30 s) intertrial intervals. The training was continued until an acquisition criterion (ten sessions with 90% of avoidances per session as a minimum) was attained. On the next day after attaining the criterion the retention test began. At the beginning of each session during this phase two trials, denoted as “antiextinction trials”¹, were run. The CS duration in these trials was shortened to 5 s. If the cat did not jump onto the shelf within this time, US was applied. The two trials were followed by ten trials with maximum CS duration of 10 s and with no US. This procedure was continued until the level of AAR performance dropped to 10% as maximum during four consecutive sessions (extinction criterion) disregarding the antiextinction trials. All animals participated in this part of the experiment. In order to assess whether the HiSt influences learning, performance or retention of the AAR, the animals were randomly divided into four groups. Group S-S (n = 8) was stimulated during the acquisition and during the retention test. Group S-NS (n = 7) was stimulated during the acquisition but not during the retention test.

¹ Some cats pay little attention to the CS during the first trial, being more interested in the smell of the predecessor. If US is not applied, a fast extinction of AAR may occur. The “antiextinction trials” were introduced to the procedure in order to normalize the animal’s emotional state before the “normal trials” and to reduce the probability of occurrence of big individual differences during the retention test.
Group NS-S \((n = 12)\) was stimulated during the retention test only. Group NS-NS \((n = 11)\) served as unstimulated, control group. The HiSt was never applied during the antiextinction trials in any group. It was expected that the comparisons of results obtained during each phase as well as the effects of changes of the experimental contingencies should reveal which of the above mentioned processes are specifically influenced by the HiSt.

(b) **Testing the disinhibitory properties of HiSt.** In order to find out whether the HiSt may produce a disinhibition eight additional sessions were performed with cats of the NS-NS group after they had attained the extinction criterion in the course of the retention test. The procedure during these sessions was similar to that during the retention test in this group, but simultaneously with CS presentation the HiSt was applied in the ten trials following the antiextinction ones. During four consecutive sessions the HiSt frequency was set at 20 cps. Since in the previous studies \((8)\) the maximum AAR facilitation was seen at 50 cps, 100 \(\mu\)A for the next four sessions the HiSt frequency was raised to 50 cps.

(c) **Testing cue properties of the HiSt.** In order to check whether the HiSt applied jointly with the tone CS during training could acquire CS properties, after attaining the extinction criterion the cats of the S-S and S-NS groups were retrained. During retraining the tone and the HiSt were applied as separate CS-i. Two new groups were formed of these cats in such a way that in each group there were three cats from the S-S group and three from the S-NS group. In one of the newly formed groups (T-S group) four sessions were performed with the use of the acoustic CS first, and then four sessions with the use of the HiSt as CS. In the second group (S-T group) the succession was reversed. The remaining procedure was the same as during the acquisition phase of the experiment i.e., the CS was presented and if within 10 s the cat did not jump onto the shelf, US was applied. The performance of the AAR or escape response terminated the CS.

After the completion of the experiment the animals were killed by an overdose of hexobarbital and their brains were subjected to a standard histological procedure for verification of the electrode placements.

**RESULTS**

**Histology.** In six cats, three from the NS-NS group, two from the S-S group and one from the NS-S group, both electrodes missed the target. Their results were excluded from the analyses. In the remaining cats the electrode scatter on both sides encompassed all main hippocam-
pal fields as well as gyrus dentatus. Owing to an assymetry in the electrode placement, usually more than one histologically distinguished area was stimulated at the same time.

Effect of HiSt on acquisition, performance and retention of AAR. No differences were found between groups in the number of sessions to the acquisition criterion, in the median response time during the criterion and in the mean number of intertrial responses per session during the criterion (Table I, Fig. 1A). On the contrary, there were large differences between groups in the number of sessions after which the cats stopped to respond to the CS in the course of the retention test \( (F_{3.24} = 148.06, P < 0.001, \text{a one way ANOVA for uncorrelated data}) \) (Fig. 1B). In group S-NS discontinuation of HiSt after training resulted in a fast decline of the level of AAR performance and a rapid extinction of the response \( (P < 0.01 \text{ in all comparisons, Duncan's test}) \). On the other hand, introduction of the HiSt after training in the NS-S group led to the maintenance of an errorless performance through a relatively large number of successive sessions, but eventually the response extinguished. This effect appeared in the majority of cats from this group \( (P < 0.01 \text{ in all comparisons, Duncan test}) \). In four cats, however, HiSt at 100 \( \mu \text{A} \) intensity produced inhibitory effects. Their results were excluded from the analysis and will be dealt with separately later on. In distinction from the above, the cats stimulated during training and during the retention test did not differ from controls. The response time increased gradually in all groups during the retention test. However, the cats from the NS-S group responded faster during the first five sessions of the retention test in comparison with the last five sessions of the acquisition criterion \( (P < 0.01, \text{Smirnov test, one tailed}) \). The reverse was true for the remaining groups \( (P < 0.05 \text{ in

