THE EFFECTS OF HIPPOCAMPAL STIMULATION IN A ONE-TRIAL APPETITIVE LEARNING SITUATION

by

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ABSTRACT

The phenomenon of retrograde amnesia has been widely investigated in connection with the study of memory consolidation. Localized electrical stimulation of the brain has been useful in examining the degree of involvement of different areas of the brain in the production of retrograde amnesia. One of the areas that has been implicated as playing an important role in the consolidation process is the hippocampus. However, it is unclear whether the hippocampus plays a general or a specific role in consolidation, since the majority of the studies examining the effects of hippocampal stimulation used the passive-avoidance paradigm. Therefore, the present study examined the generality of the role of this structure in consolidation by using a one-trial appetitive learning task which involves a different motivational system and different responses than the passive-avoidance task. Experiment 1 established the effectiveness of the one-trial appetitive training procedure in producing learning in the subjects receiving it by comparing the performance of two groups of rats on the task. It was found that rats receiving appetitive training showed superior learning as compared to rats receiving no training.

In Experiment 2, bilateral hippocampal stimulation of 115 μa was administered to subjects for 10 seconds. It was shown that this current was sufficient to consistently produce seizure activities in the hippocampus.

The third experiment compared the performance between subjects receiving bilateral hippocampal stimulation 15 seconds after training and subjects receiving appetitive training but no stimulation. No significant differences in performance were observed between the two groups.

Experiment 4 used two training-stimulation intervals of 0 second and 10 seconds after training. Rats receiving stimulation at either

interval did not differ from a trained but an unstimulated control group, nor did they differ from each other in posttraining performance. It seemed that posttrial hippocampal stimulation failed to impair memory for the appetitive learning experience.

In Experiment 5, a more severe type of amnesic treatment --ECS-- was used. Rats receiving ECS after training showed a highly significant degree of memory impairment as compared to a no-ECS control group. This showed the effectiveness of ECS in producing amnesia as well as the amenability of this type of learning to external interference.

The role of the hippocampus in consolidation was discussed in connection with the present results and the findings of other studies that examined the effects of hippocampal stimulation on consolidation.

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The mechanism whereby information is stored in the central nervous system has evoked much theorization and experimentation (Konorski, 1961; John,1967, 1971; Young, 1951; Hebb, 1959, 1961; Eccles, 1961, 1964). One central concept in this area of investigation is the consolidation of memory. The formulation of the theory concerning the nature of the process responsible for the permanent retention of information was first prompted by Müller and Pilzecker's (1900) observation that recall for a recently learned word list was significantly impaired by the interpolation of the learning of another list. Their results suggested that the formation of permanent memory involved a mechanism which is time-dependent in that its susceptibility to external interference is a function of the time interval between learning and interference. It was suggested that after an experience, memory is maintained in the form of reverberatory circuits which become transformed ("fixed" or "consolidated") into permanent memory traces (Hebb, 1949) and it is during this period of time, while memory persists in this labile form, that it is susceptible to external disruption (Glickman, 1961).

Indirect evidence for a consolidation process also comes from clinical and experimental observations of a phenomenon known as retrograde amnesia. Retrograde amnesia refers to an apparent loss of memory for events immediately or shortly preceding a trauma to the brain. Clinically, retrograde amnesia has been observed in patients sustaining head injuries. These individuals exhibited loss of memory

for events that occurred during a period varying from a few seconds to 30 minutes or more preceding the injury (Russell & Nathan, 1946). In a controlled clinical study, Cronholm and Lagergren (1960) found retrograde amnesia in patients sustaining electroconvulsive shock (ECS) treatment. In their study, groups of patients (total=230) were given a simple learning task 5 seconds, 15 seconds, and 60 seconds respectively before undergoing ECS. It was found upon later testing that these patients exhibited a differential degree of retrograde amnesia as a function of the learning-ECS interval, the shorter intervals being associated with more amnesia. These investigators interpreted their data in terms of the consolidation of memory traces occuring over time after learning.

One of the early experimental studies of retrograde amnesia under more controlled conditions was by Duncan (1949) using ECS as the amnesic agent. In this experiment, rats were trained to avoid an electrically charged grid. The treatment groups received ECS at different time intervals after training while controls were trained in the same way without posttrial ECS treatment. Retention deficits were observed in subjects with a learning-ECS interval of 20 seconds, with progressively less impairment exhibited by treatment groups with longer learning-ECS intervals (40 sec., 1 min., 4 min., and 5 min.) and groups with learning-ECS intervals of 1 hour or longer (4 hrs. and 14 hrs.) did not differ significantly from the controls.

The important observation in this as well as other studies (Hudspeth, McGaugh & Thompson 1964; Chorover & Schiller, 1965, 1966;

McGaugh, 1966; Quartermain, Paolino & Miller, 1965) is the time-dependent nature of the retrograde amnesic effect, which can be graphically represented as the retrograde amnesia temporal gradient.

The shape of the amnesia gradient has been interpreted as reflecting the time course of the underlying memory consolidation process (Glickman, 1961; McGaugh, 1966). Basically, the effect of post-learning ECS on memory was presumed to be a disruption of the process of trace formation and consolidation in the brain. If patterned firing in certain neural structures were involved in the early stages of learning and memory, the passage of a strong current through the brain would disrupt these activities and result in a partial or complete retardation of storage for the learned experience. The time interval between the occurrence of learning and the application of the amnesic treatment would therefore be an important variable in determining the shape of the gradient by varying the time during which consolidation can progress. It is now recognized that the retrograde amnesia gradient is highly variable, depending on the nature of the amnesic treatment. The current view is that this curve represents the susceptibility of memory processes to modification by external disruption (McGaugh & Dawson, 1971; McGaugh & Herz, 1972; Mah & Albert, 1973; McGaugh & Gold, 1974; Gold, Zornetzer, & McGaugh, 1974).

Retrograde amnesia can be produced by a variety of treatments including ECS, intracranial electrical stimulation, various convul-

sive or depressant drugs, hypothermia, and brain lesions. By virtue of the ease and convenience with which it can be administered, ECS has been the most commonly used amnesic agent. However, ECS has one definite disadvantage -- the current tends to have a generalized effect on much or all of the brain, and it is not possible to identify which brain areas are primarily involved in memory consolidation. In spite of the fact that the localization of memory functions in the brain is a controversial and an unresolved problem (John, 1967, 1971), there are numerous studies indicating that certain areas of the brain may be important for memory consolidation.

These areas and structures include the caudate nucleus, the mesencephalic reticular formation (MRF) and, particularly the limbic structures of the amygdala (AMG) and the hippocampus (HPC).

The Caudate Nucleus

Retrograde amnesic effects of posttrial bilateral stimulation of the caudate nucleus was demonstrated by Wyers, Peeke, Williston and Herz (1968) using a passive-avoidance task with rats. Four delay intervals (0.1, 1.0, 5.0 and 30.0 sec.) of stimulation were used but a temporal gradient was not evident. In a later study, Wyers and Deadwyler (1971) observed rats in a passive-avoidance learning situation, using three delay intervals (30, 120, and 300 sec.) combined with three current levels (300, 600, and 900 μa) of stimulation to the caudate nucleus. They were able to show amnesia for the task as well as a temporal gradient of effect. Peeke and Herz (1971)

examined the differential effects of single and multiple stimulation of the caudate-putamen complex in a food-motivated complex maze situation. They found that while both treatments have retroactive impairment effects on memory, multiple stimulation was more effective than single stimulation at a more difficult criterion of learning. Gold and King (1972) stimulated rats in the caudate nucleus at a current level of 1.5 ma (60Hz, 1 sec duration) and found retrograde amnesic effects for a passive-avoidance (step-through, inhibitory) task. They also observed a temporal gradient with amnesia produced by stimulation administered 5 seconds and 15 minutes after learning but not 60 minutes after. Using sub-seizure (350 μa, 0.5 msec single pulse) stimulation of the caudate-putamen complex Haycock, Deadwyler, Sideroff and McGaugh (1973) produced amnesia in the rat, when the stimulation was administered 15 seconds after training.

