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Modified retention of punishment with unilateral, single-pulse stimulation of amygdala but not hippocampus

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Following surgical preparation, male hooded rats were trained to descend from a platform and cross a grid floor for food. Upon reaching a performance criterion, a single treatment trial was administered. Upon descent from the platform on the treatment trial, the subjects received footshock, which was followed immediately by the delivery of a single .5-msec, 100-μA pulse of intracranial stimulation delivered unilaterally to either the amygdala or dorsal hippocampus. Performance on recall tests administered 24 and 48 h after the treatment trial revealed memory alteration in amygdala-stimulated subjects but not in hippocampus-stimulated or control subjects. These results suggest that the amygdala and hippocampus may have different roles in memory processing. The contribution of amygdala in memory processing may involve affective quality and quantity of specific events. A new interpretation of the nature of memory modification by electrical brain stimulation is discussed.

Probably one of the more promising approaches to the study of the neural mechanisms of memory is that which looks at the effects of electric current applied to restricted areas of the brain (ICS) without attending seizure discharges. Several brain structures have become prime candidates for such study, particularly the amygdala (Bresnahan & Routtenberg, 1972; Gold, Macri, & McGaugh, 1973) and the dorsal hippocampus (McDonough & Kesner, 1971; Zornetzer, Chronister, & Ross, 1973). Findings from such studies contribute ultimately toward the localization of brain processing in memory, although it is debatable as to whether memory storage loci are identified.

The amygdala and hippocampus appear not to be uniformly sensitive to the memory-altering effects of electrical stimulation; the dentate gyrus of the hippocampus (Haycock, Deadwyler, Sideroff, & McGaugh, 1973; Zornetzer et al., 1973) and, within the amygdala, the basomedial nucleus (Gold, Edwards, & McGaugh, 1975; Handwerker, Gold, & McGaugh, 1974) and the corticomedial nucleus (Bresnahan & Routtenberg, 1972) have been implicated as being more sensitive to the memory-altering effects of ICS than are other areas within these structures.

Comparisons regarding the role of the amygdala and hippocampus are difficult to make at this time, since studies of memory modification (MM) by ICS suffer from unequal treatment and, thus, unequal knowledge. For example, MM with postlearning-trial hippocampal ICS has been produced with bilateral delivery only. Asymmetrical bilateral stimulation fails to produce MM (Zornetzer et al., 1973). However, can MM occur with unilateral hippocampal ICS? The answer is not available. On the other hand, amygdala-induced MM is observed with bilateral (Gold et al., 1973) and unilateral ICS (Bresnahan & Routtenberg, 1972; Gold et al., 1975). The localization of effects reported in unilateral studies differ, in part perhaps, due to their different parameters of ICS. Both studies may be weakened in terms of localization by the somewhat prolonged ICS durations employed.

The present study was concerned with answering several related questions: (1) Is unilateral dorsal hippocampal ICS delivered following an aversive one-trial learning task capable of producing MM? (2) Is the basomedial nucleus of the amygdala the most sensitive MM area of that structure? (3) How might the above questions be answered if one employed an extremely short-duration (single-pulse) ICS in order to maximize stimulus localization?

METHOD

Subjects

Male hooded rats, 180-200 days of age, served as subjects. They were individually caged and maintained at 80% of their free-feeding weights, under a 12-h-on/12-h-off light schedule throughout the experiment.

Surgery

Surgery was performed on animals anesthetized with sodium pentobarbital (45-50 mg/kg, ip). A Kopf stereotaxic instrument

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was used to chronically implant twisted, bipolar nichrome electrodes (.26 mm diam). Using the coordinate system of König and Klippel (1963), electrodes were directed to the basomedial zone of the amygdala (anterior 5.1, lateral 2.6, vertical −3.6) or the dorsal hippocampus (anterior 3.4, lateral 2.6, vertical −3.2), depending on group assignment for each animal. Several animals were prepared with multiple electrode implants for the purpose of recording electroencephalographic (EEG) activity following ICS delivery. Recording sites included the contralateral amygdala, ipsilateral dorsal hippocampus, and cortex. In addition to the EEG implants, several animals were fitted with safety pin skin electrodes for recording heart rate (HR). All electrodes were soldered to a miniature, multiple pin connector (Winchester), which was secured to the skull with screws and dental cement.