### Table I

A comparison of data obtained during acquisition (A) and retention (R) tests

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean number of sessions to criterion</th>
<th>Median response time (in s)</th>
<th>Mean number of ITR per session</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
<td>R</td>
<td>A (last five sessions)</td>
</tr>
<tr>
<td>S-S ((n = 6))</td>
<td>13.5</td>
<td>9.4</td>
<td>4.0</td>
</tr>
<tr>
<td>S-NS ((n = 6))</td>
<td>12.7</td>
<td>6.4</td>
<td>3.7</td>
</tr>
<tr>
<td>NS-S ((n = 7))</td>
<td>12.6</td>
<td>24.4</td>
<td>3.6</td>
</tr>
<tr>
<td>NS-NS ((n = 8))</td>
<td>13.2</td>
<td>10.5</td>
<td>3.8</td>
</tr>
</tbody>
</table>
Fig. 1. Diagram showing performance curves of consecutive groups of cats during acquisition (A) and during the retention test (B). Ordinate, percentage of avoidance response performance; abscissa, experimental sessions in succession.
all cases). Summing up, the results showed that the application of HiSt during training apparently did not influence learning, but the HiSt became indispensable for a normal retention of the learned response. On the other hand, HiSt introduced after training seemed to facilitate AAR performance and to improve retention of the response.

There was a possibility, however, that the differences in the level of AAR performance during the retention test might be related to the number of US applications during the antiextinction trials. In order to test this assumption, the ratio of the number of AAR-s made during the antiextinction trials to their total number was computed separately for the four sessions consisting the extinction criterion and for the remaining sessions. The mean percentage of the AAR-s made during the antiextinction trials was 76.7% and 39.0% in the S-NS group, 81.1% and 28.8% in the S-S group, 91.2% and 64.1% in the NS-S group and 79.6% and 46.6% in the NS-NS group (for the trials performed before and during the extinction criterion, respectively). Only the differences which appeared during the extinction criterion were statistically significant (Kruskal-Wallis one way ANOVA ($H = 9.0, df = 3, 0.02 < P < 0.05$). The cats of the NS-S group performed the AAR more frequently during the antiextinction trials than the cats of the S-S and S–NS groups ($P < 0.02$, Mann-Whitney U test, two tailed), but they did not differ from the cats of the NS-NS group. The S-S, S-NS and NS–NS groups did not differ from each other. This result shows clearly that the high level of AAR performance in the NS-S group cannot be attributed to a higher number of shocks during the antiextinction trials.

Another possible cause of a long maintenance of a high level of AAR in the NS-S group might be some difficulties caused by HiSt in withholding the response. In order to test this possibility, the dynamics of extinction of AAR at the final period of the retention test was compared. The mean level of AAR performance was computed assuming the first session of the extinction criterion as the reference point on time axis (Fig. 2). Such a comparison, however, could be made only between the NS-S and the NS-NS groups, since some cats from the remaining groups stopped to respond after three or four sessions of the retention test. A factorial analysis of variances (Groups × Days, Type I) mixed design of Lindquist (16), performed on the data from five consecutive sessions preceding the first session of the extinction criterion, revealed a significant effect of Days ($F_{4,56} = 19.65, P < 0.01$) but the Group effect as well as Groups × Days interaction were insignificant. This result suggests that the stimulated cats were able to refrain from responding to the CS with the same efficiency as unstimulated animals after they had found that CS was not followed by US.
As it was mentioned above, in four cats of the NS-S group, during the retention test, the AAR was inhibited when the hippocampus was stimulated at 100 \( \mu \text{A} \) intensity. (The result obtained on these animals were excluded from the statistical analysis). In two of these cats this inhibitory effect consisted in a marked lengthening of the response time (the animal's movements were extremely slow) but the level of AAR performance was high (90–100\%). After two sessions the HiSt intensity was reduced to 75 \( \mu \text{A} \). It resulted in a disappearance of the inhibitory effect; one of these two cats attained the extinction criterion after 18 and the other one after 22 sessions of the retention test. In the remaining two cats arrest reaction followed by a series of miaows was evoked by HiSt. The conditioned response was completely inhibited in one and partially in the second animal. The reduction of HiSt intensity to 75 \( \mu \text{A} \) after two sessions eliminated the symptoms suggesting seizures and the performance returned to a high level. However, the number of sessions to the extinction criterion in these two cats was smaller than in the remaining animals of the NS-S group (15 sessions in both cases).