The Mesencephalic Reticular Formation

In an early study, Glickman (1958) showed that posttrial stimulation of the MRF resulted in amnesia for a single-trial passive-avoidance task. The current intensity was 1.25 v. delivered in a series of three 20-second stimulation with 20 seconds between each stimulation. Later studies using different stimulation parameters, however, found facilitative effects of MRF stimulation on learning. Bloch, Deweer, and Hennevin (1970) used a single post-trial MRF stimulation and obtained positive facilitative effects on the retention of a one-trial discrimination task. More data on

the facilitation of learning by MRF stimulation were provided by Bloch (1970). MRF stimulation (110-220 μa) immediately following a single learning trial for 25 consecutive days of avoidance training similarly enhanced performance in rats (Denti, McGaugh, Landfield, and Shinkman, (1970). Kesner and Conner (1973) provided interesting data showing that low level (20-45 μa) bilateral stimulation of the MRF resulted in amnesia when subjects were tested for retention 10 minutes after avoidance training but no amnesia when tested 24 hours after. This observation established the possibility that the MRF may be involved in the short-term retention of information and not long-term memory. An alternative interpretation is that the stimulation might have proactive interfering effects on performance which dissipated by 24 hours.

The Amygdala

Early studies found that the disruption of amygdaloid activities during learning impaired the acquisition of an avoidance response. Goddard (1964) used posttrial low level (3-13 μa) continuous stimulation of the amygdala for 5 minutes in a passive-avoidance learning situation and effected impairment of the acquisition of a conditioned emotional response (CER). Pellegrino (1965) found that continuous low intensity (20 μa) stimulation of the amygdala, especially its basolateral portion, impaired the acquisition of a passive-avoidance response. Stimulation was delivered unilaterally to the amygdala in both of the above studies.

Data on the effects of amygdaloid stimulation applied after training are not as clear as those on the effects of stimulation applied during training. McIntyre (1970) showed that bilateral, kindled convulsions elicited through unilateral amygdaloid stimulation. in rats were highly effective in impairing the acquisition of a passive-avoidance response. It was also shown in the same study, however, that unkindled, unilateral amygdaloid stimulation with low level current (50 μa) applied after training trials did not result in retention deficits for the same task. This result seems to be in discord with that of Goddard's (1964) study which demonstrated the retrograde amnesic effects of low level unilateral amygdaloid stimulation applied also immediately after training and lasting for 5 minutes. On the other hand, it should be noted that stimulation parameters were different between the two studies, which may account for the conflicting results. McIntyre (1970) used a higher current level of 50 μa with a brief duration of 15 seconds whereas Goddard used a lower level but for a much longer duration of 5 minutes. Also, in the Goddard study, subjects were liberally stimulated for threshold gauging before testing as well as for a large number of trials (blocks of 20) during testing, thus the possibility of kindling effects exists. In McIntyre's study, subjects receiving posttrial stimulation had never been stimulated prior to the application of treatment. Lidsky, Levine, Kreinich and Schwartzbaum (1970) also found that low level (20-30 μa) post-

trial amygdaloid stimulation for brief (30 sec.) as well as prolonged (5 min. as in Goddard, 1964) durations, and intermediate level (40-60 μa) stimulation for brief intervals failed to produce retrograde effects on avoidance learning. Retrograde impairment was produced only when high level stimulation (100-200 μa) was used with accompanying behavioural seizure and afterdischarges (ADs). It is not clear which factors contributed to the discrepant results, though the use of bilateral stimulation and the apparent proactive effects lasting up to 24 hours in the subjects receiving seizure level stimulation (Lidsky et al., 1970) may play a role. However, recent studies all showed that low level (25-50 μa, for 10 sec.) sub-seizure, bilateral stimulation of the amygdala is effective in affecting posttraining memory processes. Such stimulation produced amnesia for an active-avoidance task (Handwerker, Gold, and McGaugh, 1975) when administered 1 minute after, and for a passive-avoidance task when administered 1 hour after training (Gold, Marci, and McGaugh, 1973b). Also, Gold, Hankins, Edwards, Chester, and McGaugh (1975) showed that low intensity amygdaloid stimulation has amnesic effects for a passive-avoidance task under the condition of high intensity (2 ma, 2 sec.) footshock in training, but facilitative effects under the condition of low intensity footshock (0.5 ma, 0.5 sec.).

Two studies by Kesner (Kesner & Doty, 1968; McDonough & Kesner, 1971) using cats as subjects in a one-trial passive-avoidance

situation, examined the effects of amygdaloid and hippocampal stimulation. Using seizure (ADs) threshold posttrial unilateral stimulation of the amygdala or the dorsal hippocampus, these investigators demonstrated amnesia for the aversive experience in the subjects. On the basis of a more detailed analysis of the data, Kesner and Doty held that the amygdala played a more important role in consolidation than the hippocampus, since amygdaloid stimulation consistently produced amnesia whereas stimulation at other sites including the ventral hippocampus (and the dorsal hippocampus in several cases) failed to do so. In the McDonough and Kesner (1970) study, however, it was found that brief (5 sec.) low intensity bilateral stimulation of either the amygdala (at 0.07-0.7 ma) or the hippocampus at (0.04-0.5 ma) each produced amnesia for the aversive experience in the passive-avoidance task.

The Hippocampus

One of the earlier works that observed possible relationships between the hippocampus and memory functions was a clinical study by Penfield and Milner (1958). They derived their data from observations of patients sustaining lesions of the hippocampus and the hippocampal gyrus who showed difficulty in storing new information, as well as retrograde amnesia which sometimes extended preoperatively for a period of several months. This prompted increased interest in the effects of hippocampal lesions on learning in more controlled situations using animals as subjects. Kimble (1963) observed rats with bilateral hippocampal lesions in a passive-avoidance situation and found that these subjects showed significantly impaired performance of the avoidance response. Similar findings were provided by Kimble, Kirby, and Stein (1966) showing that bilaterally hippocampectomized rats were impaired in the performance of a previously learned passive-avoidance response. Hostetter and Thomas (1967) presented data showing that hippocampal lesions in rats did not interfere with post-operative learning of a passive-avoidance response. Such lesions, however, attentuated the amnesic effects of ECS on the task. The authors offered the interpretation that the hippocampal structures were involved with memory consolidation processes.

On the basis of the above data, it is reasonable to expect that posttrial temporary disruption of hippocampal activity by

electrical stimulation will disrupt the consolidation process. Lidsky and Slotnick (1970) studied the effects of posttrial stimulation of the dorsal hippocampus as well as ECS on learning and memory in mice in a one-trial passive-avoidance situation. They used low level (3 v.) stimulation which produced mild behavioural orienting responses but no convulsions. Hippocampal stimulation and ECS were both administered an average of 40 seconds after training. Hippocampal stimulation was delivered bilaterally and for a duration of 6 seconds. Their results clearly showed that both hippocampal stimulation and ECS produced a retention deficit for the aversive experience of footshock received during training. It should be noted that the amount of amnesia produced by hippocampal stimulation was comparable to that produced by ECS. No correlation was found between the placement of electrodes and the degree of amnesia produced. Another study (Barcik, 1970) also showed the amnesic effects of posttrial hippocampal stimulation to be comparable to that of ECS. Afterdischarge-eliciting bilateral stimulation was delivered to the dorsal hippocampus shortly after training. The 10-second, 3.0-3.6 v. stimulation resulted in a significant amount of retention deficit which was comparable to that resulting from ECS.

Brunner, Rossi, Stultz, and Roth (1970) reported the effectiveness of posttrial low level unilateral stimulation of the dorsal hippocampus in the production of retention deficit in a one-trial

passive-avoidance task for rats. The subconvulsive stimulation used was a 1.5-second train of current at 25-50 ma delivered immediately following the conditioned stimulus of footshock and an identical train 10 seconds later. Vardaris and Schwartz (1971) also reported that unilateral stimulation of the hippocampus given within 3 seconds after passive-avoidance training interfered with the retention of an aversive experience. The current intensity was 120% above individually determined thresholds to ensure the elicitation of afterdischarge activities without gross motor convulsions. On the basis of the observation that the stimulated subjects showed some residue of memory for the aversive experience, these authors concluded that the stimulation only produced a relative retention deficit rather than total amnesia.