Four groups of animals were employed. The amygdala/ICS (AI) group ($n = 6$) received a unilateral amygdala implant. The hippocampus/ICS (HI) group received a unilateral hippocampal implant ($n = 6$). The ICS/control (IC) group ($n = 6$) were anesthetized and secured in the stereotaxic instrument, received a scalp incision, and had two burr holes drilled through the skull, but received no electrode penetration. Pilot data revealed no differences between operated and unoperated control subjects for the procedures employed in the present study. Three subjects in the footshock control (FC) group ($n = 6$) received an amygdala implant. The other three subjects in this group received no surgical treatment.

Apparatus

A step-down apparatus consisted of a larger chamber (31 × 35 cm) and an adjacent smaller platform chamber (12 × 18 cm), separated by a sliding door. The platform chamber floor was elevated 10 cm above the floor of the larger chamber. When the door was lowered, the animal was free to step down to the stainless steel rod floor (1.3-cm rod separation). A shock generator and scrambler (Davis Instruments) were connected and capable of delivering footshock current through the rods. A food cup supplied with sweetened rat mash was available on the far wall of the large chamber, opposite to the small chamber. A microswitch located beneath the floor of the platform chamber activated an electric timer (Hunter, Model 120A) when the door was lowered. The timer measured the latency between the opening of the door and the animal's descent from the platform. A second microswitch located beneath the platform disrupted the timing circuit when the subject stepped down from the platform.

Intracranial stimulation (ICS) was produced by a Grass stimulator (Model S-8), fed through a Grass stimulus isolation unit (SIU 478-A) and constant current unit (Model CCU-1A), and monitored on an oscilloscope (Tektronix, Model 502).

Procedure

Acquisition trials were given in blocks of six per daily session. On each trial, the animal was placed on the platform and, following an average delay (intertrial interval) of 90 sec, the sliding door was lowered. The animal was able to descend and feed from the food cup for 15 sec before being returned to the platform chamber. Upon reaching the criterion of five consecutive descents from the platform with a latency of 8 sec or less per trial, and immediately after the descent on the next trial (treatment trial), members of various groups received the following treatments: AI group, footshock (2-sec duration, 2.5-mA intensity) followed immediately by ICS (single pulse of .5-msec duration, 100-μA intensity) delivered unilaterally to the amygdala; HI group, footshock (2-sec duration, 2.5-mA intensity) followed immediately by ICS (single pulse, .5-msec duration, 100-μA intensity) delivered unilaterally to the dorsal hippocampus; IC group, footshock (2-sec duration, 2.5-mA intensity); half the FC group, only ICS (single pulse of .5-msec duration, 100-μA intensity) unilaterally to the amygdala; other half of the FC group, neither footshock nor ICS. Following this single treatment trial, the animals were returned to their home cages.

Single-trial recall tests were given 24 and 48 h after the treatment trial. The procedure used for recall tests was the same as that used in the treatment trial, except that footshock and ICS were not delivered. During the test trials, the criterion for "no descent" was defined as 3 min on the platform without descent and was scored as a step-down latency of 180 sec.

Electrographic Recording

Subjects surgically prepared for EEG recording were placed in the large chamber of the step-down apparatus after behavioral testing was completed. Polygraphic recordings were made of EEG activity in several brain structures prior to and following unilateral delivery of a single .5-msec pulse of 100-μA intensity to the amygdala. Polygraphic recordings were scrutinized for possible localized seizure activity or changes in HR activity resulting from ICS delivery.

Histology

Following the 48-h recall test, all animals were sacrificed with an overdose of sodium pentobarbital and perfused with 10% formol-saline solution. Marker lesions were produced at the electrode tips (anodal dc current of .5 mA, 10 sec) to aid in the identification of electrode placements. All brains were stored in formol-saline, stereotaxically blocked, and then sectioned at 50 μ on a cryostatic microtome (Spencer). Sections were stained for cell bodies with thionine and examined microscopically to locate electrode placements.