![Diagram showing the extinction rate in stimulated (NS-S group) and unstimulated (NS-NS group) cats at the final phase of the retention test.](image)

The group means for each session were calculated assuming the first session of the extinction criterion as the reference point. Thus, the left parts of the curves represent the AAR performance during four successive sessions just preceding the extinction criterion. The right parts represent the extinction criterion.
common feature of these four animals was a very symmetrical placement of electrodes. Both, were located within CA-1 field, near the subiculum border at the same stereotaxic plane. It might be the factor responsible for the high sensitivity to stimulation.

Effect of HiSt on the extinguished AAR. Stimulation at 20 cps as well as at 50 cps frequency, applied jointly with the CS after extinction of AAR in cats of the NS-NS group, had no effect on behavior in six animals. No signs of fear or motor agitation were observed and the AAR did not appear. In two cats, however, the extinguished response reappeared at 50% and 60% level in the first session when 20 cps HiSt was applied. During successive three sessions with the same parameters the level of AAR decreased to 20% but it increased again (up to 100% in one animal and to 40% in the other) when the stimulation frequency was raised to 50 cps. However, the response extinguished rapidly during the following three sessions.

Retraining. In this test there were no differences between cats belonging to different groups (S-S or S-NS) during the main part of the experiment. Application of HiSt as CS in the S-T group resulted in a gradual increase of the number of intertrial responses (ITR). The number of jumps onto the shelf during HiSt increased slowly. When the HiSt CS was replaced by the acoustic CS, the level of AAR performance increased to 90–100% in the first session with this CS and remained that high in the successive three sessions (Fig. 3A). The results obtained in the T-S group were similar, but the level of AAR to the tone CS increased more gradually than in the S-T group (Fig. 3B). A factorial ANOVA (Groups × Days × CS type, Type VI) mixed design of Lindquist (16) showed that the effects of all three factors on the level of AAR performance were significant (effect of Days: $F_{3,24} = 12.37$, $P < 0.01$; effect of CS type: $F_{1,8} = 6.51$, $P < 0.05$; effect of Groups: $F_{1,8} = 8.97$, $P < 0.025$). The Days × CS type and the Groups × CS type interactions were also significant ($F_{3,34} = 6.75$, $P < 0.05$, and $F_{1,8} = 138.60$, $P < 0.01$, respectively). As for the ITR data, the analysis revealed only a significant Days × CS type interaction ($F_{3,34} = 4.10$, $P < 0.025$) and a significant Groups × CS type interaction ($F_{1,8} = 26.88$, $P < 0.01$). A regression correlation analysis showed the existence of a positive correlation between the level of AAR performance and the number of ITR-s when the HiSt CS was used ($r = +0.7$, $P < 0.02$), but not when the acoustic CS was applied ($r = +0.3$, $P > 0.05$). It suggests that the avoidances made during HiSt might be in fact spontaneous jumps and that the HiSt was not recognized by the animal as a conditioned signal.
The results obtained in the present study have shown that the application of HiSt during trials in an active avoidance situation may exert a strong facilitatory influence on the performance and on retention of the conditioned response. However, they have shown that the appearance or non-appearance of this facilitatory effect depends on several factors. As we have seen in the present as well as in our previous study (9), the HiSt produced AAR facilitation may be easily replaced by AAR inhibition (and vice versa) by a change of the HiSt parameters. It proves that the type of effect depends on the HiSt strength. Location of electrodes seems to be of no importance for obtaining the two type of effects, although at some electrode location (or configuration of electrodes when a bilateral HiSt is applied) the inhibitory effect may appear
at lower current intensities. The question arises why this facilitatory effect of HiSt applied during trials has not been noted in experiments of other authors. A possible answer lays in its duration. The inhibition of responding occurring during a strong HiSt is a short-lasting effect. If seizure activity is not precipitated, it ends almost immediately after the HiSt termination, being frequently followed by a facilitatory rebound (5, 12, 17). On the contrary, as our previous data have suggested (8), the response facilitation produced by a weak HiSt may outlast the HiSt even by several minutes. It allows to understand why this effect might have passed undetected in stimulation experiments in which comparisons were made between stimulation and nonstimulation trials presented in a random order during the same experimental session (e.g., 5, 17). Under such conditions no effect or only the response inhibition produced by a strong HiSt might appear as a reliable outcome. On the other hand, the facilitatory influence may be easily detected when the comparisons are made between stimulation and nonstimulation sessions spaced a day apart (8), or between stimulated and nonstimulated animals as in the present experiment.