Shinkman and Kaufman (1969) obtained impairment of CER acquisition, using bilateral hippocampal stimulation given daily following each CER trial, for 7 days. In a subsequent study, Shinkman and Kaufman (1970) found that low level (2-12 v.) bilateral stimulation of the dorsal hippocampus did not affect the acquisition of CER. The current level was predeteremined to produce behavioural orienting responses and was delivered in three 15-second bursts each separated by 5 seconds. Three training-stimulation intervals from 0 second to 5 minutes were used to detect any temporal effect but there were no significant differences among the groups which all showed retention for the aversive experience. However, when higher level stimulation (7-21 v.), sufficient to produce hippocampal afterdischarges

was used, retention deficits were observed. Their data showed that seizure threshold hippocampal stimulation given immediately after each training trial significantly impaired CER acquisition whereas delayed stimulation given 30 seconds or longer (5 min.) after were ineffective. Thus an "all-or-none" time-dependent effect of posttrial hippocampal stimulation on rentention was observed, though a temporal gradient of effect was not demonstrated. To examine the possibility of a temporal gradient of amnesic effect was the purpose of another of Shinkman and Kaufman's studies (1972b). At first, they used low level posttrial stimulation (50-187 μa, $\bar{X}$=147 μa; duration=30 sec.) which failed to interfere significantly with CER learning, even when given immediately after training. Consequently, stimulation at the higher level of 200 μa for a duration of 10 seconds was used. Six training-stimulation intervals were adopted to examine the temporal gradient effect, these being 0 sec., 10 sec., 20 sec., 30 sec., 40 sec., and 120 sec. after the receipt of footshock in CER training. Their results showed a clear temporal gradient effect, demonstrating an inverse function between retroactive effects of hippocampal stimulation and training-stimulation time intervals. Retention deficit was found to be complete when stimulation was administered immediately after footshock, partial 10-40 seconds after, and absent 120 seconds after. Afterdischarges were consistently observed in subjects receiving either the threshold (50-187 μa) or the 200 μa stimulation, with the latter group showing occa-

sional mild behavioural seizure without convulsions. No correlation between electrode locations and amnesic effects was observed. This study is the first to demonstrate systematically a clear temporal gradient of the effect of posttrial hippocampal stimulation in rats, though other studies have shown a less clear-cut and less robust gradient in cats (McDonough & Kesner, 1971), or a longer gradient in mice (Lidsky & Slotnick, 1971). McDonough and Kesner (1971) used low level stimulation (0.04-0.5 ma) and found two out of four subjects receiving stimulation 30 minutes after training showed amnesia and one out of two subjects stimulated 60 minutes after showed amnesia. Lidsky and Slotnick (1971) used an intermediate level stimulation (100 μa) and found amnesia in subjects receiving stimulation up to 2.5 hours after training.

There are several studies examining the amnesic effects of hippocampal stimulation along with those produced by amygdaloid stimulation (Kesner & Doty, 1968; McDonough & Kesner, 1971; Lidsky & Slotnick, 1971; Bresnahan & Routtenberg, 1972). Kesner and Doty (1968) held that the amygdala played a more critical role in mnemonic processes than the hippocampus, since they found that stimulation of the ventral hippocampus, fornix or septum which induced ADs in the dorsal hippocampus all failed to produce amnesia, and likewise in five cases stimulation of the dorsal hippocampus was ineffective. On the contrary, stimulation of the amygdala was consistently effective

in amnesia production. They suggested that when stimulation of the dorsal hippocampus was successful in producing amnesia, it was due to propagation of ADs to the amygdala. Stimulation was delivered unilaterally 4 seconds posttrial. The current used was of threshold intensity (1.0-2.0 ma) eliciting AD activities but not overt convulsions.

The following information from other studies, however, severely limits generalization about differential disruptive actions of hippocampal vs. amygdaloid stimulation. (1) A study by Lidsky and Slotnick (1971) found that while bilateral amygdaloid stimulation resulted in a clear deficit in retention, the degree of amnesia was significantly less than that produced by stimulation of the hippocampus or ECS, the latter two conditions being equally effective in amnesia production. The authors pointed out that it was significant that bilateral hippocampal stimulation was equally as effective as ECS in amnesia production, since they may have a common basis of action, namely hippocampal seizure activities. In contrast to the view of Kesner and Doty (1968), they suggested further that the amnesic effects of amygdaloid stimulation may be the result of induced hippocampal activities. Furthermore, it was found in Lidsky et al. (1970) that subthreshold (20-30 μa, 5 μa below individually determined thresholds) amygdaloid stimulation for 30 seconds or 5 minutes after training failed to produce any retention deficits. An intermediate current at self-stimulating level (50-80 μa), administered in 10

intermittent bursts of 0.5 second each, still failed to have any disruptive effect. It was only when a high level current of 100-290 μa delivered immediately after training was used that a retrograde amnesic effect was observed. Stimulation at this level was shown to produce motor seizure as well as massive amygdaloid ADs followed by postictal depression which was propagated to the neocortex to produce cortical depression. It seems, then, that seizures initiated in the amygdala by such high level stimulation would have also involved the hippocampus. (2) Using cats, McDonough and Kesner (1971) showed that brief (5 seconds) low level, bilateral stimulation of either the amygdala or the hippocampus was consistenly effective in producing amnesia for a passive-avoidance task and there were no significant differences between the amount of amnesia in the two conditions. No ADs were elicited as a result of the low level intensity used in this study. It is unlikely, therefore, that when ADs were absent in the hippocampus, there should be any propagation of disruptive activities to the amygdala, the latter condition being presumed by Kesner and Doty (1968) to be important in amnesia production. In view of these results, the investigators (McDonough & Kesner) suggested that neither structure was the site for long-term storage of information, while both may be part of a critical system. (3) Zornetzer and McGaugh (1970) produced amnesia (in a magnitude comparable to that produced by ECS) by stimulating the frontal cortex in amygdalectomized rats. This finding suggests that the amygdala

is not critical in the disruption of consolidation processes. (4) Kesner and Doty (1968) used cats in their study, and differences, anatomical or otherwise, exist between the amygdala and the hippocampus of the cat and those of the rat (Fonberg, 1967). Most data in this area of research are obtained from studies using the rodent (rats or mice) as subjects.

On the other hand, support for Kesner and Doty's position comes from the data provided by Bresnahan and Routtenberg (1972). They showed that sub-seizure (5 μa) unilateral stimulation of the hippocampus did not result in memory disruption, whereas similar stimulation of the amygdala did. Again, important paradigmatic differences preclude direct comparisons between these two studies, especially with regard to the fact that continuous rather than posttrial stimulation was used in Bresnahan and Routtenberg's experiment.

Zornetzer, Chronister and Ross (1973) found in their study on the effects of hippocampal stimulation in mice that there is a strong relationship between electrode location and amnesia production for a passive-avoidance response. Employing a current level 25% below individually determined AD thresholds (20-175 μa, $\bar{X}$=70 μa for 1 sec.) they showed that such treatment resulted in a significant degree of amnesia only when both electrodes were located in the dentate region of the dorsal hippocampus, stimulation with electrodes in other areas of the hippocampus or surrounding tissue being ineffective. It was further shown that suprathreshold stimulation (25% above seizure threshold: 12.5-137μa, $\bar{X}$=49 μa) produced a greater degree of amnesia than was produced by subthreshold stimulation, with bilaterally symmetrical location of electrode tips in the dentate regions of both hippocampi as a pre-condition. The authors recognized that in the case of asymmetrical electrode placement the stimulation may spread bilaterally to interfere with normal neural activities in the contralateral hippocampus, but suggested that such action is ineffective in disrupting consolidation, whereas bilateral interference of neural functioning as a result of "initiation-in" a structure, as opposed to "propagation-to", is effective in this respect. However, the data were not discussed in consideration with the existing evidence on the effectiveness of unilateral stimulation in amnesia production, notably the findings of Lidsky and Slotnick (1971) and others (Kesner & Doty, 1968; Bresnahan & Routtenberg,

1968). Furthermore, several investigators also have observed, on the basis of histological analysis, that there seems to be no systematic relationship between electrode placement within the hippocampus and the magnitude of amnesia produced (Shinkman and Kaufman, 1970, 1972b; Lidsky & Slotnick, 1970). Of special relevance are the findings of Sideroff, Bueno, Hirsh, Weyland and McGaugh (1974) that sub-seizure (175-200 μa, 0.25 msec., 1 pps for 5 sec.) unilateral or bilateral stimulation of the dorsal hippocampus at CA1, CA3, or the dentate area is equally effective in producing amnesia for a passive-avoidance task. Both unilateral and bilateral stimulation produced amnesia when administered 10 seconds or 3 hours but not 6 hours after training. Nevertheless, the data of Wyers et al. (1968) on posttrial hippocampal stimulation, though somewhat incomplete, seem to offer some support for Zornetzer et al.'s conclusion. It was shown that subseizure bilateral stimulation of the dentate regions of the ventral hippocampus of rats produced a significant degree of retention deficit for a passive-avoidance task, while two subjects receiving stimulation of the dorsal hippocampus did not exhibit amnesia. It is difficult, in view of the variability in experimental paradigms and stimulation parameters, as well as species differences in experimental subjects, to precisely account for the discrepancies between the results of these studies.