RESULTS

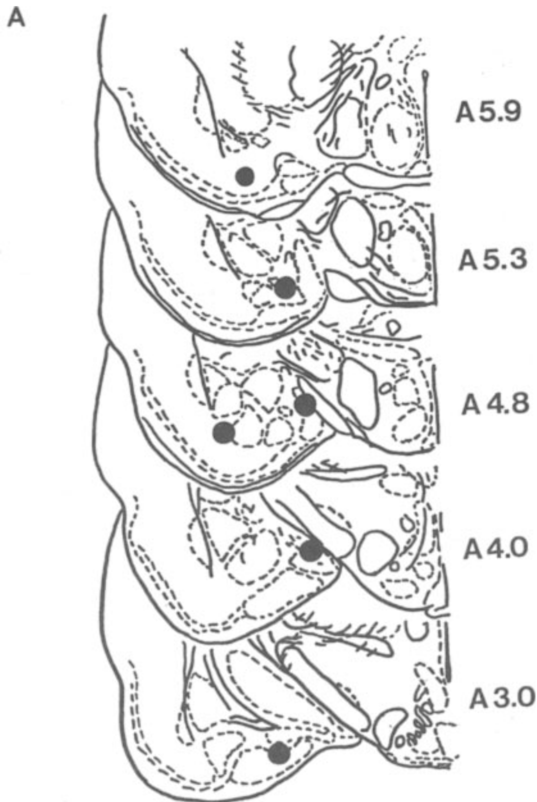
Histology

Microscopic examination of stained brain sections found to contain stimulation electrode tracts revealed electrode placements within or adjacent to intended areas. The histological findings for the AI group are summarized in Figure 1A. Stimulation sites for this group ranged from anterior 4.8 mm to anterior 3.0 mm (König & Klippel, 1963). Electrodes were found to have been implanted in a region including the basal nucleus, the medial nucleus, and the cortical nucleus of the amygdala.

The histological findings for the HI group are summarized in Figure 1B. Stimulation sites for HI subjects ranged from anterior 5.1 mm to anterior 3.2 mm. Four of the stimulation sites were found to lie in the dorsal hippocampus, while the other two electrodes were implanted in the dentate gyrus of the hippocampus.

Behavior

Step-down latency data for the 24- and 48-h tests are shown in Figure 2. An analysis of variance (ANOVA) for a completely randomized design was used to compare the number of training trials necessary to reach the performance criterion for all groups. The results of this analysis revealed that there was no significant difference between the groups in the number of training trials to criterion [$F(3,20) = 1.7$, $p > .05$]. This indicates that the surgical preparations employed had no effect on the acquisition of the food approach response.



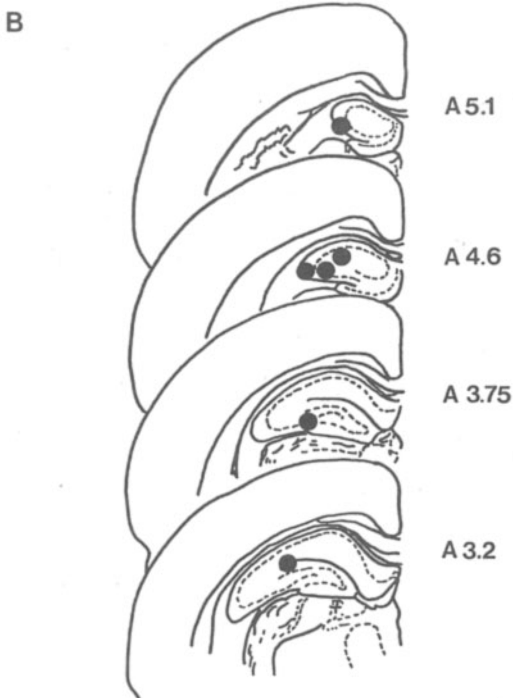


Figure 1. Histologically verified electrode tip placements represented in schematic serial sections: (A) locations of electrodes placed within or near the amygdala and (B) location of electrodes placed within or near the dorsal hippocampus.