Another factor which has not been discovered previously, and which determines the occurrence of the facilitatory effect of HiSt on AAR performance is the phase of experiment at which it is introduced to the experimental procedure. As we saw, the 20 cps, 100 μA HiSt introduced after training (NS-S group) shortened temporarily the response time and helped to maintain a high level of AAR performance during many successive sessions. On the other hand, the same HiSt applied from the beginning of training (S-S group) influenced neither the acquisition rate nor the response performance during the criterion phase of training and during the retention test. The lack of effect on the acquisition rate is in contrast with the facilitation of learning obtained in a shuttle box (9, 10). However, normal cats in the one way active avoidance situation attain 90–100% level of AAR performance after two or three training sessions, which may be an effective ceiling for this task. In such a case an improvement of learning may be simply impossible. The lack of effect of the HiSt applied during training was apparent. Its significance for the learned behavior became obvious when, after the HiSt discontinuation in the S-NS group, a rapid decline of the level of AAR performance occurred. This latter result is similar to the dissociation phenomena know from drug studies (20). A similar effect may be also observed when after training to a compound CS the performance is being checked to each of the compounds presented separately (9). However, as the data have shown, the HiSt used in the present studies did not acquire CS properties. It also produced no signs
of fear or motor agitation when it was presented after the extinction of the AAR. Therefore, it seems justifiable to assume that the HiSt effect might consist in an unspecific subthreshold activation of some neuronal areas involved in the process of conditioning. The lack of overt manifestation of this effect during training and the fast AAR extinction after the HiSt discontinuation in the S-NS group makes it possible to assume that either the excitability changes due to the process of learning grew smaller in the constant presence of the excitatory influence, or that an inhibitory process developed which counterbalanced the HiSt produced surplus excitation within the AAR circuitry. The gradual disappearance of the facilitatory effect and the fast decline of responding at the end of the retention test in the NS-S group may confirm the latter supposition.

The main question concerns the processes which may be directly influenced by HiSt. As we have seen in the present study HiSt introduced after training facilitated the AAR, but it had no effect on behavior in the majority of cats of the NS-NS group when it was applied after AAR extinction. This confirms our previous observations (11) which showed that the effect of HiSt on behavior depends on the animal's previous experience with the situation in which it is stimulated. It looks like the HiSt, having no cue properties and motivational value of its own, might somehow amplify the motivational value of the stimuli present in the environment according to the animal's experience. Thus, it may be assumed that HiSt influence directly sensory and/or associative processes, or, according to Konorski's model of the reflex arch type II (13), the cortical CS representation and its associative links with the motivational (drive) centers on the one hand and with the cortical representation of US on the other. From this point of view the reappearance of the extinguished AAR during HiSt in the two cats from the NS-NS group may be interpreted as a result of the ambiguous motivational significance of the CS caused by the use of the two antiextinction trials at the beginning of each session during the retention test.

It cannot be stated at present whether the facilitatory effects of HiSt are due to an enhancement or to an interference in the physiological function of the hippocampus. In any case, they do not resemble the disruptive effects of hippocampal lesions or cutting the hippocampal connection on the retention of one way AAR found in the cat (6, 18, 25). It is worth mentioning here that a moderate HiSt may facilitate the generation of theta rhythm in the hippocampus (15, 19). Moreover, the changes in the amplitude of cortical evoked potentials produced by a low intensity and low frequency HiSt resemble those appearing during theta rhythm (21). It is possible that the behavioral effect of a weak HiSt may be related to the electrophysiological ones mentioned above.
REFERENCES


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