In contrast to the importance of precise localization within the hippocampus as proposed by Zornetzer et al. (1973), Shinkman and Kaufman (1972a) provided evidence which seems to suggest that large, rather then restricted areas within the hippocampus have to be involved in order for consistent disruptive effects of the stimulation to occur. They showed that while localized posttrial hippocampal stimulation in the dorsal region was effective in amnesia production, large intra-group variability was observed. However, when two pairs of electrodes(an anterior pair located bilaterally in the dorsal hippocampus, and a posterior pair in the ventral hippocampus) spanning a large region of the hippocampus were used, such variability was reduced to a minimum. Data showed that with threshold stimulation (150-260 μa, $\overline{X}$=190 μa, for 30 sec.) which produced ADs as well as behavioural responses, none of the subjects showed clear retention. The use of subthreshold (45-70 μa, $\overline{X}$=60 μa, for 90 sec.) stimulation which produced neither ADs or behavioural effects, was also successful in producing amnesia in six of seven subjects. Again, discrepant data were observed in a later study by the same investigators (Shinkman & Kaufman, 1972b). Using identical experimental paradigms, they found that stimulation of the hippocampus with two pairs of electrodes as in their previous study, but at a high level of current (50-187 μa, $\overline{X}$=147 μa, for 30 sec.) failed to produce amnesia, even when administered immediately after learning. Such stimulation was intense enough to produce both orienting

responses and brief periods of ADs in the subjects. They found that the degree of amnesia in different subjects, though non-significant, was related to the intensity of the individually determined current. Subsequently in a second experiment, a higher level stimulation of 200 μa for 10 seconds was used uniformly for all subjects and was found to be effective in causing retention deficits. These results seem to be inconsistent with their earlier data showing the effectiveness of low level stimulation in producing amnesia. The experimental paradigm was identical in both studies and the only difference which may be important was that in the earlier study (1972a), the effective lower level current ($\bar{X}$=60 μa) was on for a longer duration of 90 seconds than the higher level stimulation ($\bar{X}$=60 μa) of 30 seconds duration in the latter study.

A recent study by Kesner and Conner (1973) attempted to examine whether consolidation involved two processes (short-term and long-term memory) subserved by different neural systems. It was shown that subseizure (15-32 μa for 5 sec.) stimulation of the hippocampus delivered bilaterally 4 seconds after training produced amnesia for an aversive experience in rats. Such amnesic effects were detected only when the subjects were tested after a long delay of 24 hours (as in most studies in this area of investigation). When the subjects were tested after a short delay of 64 seconds after stimulation, no amnesia could be recorded. On the other hand, MRF stimu-

lation produced amnesia at the 64-second retest but not at the 24-hour retest. Assuming that short-term memory is being measured at the 64-second treatment-test interval and long-term memory at the 24-hour interval, these authors suggested that the MRF is involved in short-term and not long-term memory while the hippocampus is involved in long-term and not short-term memory. However, it is possible that the amnesia found at 64 seconds was simply a proactive disturbance produced by MRF stimulation.

Hippocampal stimulation has also produced facilitative effects in several cases (Stein & Chorover, 1968; Landfield, Tusa, & McGaugh, 1973; Erickson & Patel, 1969). Stein and Chorover (1968) used stimulation of the hippocampus in the rat (5.5-10.5 μa) immediately after an appetitive maze task and found that learning was enhanced. Landfield et al. (1973) showed that bilateral stimulation of the ventral hippocampus 5 seconds after training facilitated learning of an avoidance response as well as an inhibitory avoidance response. Such facilitative effect was time-dependent, strongly suggesting that the stimulation acts on posttraining processes. Using low intensity (30 μa for 3 sec.) posttrial stimulation, Erickson and Patel found significant enhancement of discriminative avoidance learning. These results suggest that the hippocampus may be involved in learning and memory and that the facilitation of learning was due to the "arousal" effect of the stimulation (Erickson & Patel, 1969, p. 405) on post-training memory storage process" (Landfield et al., 1973, p. 490).

Task Differences

The basic paradigm of a passive-avoidance task ordinarily involves a situation wherein a subject is first allowed to naturally (as in step-through or step-down tasks) or through training (as in bar-pressing tasks), approach a particular stimulus or make certain operant responses to it, and then later trained through punishment to withhold the same responses, thus resulting in a conflict situation. Such a situation may introduce processes (e.g. inhibitory mechanisms) adding to the complexity of the experimental variable under study. On the other hand, an appetitive learning task is comparatively simpler. An appetitive approach response is based on appetitive reinforcement such as food or water for deprived subjects. In requires the subject to actively approach a desired stimulus rather than to withhold or inhibit a response as in a passive-avoidance situation.

One of the early experiments making use of the single-trial appetitive learning task was undertaken by Tenen (1965). In this experiment, water-deprived rats were trained in a chamber with a niche in one of the walls wherein the spout of a water bottle could be inserted for drinking by the subject. Experimental groups of subjects included rats which were trained in an appetitive task (where water could be obtained from the niche) and given ECS at the end of training. Control subjects were not given appetitive training, or were trained without posttrial ECS. Retention was measured in terms of the number of niche-exploration during the testing session

between groups. Upon testing, it was found that subjects that had previously found water at the niche increased their frequency of niche-exploration. Rats receiving appetitive training but also posttrial ECS explored the niche significantly less frequently than non-ECS controls. Having ruled out the aversive and the proactive effects of ECS as major contributing factors to retention deficits, the author interpreted the data to be evidence for the retrograde amnesic effect of ECS.

Herz (1969) found that ECS administered within 20 seconds after water-reinforcement produced an almost total attenuation of the retention of this experience. Animals administered ECS up to 30 minutes after learning showed partial attenuation, strongly suggesting a graded interference effect. Comparison of results for the two experiments in this study, using aversive and appetitive motivation respectively, demonstrated that when the reinforcement was administered in very similar experimental conditions, the degree of amnesia produced is a function of the type of motivation, among other variables. Graded amnesic effects of ECS on appetitive learning were also observed in Pinel (1969), though only subjects receiving ECS 10 seconds and 1 minute after learning showed significant degrees of amnesia.

The effects of localized stimulation of the hippocampus has also been investigated in an appetitive learning situation. Stein and Chorover (1968) studied rats in a maze-learning situation, using food as reinforcement. Their data showed that when AD-inducing posttrial

hippocampal stimulation was administered in a spaced-trial condition, performance in the maze was enhanced. When stimulation was used under a massed-trial condition, however, disruption of learning was observed. Due to the short interval (6 hrs.) between stimulation and testing and the massed stimulation condition, this disruption may have been caused by proactive effects of the stimulation. Gilman (1970) used an appetitive task to study the disruptive effects of hippocampal stimulation. Bilateral stimulation of the hippocampus was shown to result in an impairment in acquisition of an alternation task in a maze situation. However, continuous rather than posttrial stimulation was used in his experiment, thus precluding conclusions about retrograde effects of the stimulation. A recent experiment by Zornetzer and Chronister (1973) studied the effects of posttrial ventral hippocampal stimulation in mice in an aversive as well as an appetitive situation. Using single pulse stimulation at 500 μa for a duration of 0.05 second delivered bilaterally within 15 seconds after training, they found retention deficits for the appetitive experience in the maze, but no impairment of a passive-avoidance task. The authors suggested that this may be due to the differential thresholds (susceptibility) of the ventral hippocampus to disruption in the aversive as compared to the appetitive situation. However, in an earlier study, Zornetzer et al. (1973) using an identical step-through task, found that stimulation of the dentate dorsal hippocampus was effective in producing amnesia for aversive learning. These

conflicting results may be due to the different current parameters (20-175 μa and 12.5-137 μa for sub-seizure and supra-seizure stimulation respectively) and the different sites of stimulation (ventral hippocampus) in Zornetzer et al. (1970).

Rationale and Method of the Present Study

In summary, the hippocampus appears to be an important structure involved directly, or indirectly as part of a larger system including the amygdala, caudate nucleus and the mesencephalic reticular formation, in the consolidation of memory. It seems clear that learning and memory in a passive-avoidance situation are generally susceptible to the amnesic effects of posttrial hippocampus stimulation, whereas less data is available on the effectiveness of such treatment in an appetitive situation. In view of this, the present study was undertaken to investigate the generality of the role of the hippocampus in memory function, using posttrial stimulation in a one-trial appetitive situation.