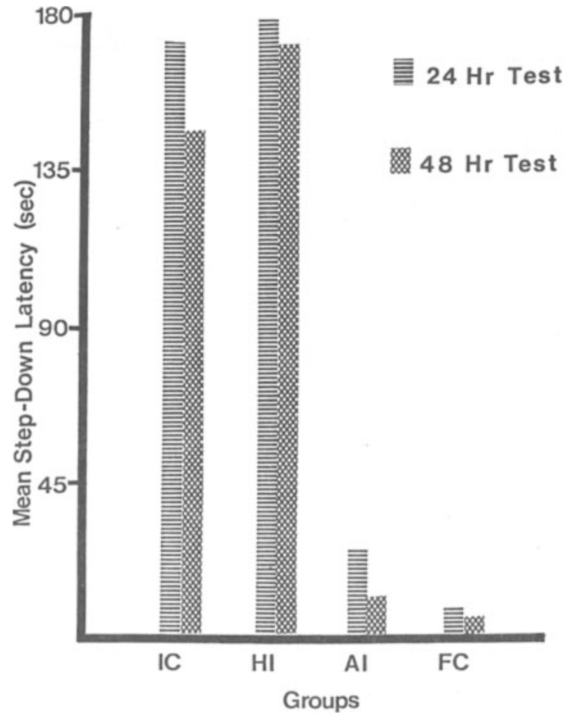


Figure 2. Mean step-down latencies for 24- and 48-h recall tests for animals in the hippocampus-ICS group (HI), amygdalar-ICS group (AI), footshock control group (FC), and ICS control group (IC).

The step-down latencies for both the 24- and 48-h tests were also analyzed using an ANOVA. This analysis of the 24-h latency scores revealed a significant difference between groups [$F(3,20) = 173.9$, $p < .001$]. The source of this variation was identified by a Duncan's multiple range test. The Duncan's test revealed significantly ($p < .05$) longer step-down latencies for the IC and HI groups than were found in the AI or FC groups. Long step-down latencies are indicative of recall of the aversive footshock, while short step-down latencies indicate modified recall of the footshock.

The results of the ANOVA for the 48-h data indicated a significant difference between groups [$F(3,20) = 170.61$, $p < .001$]. The Duncan's multiple range test was employed to identify the source of the variation. This test revealed five significant ($p < .05$) pairwise comparisons. The results of the analysis of the 48-h test data reveal that the AI group had significantly shorter test latencies than the IC, FC, and HI groups. This suggests that there is still a deficit in the recall of the aversive footshock in subjects receiving ICS to the amygdala, but not in the subjects receiving ICS to the hippocampus. The step-down latencies of the HI and IC groups differed significantly on the 48-h test, but this change can be attributed to the performance of one IC subject that stepped down on the 48-h but not on the 24-h test.