The first experiment sought to establish the efficacy of the one-trial appetitive learning procedure in producing learning in the subjects. The second experiment was conducted to confirm that the current selected to be used for hippocampal stimulation in subsequent experiments was sufficient to consistently initiate afterdischarges in the hippocampus. The third experiment compared the performance of subjects receiving bilateral hippocampal stimulation 15 seconds after training to those receiving only training but not stimulation. Since no amnesia was observed, a fourth experiment was conducted to investigate whether stimulation administered concurrently with or at a short interval after training would result in amnesia. Again, no clear-cut amnesia was recorded. The

last experiment, therefore, was included to examine if amnesia would be produced by the application of ECS, since it is a more severe type of treatment presumed to affect more extensive areas of the brain than localized stimulation would.

EXPERIMENT 1

In order to evaluate whether an amnesic treatment disrupts memory consolidation, it is first necessary to show that learning has actually occurred as the result of training. Thus this experiment was conducted to examine the effectiveness of the one-trial appetitive training procedure in producing learning.

Method

Subjects

Subjects were 20 male hooded rats obtained from Canadian Breeding Farm and Laboratories, LaPrairie, Quebec. They weighed 275-340 grams at the beginning of the experiment. Each subject was housed separately in an individual cage, with ad lib supply of Purina rat chow and tap water except when it was on the restrictive drinking schedule.

Apparatus

All behavioural observations and testing were carried out in a lidless plywood box with a mesh-wire floor. This box measured (45x45x45) cm. and was painted black. One of the walls contained a small niche (8x5x9 cm)3.5 cm. above the floor, into which the spout of a water bottle could be inserted from outside when desired. This adjunction was equipped with electric photo-cells which activated a counter whenever the subject poked its head into the niche,

so that the number of niche-explorations (head-pokes) by the subject could be automatically recorded. Latency for the first head-poke was also recorded by a timer started manually when the subject was first put into the box and which was connected to the electric photocells so that it would stop upon the subject's first poke as a result of the disturbance of the light beam across the niche.

Procedure

Two groups of unoperated subjects were used in this experiment: one trained group and one untrained group, including 10 subjects each.

Before the start of behavioural observations, all subjects were habituated for three days to a restrictive drinking schedule of 23-hour deprivation and 1-hour supply of water each day. During this period, each subject was handled each day for 5 minutes as a gentling procedure.

The experiment consisted of 5 daily sessions. Days 1, 2, and 3 were habituation days in which the subjects were familiarized with the apparatus, and the basal rate of activities on the two measures were recorded. On Day 4, the subjects were randomly divided into treatment and control groups. On this day, appetitive training was administered. Behavioural testing occurred on Day 5, approximately 24 hours after training.

On Days 1, 2, and 3 each subject was placed in the apparatus in the middle of the mesh-wire floor, its head facing the wall

opposite the niche. The subject was allowed to stay in the box and explore for a period of 10 minutes during which the latency for the first-head poke and the frequency of niche-exploration (total number of head-pokes) were recorded for the session. At the end of this 10-minute period, the subject was taken out of the box and conveyed back to home-cage where it was given free access to water for a period of 45 minutes to 1 hour.

Appetitive training was given on Day 4. A water spout (same kind as those used in home-cages) out of which the subject could easily drink was inserted into the niche from outside the box for subjects receiving appetitive training and was absent for the controls. At the start of the session, the subject was placed in the apparatus, its head facing the wall opposite the niche. For subjects receiving appetitive training, upon the first poke into the niche, a period of 15 seconds was allowed to elapse during which the subject might or might not have drunk from the water spout. It was assumed that by inserting its head inside the niche, the subject would have at least discovered the presence of the familiar water-spout which had been associated with water because the same kind of spout was also used in home-cage. The mere discovery of the water-spout inside the niche, without actually having drunk from it, has been found to result in significant learning (Albert and Mah, 1973). This procedure constituted appetitive training. At the end of this 15 seconds of training, the subjects were taken out of the appa-

ratus and conveyed back to home-cage.

The untrained controls were not given appetitive training on Day 4 but were left for 15 seconds after the first poke into the niche without the spout in it before being taken out of the apparatus.

Retention testing was undertaken on Day 5. Procedures were identical to those of Days 1, 2, and 3, where subjects were put in the box for a period of 10 minutes without the presence of the water spout in the niche. The 2 behavioural measures of latency and frequency were recorded for the session.

Two experimental measures (dependent variables) were used as operational indicators of memory for the training experience on Day 4: (1) the number of niche-explorations (head-pokes into the niche) during the 10-minute stay in the apparatus, and (2) latency for the first-poke, which was the length of time taken for the subject to make the first poke after being placed inside the experimental box. The rationale behind this is that if a thirsty subject had found water from the water spout inside the niche on training day, it would show its memory for that experience on testing day by going to the niche and poking into it within a shorter time after being placed inside the box (shorter latency for first head-poke) and by poking inside the niche more frequently (increased frequency of niche-exploration) during the 10-minute stay inside the box on the testing day.

Results

One subject in the control group did not make the poking response on training day and data from this subject was discarded. The results from this experiment therefore included data of 10 subjects in the trained group and 9 subjects in the untrained group.

The mean scores of the latency and frequency measures were calculated for the habituation and testing days, as shown in Table 1.

Comparisons by the Mann-Whitney U-test between groups for testing day behaviour revealed a significant difference ($U=18$, $p<0.05$) on the frequency measure (total no. of pokes) but non-significant difference on the latency measure (latency for 1st poke). Within group comparisons by the Wilcoxon Test across days (Day 5 over Day 3) showed that the trained group had an increased frequency ($T=0$, $p<0.005$) and a lowered latency ($T=7$, $p<0.025$) of response. The untrained controls showed no significant differences between Day 3 and Day 5. (Statistical procedures for this as well as subsequent experiments followed Siegel, 1956; all critical values are for one-tailed tests.)

TABLE 1

Performance Data of Experiment 1, Means (& ranges)

A) Latency (in sec.)

	Days			
	1	2	3	5
Trained	32.4 (5-101)	81.1 (6-209)	145.9 (5-600)	37.3 (2-102)
Untrained	99.4 (27-600)	115.5 (10-600)	75.5 (3-364)	109.5 (3-600)

B) Frequency (total no. of response)

	Days			
	1	2	3	5
Trained	10.2 (3-21)	4.1 (1-12)	3.9 (0-7)	10.6 (1-19)
Untrained	9.0 (0-20)	5.5 (0-13)	3.7 (2-8)	3.9 (0-11)

Discussion

These data show that the appetitive training procedure was effective in producing learning in subjects that found water in the niche on training day. These subjects poked in the niche significantly more times on testing day than subjects that did not receive appetitive training. The trained subjects also showed retention of the appetitive experience by making their first pokes within a shorter period of time and made more head-pokes upon post-training testing as compared to their own behaviour on pretraining day, whereas subjects receiving no appetitive training showed no learning in this respect.

Since the between group comparison on the latency measure was non-significant, while the between group comparison on the frequency measure was significant (within group comparison on both frequency and latency showed significant differences), the latter measure (frequency) seems to be a more reliable indicator of learning. Latency values tend to show a high degree of variability among subjects. An early study by Tenen (1965) using the one-trial appetitive task in a condition similar to the present experiment also found that a comparison between groups on the frequency of niche-exploration was the more reliable measure of learning than latency. In the passive-avoidance situation, it was also observed that the frequency of response was the more sensitive measure of amnesia. In a study by Shinkman and Kaufman (1972a), the difference in response latency

between hippocampal-stimulated and control subjects showed the same trend as the frequency measure, but the former did not reach statistical significance as the frequency measure did.

EXPERIMENT 2

This experiment was conducted to assure that the current level to be adopted for hippocampal stimulation in subsequent experiments would be of such an intensity and duration as to consistently produce seizure activities in the hippocampal structure. Such information is necessary in order to infer that the normal neural activities and thus functioning of the hippocampus are disrupted.

Method

Subjects

Eleven naive, male hooded rats obtained from Canadian Breeding Farm and Laboratories, LaPraire, Quebec, served as subjects for this experiment.

Apparatus

Hippocampal stimulation was generated from a 60 Hertz, 115 volt source in series with a 470,000 ohm resistance. The current was of approximately 230 microamps intensity and 10 seconds duration, and was divided between the two bilateral electrodes, yielding approximately 115 μa to each electrode.

Recordings were made on an AC channel of a Beckman Type RS dynograph with a time constant of 0.03, a chart speed of 5mm/sec. and a sensitivity of 100 μv/cm.

Surgery

Stainless steel wires (diameter=0.25 mm) were twisted to make bipolar electrodes for stimulation. The electrodes were insulated except 0.5 mm at the stimulating tips and also were neatly soldered onto stainless steel pins. They were tested for insulation and short-circuit before use. Coordinates for the bilateral implantation were: 3.2 mm posterior to bregma, 2.5 mm lateral to midline, and 2.7 mm below the surface of the dura. The interauralline was 5 mm below the level of the upper incisor bar. Each operated animal was anaesthetized with sodium pentobarbital (Diabutal, 60 mg/kg), mounted on to a stereotaxic surgical instrument and had its skull exposed. The electrodes were implanted through holes in the skull made by a dental drill. They were secured in position by a thin layer of dental acrylic which covered the entire exposed area including three supportive screws anchored in the skull. The electrode leads protruded approximately one half of an inch perpendicularly above the surface of the acrylic mass. Immediately after the operation each animal was administered 0.1 ml streptomycin-penicillin (Crystamycin) intramuscularly. All animals were given a week to recover from the surgery before the commencement of behavioural observation.

Procedure

On the basis of pilot data, the stimulating current was set at 230 μa. Each subject was only administered hippocampal stimu-

lation once for a duration of 10 seconds. During the passage of current, the polygraph leads were shorted to ground and, immediately after current offset, were switched back to the animal. Each subject was taken back to home-cage immediately after recordings were made.

The animals were later sacrificed, intracranially perfused with saline and then 10% formalin and had their brains removed. Each brain was fixed in 10% formalin for at least 48 hours before being sectioned at 40-μ through the electrode tracks by a freezing microtome. Every 5th section was mounted on a glass slide for the examination of the location of the electrode tips within the brain. This was accomplished with the use of a projection microscope (manufactured by Bausch & Lomb) and verification of electrode sites was based on stereotaxic coordinates from the atlas of Pellegrino and Cushman (1967).

The criterion for a correct placement was that both the left electrode and the right electrode were inside the corresponding hippocampus. The histological verification was carried out according to a "blind" procedure.

Results

The results of this experiment are summarized in Table 2. Of the 11 subjects that received hippocampal stimulation, all except one showed primary afterdischarge activities lasting on the average

12 seconds, as well as postictal depression. Recordings were usually lost for about two to three seconds immediately after stimulation was turned off due to polygraph blocking. Primary seizure activities were of a frequency of approximately 4 cycles per second, ranging from 3 to 5 cycles per second. The peak to peak amplitude averaged 280 μv, ranging from 100 to 450 μv. Ten out of the eleven subjects showed postictal depression lasting approximately 3 minutes. Seven subjects showed secondary seizure which appeared approximately 60 seconds following stimulation, with a frequency of 2 to 4 cycles per second and an average amplitude of 150 μv peak to peak.

Recordings of primary afterdischarges in one subject were not obtained due to the fact that the polygraph was blocked for an unusually long period of time after the stimulation. However, postictal depression and secondary seizure activities were recorded in this same subject. It is reasonable to assume that primary afterdischarges may also have been present in this animal.

TABLE 2

Primary Afterdischares (PAD) and Secondary Afterdischarge (SAD) Parameters for Subjects receiving Hippocampal Stimulation

Ss	PAD duration (in sec.)	PAD amplitude (in μv)	PAD frequency (spikes/sec.)	SAD (yes/no)	Postical Depression (yes/no)
1	6	200	4	yes	yes
2	0*	-	-	yes	yes
3	22	125	4	no	yes
4	13	100	4-5	yes	yes
5	10	300	6	no	yes
6	8	325	4-5	yes	yes
7	12	300	4	yes	no
8	13	300-400	4	yes	yes
9	15	350	4	yes	yes
10	9	300	3	no	yes
11	14	450	4	no	yes

*Polygraph blocked for 6 seconds; no seizure seen after this time.

Discussion

On the basis of these results it is reasonable to assume that bilateral stimulation at the intensity of 115 μa will reliably produce seizure activities in the hippocampus, particularly primary ADs and postictal depression. The initiation of these abnormal activities in the hippocampus seems to indicate a disruption of the normal functioning of this neural structure (Doty, 1969), forming the basis for the possible disruptive effects of the stimulation on consolidation processes if they occurred in the hippocampus.

EXPERIMENT 3

This experiment was designed to examine the effects of post-trial hippocampal stimulation on memory for the appetitive experience. The design essentially consisted of training subjects in an appetitive task and then applying stimulation to the hippocampus after the learning experience. If such posttraining stimulation had a disruptive effect on the consolidation process, this effect would be demonstrated as a retention deficit in later testing.

Method

Procedure

Two groups of subjects (naive, male hooded rats obtained from the same source as previous experiments) were included in this experiment, a trained-stimulated group (n=8) and a trained-non-stimulated group (n=8) serving as controls. All subjects in both groups were operated upon and implanted with bilateral stimulating electrodes.

The apparatus, equipment and current parameters were the same as in previous experiments. Procedures for implantation of electrodes and histological examination were the same as those in Experiment 2.

Both groups of subjects were treated according to identical procedures on Days 1, 2, 3, and 5, as described in Experiment 1.

Procedural differences occurred on the training day (Day 4). On this day, just before it was lowered into the apparatus, each subject had the cable from the stimulation equipment attached to its electrode leads. The cables remained attached to the subject during its stay in the apparatus and were detached before they were returned to home-cage. Also, subjects in both groups were given appetitive training. That is, each subject was put into the apparatus and, upon its first poke into the niche, was given 15 seconds to drink or otherwise explore the niche with the water spout in it. At the end of this learning period, each subject was taken out of the apparatus and put into a carrying box. At this point, each subject belonging to the stimulated group received bilateral hippocampal stimulation of 115 μa of constant current for a duration of 10 seconds and was then returned to its home-cage. The unstimulated subjects stayed in the carrying box for the same period of 10 seconds but were given no stimulation before returning to home-cage.

The two dependent variables used here were the same as those in Experiment 1, namely, the frequency and the latency of responses.

Results

The data from one subject in the trained-stimulated group were discarded because this subject's electrode became detached during the session on the training day thus precluding the delivery of

hippocampal stimulation to it. Thus the results of this experiment consisted of data from only 7 subjects in the trained-stimulated group and 8 subjects in the trained-non-stimulated group. (See Appendix A for electrode placements)

Table 3 contains the mean scores for the frequency of niche-exploration and latency for the first pokes into the niche.

Mann-Whitney comparisons of testing day behaviour between the stimulated and the non-stimulated groups showed no significant differences in terms of either latency for first poke or the frequency of niche-exploration. Within group comparisons between Days 3 and 5 for the stimulated subjects showed no significant differences on the latency measure while the frequency of niche-exploration had significantly increased on testing day (Wilcoxon Test: $T=3$, $p<0.05$). For the non-stimulated controls this comparison yielded significant differences on both measures ($T=0$, $p<0.005$ for frequency; $T=0$, $p<0.005$ for latency).

TABLE 3

Peformance Data of Experiment 3, Means (& ranges)

A) Latency (in sec.)

	Days			
	1	2	3	5
Stimulated	74.0(10-152)	64.4(3-202)	70.1(29-116)	29.0(2-168)
N-stimulated	53.1(14-130)	107.5(29-271)	27.4(9-50)	4.4(2-8)

B) Frequency (total no. of response)

	Days			
	1	2	3	5
Stimulated	10.1(3-17)	8.3(2-14)	4.7(1-9)	14.1(5-19)
N-Stimulated	9.1(5-13)	4.9(2-7)	6.8(2-11)	14.5(8-19)

Discussion

These data show that testing performance between the stimulated and non-stimulated subjects did not differ significantly. That is, the stimulated subjects did not show a significant amount of retention deficit or retrograde amnesia when compared to subjects that had received no stimulation to the hippocampus. The within group data also show no retention deficits for subjects in either group. On the contrary, with the exception of the latency measure for the stimulated group, the data show significant retention for the appetitive experience in both stimulated and non-stimulated groups. Thus hippocampal stimulation treatment failed to demonstrate disruptive effects on retention in the present learning situation.

EXPERIMENT 4

In Experiment 3, the training-stimulation interval was 15 seconds after the start of learning, a period long enough for memory to at least partially consolidate as reflected in the progressive decrease in the effectiveness of amnesic treatments to disrupt memory over time (e.g., Chorover & Schiller, 1965). In this experiment, therefore, shorter training-stimulation intervals were examined. By decreasing the time lapse between the occurrence of learning and stimulation to 10 seconds, it was intended that the disruptive effects of hippocampal stimulation upon consolidation, if any, would be more clearly observed. In addition, the effects of stimulation upon learning was also examined by adopting a 0-second training-stimulation interval, wherein stimulation was administered immediately upon and simultaneously with the occurrence of training. If the stimulation did have a disruptive effect on the learning processes in the appetitive situation, this disruption would result in performance deficits in posttreatment behaviour.