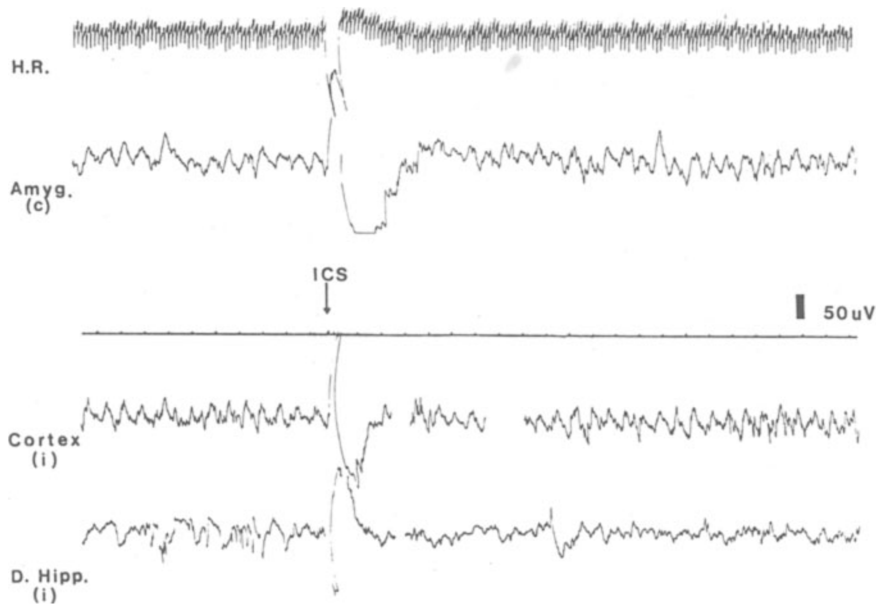


Figure 3. Sample record from one subject of electrographic recordings of EEG and heart rate before and after amygdalar ICS was delivered. Recording sites were: contralateral amygdala (amyg., c), ipsilateral hippocampus (D. Hipp., i), ipsilateral cerebral cortex (Cortex, i), and heart rate (H.R.). Vertical markers on time scale indicate 1-sec intervals.

Electrography

Figure 3 represents a sample of an electrographic record before and after ICS delivery. No electrical afterdischarges were observed in any subject at any of the EEG recording sites following ICS delivery; and there were no changes in autonomic activity (heart rate).

DISCUSSION

The present study found that unilateral amygdalar stimulation resulted in altered recall of a punishing experience in the rat. These animals returned rather rapidly to the area where punishment was delivered 24 and 48 h earlier, whereas the control animals did not. Unlike several studies which applied unilateral stimulation during the learning trial (Bresnahan & Routtenberg, 1972; Goddard, 1964), the memory-disrupting stimulus followed the learning trial in the present study. To this extent, these results are similar to effects reported by Gold et al. (1975). They stated that the most effective memory-disrupting stimulation points were in the basomedial amygdala, whereas in the present study, effective memory disruption resulted from stimulation sites within and outside the basomedial area with step-down latencies not related to the distance of the stimulation site from the basomedial nucleus. A possible explanation for this discrepancy may lie in the difference in ICS intensities employed in the two studies: the present study employed a 100-μA current, while Gold et al. (1975) used 50-55 μA. This turns out to be an improbable explanation, since a subsequent study in our laboratory (Cross & Goodman, 1979), which also employed unilateral single-pulse ICS as low as 25 μA, also noted a widespread array of effective memory-altering sites within the amygdala, some clearly outside the basomedial area and in the piriform cortex.

Our behavioral findings cannot be accounted for by tissue destruction resulting from electrode penetration through the brain. Also, the electrographic data show an absence of electrical afterdischarges in various areas of the brain or autonomic (heart-rate) disturbances following brain stimulation and thus eliminates these as correlates or causes of altered memory.

One must be prepared to recognize that the localization of a vulnerable area of the brain for memory alteration may not be leading one to the "locus of memory." It might be speculated, however, that such a memory-altering area may be a source of influence in normal memory processing in the intact organism. The nature of that influence, in our view, is a basic question. Does amygdalar ICS, for example, render memory more or less complete or, perhaps in the case of an aversive experience, more or less aversive? The latter option may be more attractive, since the amygdala has been associated with emotionality (Goddard, 1964) and thereby may influence the affective quality of memory. Evidence for this argument was provided

by Cross and Goodman (1979), who used graded ICS intensities and heart rate, in addition to step-down latency, as dependent measures.

While this study can report memory-altering effects of unilateral, single-pulse ICS within the amygdala, it has failed to find such effects when the stimulus was delivered to the dorsal hippocampus. This contradicts no previously reported evidence of which the authors are aware. It is compatible with the report of Zornetzer et al. (1973), who found retrograde amnesia produced with bilaterally symmetrical stimulation of the dentate gyrus but not with asymmetrical dentate or nondentate stimulation. The identification of unilateral hippocampal pathology in humans with memory disturbances is well documented (Milner, 1972, 1974); however, its adverse effects in rodents has yet to be demonstrated.

With respect to MM effects of ICS at one or another brain location, it is important to appreciate the role of behavioral task demands. The memory-altering effects of unilateral amygdalar ICS need to be tested for appetitive as well as aversive tasks. Likewise, unilateral hippocampal ICS may, indeed, provide MM for tasks that are more akin to those in which deficits are shown in humans.

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