Method

Procedure

Subjects were 33 naive, male hooded rats obtained from the same source as previous experiments. They were divided into a stimulated-immediate group (n=14), a stimulated-delayed group (n=12), and a

group of implanted controls (n=7).

Apparatus, equipment, implantation, histology and other procedures were the same as previous experiments.

Trials of habituation and testing were also the same as the previous experiments, while training and stimulation conditions necessitated variation on Day 4 procedures between groups. The stimulated-immediate group consisted of 14 subjects that received hippocampal stimulation immediately upon the first head-poke into the niche, the treatment being administered inside the apparatus for a duration of 10 seconds. For the stimulated-delayed group, 10 seconds were allowed to elapse upon the first poke before stimulation was administered for 10 seconds. The current intensity was 115 μa, same as in Experiment 3. The implanted controls were also given 10 seconds of appetitive training, and 10 seconds of waiting period during which no stimulation was administered before being taken back to home-cage.

Results

As the result of histological evaluation, 3 subjects from each of the stimulated groups were discarded due to incorrect placement of electrode tips. The results presented here included 11 subjects from the stimulated-immediate group, 9 subjects from the stimulated-delayed group and 7 subjects from the control group.

Mean scores for the latency and frequency measures for the

groups are presented in Table 4.

Mann-Whitney comparisons between the 3 groups (stimulated-immediate & controls; stimulated-immd & stimulated-delayed; stimulated-delayed & controls) yielded no significant differences on either measures. For within group comparisons none of the 3 groups showed any differences across days on the latency measure. For the frequency measure, the stimulated-immediate subjects showed significant retention (Wilcoxon T=4.5, $p<0.01$). While the stimulated-delayed subjects also showed an increase on the frequency measure, this increased only approached significance (T=10, $p<0.1$). Significantly increased retention was observed in the control subjects on testing day as compared to Day 3 (T=1, $p<0.05$).

TABLE 4

Performance Data of Experiment 4, Means (& ranges)

A) Lantency (in sec.)

	Days			
	1	2	3	5
Stimulated-Immd.	78.6(16-195)	47.2(5-220)	89.2(6-544)	41.6(2-302)
Stimulated-Del.	44.9(12-96)	95.2(15-495)	53.1(1-185)	122.7(3-600)
Non-Stimulated	52.1(11-125)	96.3(4-600)	98.0(3-259)	76.6(4-485)

B) Frequency (total no. of response)

	Days			
	1	2	3	5
Stimulated-Immd.	6.8(2-9)	4.6(1-10)	4.0(1-9)	11.4(1-50)
Stimulated-Del.	8.6(3-14)	5.8(2-11)	6.4(1-15)	9.7(0-22)
Non-Stimulated	11.0(3-21)	4.9(0-10)	4.0(2-7)	18.3(2-13)

Discussion

These results indicate that hippocampal stimulation did not have any significant disruptive effect on memory for the appetitive experience. Both the stimulated-immediate subjects and the non-stimulated controls showed significant retention on testing day in terms of the frequency but not the latency of responses. The stimulated-delayed group, on the other hand, showed no significantly increased retention as compared to their own performance on pre-training. However, since six of the nine subjects did show an increase in their frequency of responses and such an increase approached significance, it is not likely that the statistically non-significant difference between Day 5 and Day 3 is actually indicative of the disruptive interference of hippocampal stimulation. Furthermore, these subjects did not show any retention deficits when their performance on testing day was compared to that of the stimulated-immediate and that of the control groups; that is, these subjects performed as well as the other groups. There were no significant differences on either frequency or latency between the three groups on testing day. It seems that the stimulation was not effective in producing significant disruption of learning and memory when applied concurrently with, or 10 seconds after training in the appetitive task.

EXPERIMENT 5

In Experiments 3 and 4, hippocampal stimulation administered concurrently with, 10 or 15 seconds after appetitive training failed to disrupt retention for the learning task. In the present experiment a more severe type of amnesic treatment --ECS-- was employed. The amount of amnesia has been found to increase with an increase in the severity of the amnesic treatment, whether by increasing the intensity (Miller, 1968; Haycock & McGaugh, 1968; Ray & Barrett, 1969; Kral, 1972), the duration (Alpern & McGaugh, 1968), or the number (Mah, Albert & Jamieson, 1972; Jamieson, 1972) of ECS. The same phenomenon also occurred with localized stimulation of the cortex (Zornetzer & McGaugh, 1972) and the caudate nucleus (Peeke & Herz, 1971). These findings could be interpreted as indicating that an increase in the severity of the amnesic treatment would produce more severe disruption on neural activities in the brain, since increased electroshock intensity has been correlated with increases in the duration and frequency of seizure discharges (Zornetzer & McGaugh, 1970, 1972). High intensity ECS invariably produces clonic-tonic convulsions in the subject and appears to result in disruption of a higher magnitude and more general involvement of the brain. If ECS applied on training day did result in retention deficits on testing day, then it is plausible that the disruptive effect of ECS is due to the involvement of areas of the brain other than or in addition to the hippocampus. In other words, if the

one-trial appetitive learning task in the present study was amenable to disruption by ECS but not by hippocampal stimulation, then the data could be interpreted to support the view that the failure of hippocampal stimulation to produce amnesia lies in the differences in the amnesic treatment used; that is, the effectiveness of the two types of stimulation (hippocampus vs ECS) is disrupting the consolidation process in this particular situation.

Method

Subjects

Sixteen naive, male hooded rats served as subjects in this experiment. These animals were obtained from the same source as those in previous experiments.

Apparatus

Behavioural testing was undertaken in the same apparatus used in previous experiments.

ECS was administered through a 740 volt transformer in series with a 44,000 ohm resistance and a Hunter timer. The current produced was approximately 16 ma and lasted 0.5 second.

Surgery

Each ECS animal was anaethetized with sodium pentobarbital (Diabutal, 60 mg/kg) and, immediately after the operation, received 0.1 ml streptomycin-penicillin(Crystamycin) intramuscularly. ECS

electrodes were made from small stainless steel screws neatly soldered onto stainless steel pins. For each animal, two ECS electrodes were implanted bilaterally in the skull, piercing it to touch the dura each in a position 1-2 mm posterior to bregma and 3-4 mm lateral to midline. Two additional supportive screws were implanted and the entire exposed area of the skull was then covered with dental acrylic so that the electrode leads would be protruding approximately one half of an inch from the surface. The procedure is similar to that used in Jamieson and Albert (1970).

Procedure

Housing, feeding, restrictive drinking schedules and gentling procedures were identical to those found in previous experiments. Procedures for habituation and testing were also identical to those in previous experiments.

On training day (Day 4), subjects in the ECS group (n=8) were given ECS immediately upon the first head-poke, stimulation being administered inside the apparatus. These subjects were taken out of the apparatus and put into a carrying box while still unconscious and were conveyed back to home-cage when they began standing on their feet. The implanted controls (n=8) were immediately taken out of the apparatus upon the first poke, given no ECS and returned to home-cage.

Results

The electrodes of 1 subject became detached during one of the habituation sessions and this subject was excluded from the experiment. The results presented here included data from 8 stimulated and 7 control subjects.

The mean scores for habituation and testing day behaviour are shown in Table 5.

The ECS subjects showed significantly longer latency (Mann-Whitney U=9, $p<0.02$) and lowered frequency of niche-exploration (U=1.5, $p<0.001$) than the controls. Within group comparisons across days yielded no significant differences between Day 3 and Day 5 for the ECS group on either measure. In the control group, while no significant differences were observed on the latency measure, the frequency of niche-exploration significantly increased on Day 5 over Day 3 (Wilcoxon T=0, $p<0.01$).

TABLE 5

Performance Data of Experiment 5, Means (& ranges)

A) Latency (in sec.)

	Days			
	1	2	3	5
ECS	25.3(13-58)	43.3(6-213)	31.5(5-92)	125.8(9-493)
No-ECS	35.2(20-57)	35.1(10-107)	51.9(5-138)	15.7(2-36)

B) Frequency (total no. of response)

	Days			
	1	2	3	5
ECS	8.3(3-19)	6.8(2-11)	5.4(2-21)	3.4(1-7)
No-ECS	12.9(5-19)	7.4(3-19)	5.9(1-18)	19.3(5-44)

Discussion

The disruptive effects of ECS on the learning and retention of the appetitive experience was demonstrated. Subjects receiving ECS on training day showed a significant retention deficit upon testting as compared to subjects that did not receive ECS. Also, analysis of ECS subject's behaviour across days showed that they did not show any learning since their posttraining performance did not differ from their pretraining performance, even though they had received appetitive training. Subjects that received no ECS and only appetitive training, however, showed significant retention of that experience when tested on Day 5. It should be noted that for subjects in the control group the learning period was very short as they were taken out of the apparatus immediately upon the first poke into the niche. However, they still showed a significant amount of learning for the appetitive experince, while the ECS subjects did not.

Since highly significant differences were observed between ECS-treated and control subjects in this experiment, and the direction of change in behaviour in terms of impaired performance would have been the same if ECS had punishing effects, it is necessary to consider whether this is a likely hypothesis for the interpretation of the data. It has been shown that aversive effects of ECS do not result from a single application (Hudspeth, McGaugh & Thompson, 1964). Moreover, it was shown by Kesner, Gibson and LeClair (1970) that the punishing property of ECS is related to the route of ad-

ministration. Rats given multiple ECS through ear-clip or ear-snaps showed fear behavior but when ECS was administered transcranially (as in the present experiment), no aversive effects were apparent.

GENERAL DISCUSSION

The results of this study showed that posttrial bilateral stimulation of the hippocampus failed to produce any retrograde amnesic effect for a one-trial appetitive learning task. Moreover, this stimulation did not have any disruptive effect even when applied concurrently with learning. These results appear to indicate that the hippocampus is not critically involved in mediating the consolidation of the kind of learning task used in this study -- the one-trial appetitive task. However, there are several alternative possibilities. Before accepting this conclusion it is necessary to consider issues of current parameters, the site of stimulation, the time of the application of the treatment, and the possible reinforcing effects of hippocampal stimulation. Furthermore, the generality of the role of the hippocampus in memory functions will also be examined in connection with, and on the basis of, data derived from stimulation studies.

Current Parameters

The current level for hippocampal stimulation in the present study was of an intensity high enough to consistently produce postictal seizure activities in the hippocampus, as was demonstrated in Experiment 2. The presence of such abnormal activities in the hippocampus is generally assumed to be indicative of a disruption of normal activities and functioning of this neural structure as

the result of externally applied interference through electrical stimulation. Yet, no disruptive effects on consolidation in terms of performance deficits were observed in the present study. However, threshold stimulation of the hippocampus applied either posttrial or concurrently with learning has been observed to retard memory for aversive learning in many cases (Zornetzer et al.,1973; Vardaris & Schwartz, 1971; Shinkman & Kaufman, 1970, 1972a, 1972b; Barcik, 1970). Of special interest are the findings of Arthur (1975) where the acquisition of a conditioned taste aversion was disrupted as a result of bilateral amygdaloid stimulation using identical current parameters as the present study. This shows the effectiveness of the present current parameters in the disruption of learning and consolidation, although in a different situation. Furthermore, it has been found that hippocampal-evoked ADs were not a necessary condition for amnesia production, since disruptive effects were observed, whether the stimulation was below hippocampal seizure level (Zornetzer et al., 1973; Shinkman & Kaufman, 1972a; McDonough & Kesner, 1971; Zornetzer & Chronister, 1973; Wyer et al., 1968,; Haycock et al., 1973; Sideroff et al., 1974), or below behavioural seizure level (Kesner & Conner, 1973; Brunner et al., 1970; Stein & Chorover, 1968; Shinkman & Kaufman, 1972a; Gilman, 1970; Lidsky & Slotnick, 1970; Wyers et al., 1968). Therefore, if subthreshold stimulation was

effective in producing disruption, suprathreshold stimulation should also be likely to do so.

Site of Stimulation

Stimulation in the present study was applied bilaterally to the dorsal hippocampus. Although the importance of intrastructural specificity of stimulation sites in terms of bilaterally symmetrical location of electrode tips in the dentate region of the hippocampus (Zornetzer et al., 1973), and the superior effectiveness of stimulating a large amount of hippocampal tissue (Shinkman & Kaufman, 1972a) have been suggested, neither condition seems to be necessary for amnesia production. Most studies reviewed in the Introduction (with the exception of Zornetzer & Chronister, 1973) have shown the effectiveness of bilateral stimulation to the dorsal hippocampus and they did not report any systematic relationship between amnesia production and electrode location within the dorsal hippocampus. Stimulation of the CA1 or CA2 areas of the dorsal hippocampus was found to be equally effective as stimulation of the dentate area of the dorsal hippocampus in producing amnesia for a passive-aversive task (Sideroff et al., 1974). In fact, it has been shown that unilateral stimulation is also effective in producing significant degrees of retention deficits (Lidsky & Slotnick, 1970, 1971; Kesner & Doty, 1968; Bresnahan & Routtenberg, 1972; Sideroff et al., 1974). Stimulation within the hippocampus (e.g., ventral hippocampus) other than the

dorsal area likewise resulted in disruption of learning and memory in rats and mice (Wyer et al., 1968; Zornetzer & Chronister, 1973). It is possible, then, that memory consolidation may occur through the involvement of the ventral hippocampus, and this interpretation may account for the failure of the stimulation of the dorsal hippocampus in producing amnesia. Again, discrepant findings are observed. Kesner and Doty (1968) produced amnesia by stimulating the dorsal hippocampus of cats but failed to do so using stimulation of the ventral hippocampus. In this case, however, the species difference may account for the conflicting results.

Time of Application of the Stimulation

It has been shown in a passive-avoidance situation using hippocampal stimulation that amnesic effects can be obtained consistently as long as 5 minutes after training (Shinkman & Kaufman, 1970), though most studies showed the effectiveness of stimulation administered within 30 to 40 seconds after training. In the one-trial appetitive learning situation, the effective training-stimulation intervals were similar. ECS administered within 20 seconds (Tenen, 1965; Herz, 1969; Pinel, 1969) and in one case up to 1 minute (Pinel, 1969) was effective in disrupting consolidation. With hippocampal stimulation, the effective intervals were immediately or 15 seconds after training (Stein & Chorover, 1968; Gilman, 1970; Zornetzer & Chronister, 1973). In view of these findings, the learning-stimulation intervals used in the present study, the longest of which was

15 seconds, should also be appropriate for the stimulation to exert its disruptive effects within the period of susceptibility of consolidation to disruption by external interference. This has not been the case in the present study. One possibility is that the mere discovery by the subject of the presence of the familiar water spout in the niche without actually poking into it constituted learning before the onset of hippocampal stimulation upon or after the first poke. Albert and Mah (1972) found that rats reinforced by an empty spout in the niche in a learning situation similar to the task used in the present study learned as much as rats reinforced by a full water spout. They suggested that the drinking spout acted as a strong reinforcer as the result of its continuous close association with water. In the present study, if subjects had sighted the water spout in the niche and waited a period of time before poking into it, consolidation of this experience would have time to progress before the application of stimulation which was contiguous upon the first poke into the niche by the subject. Stimulation applied at this time might be at a point where the susceptibility of the consolidation process becomes minimal. It is unfortunate that the paradigm of the present study did not provide for controlled observation to ascertain whether this was actually the case or not. Whereas it is difficult to completely disregard this as a possible interpretation of the present data, it is of low probability for the following reasons. Firstly, in Albert and Mah's (1972) study, the subjects had actually poked

into the niche and some also appeared to lick and bite at the spout. This seems to have ensured learning whereas in the present study, it was uncertain whether the subjects had actually discovered the water spout before poking into the niche. Considering their deprived state, it is reasonable to expect the subjects to poke into the niche immediately upon or soon after sighting the spout. In such a case, the time for consolidation before stimulation upon the first poke would have been minimized but no amnesia could be recorded even in the stimulated-immediate group in Experiment 4. Secondly, if this kind of "incidental" learning and its consolidation did occur, it was equally probable to have occurred also in the ECS experiment (Exp. 5). However, the findings of Experiment 5 showing clear amnesia in the subjects receiving ECS tend to contradict this hypothesis. Moreover, in a similar learning situation, Pinel (1969) observed clear-cut amnesic effects in spite of the possibility of such "incidental" learning, since a water spout was also used in a niche in his experiment.

The possible Reinforcing Effects of Hippocampal Stimulation

The data of the present study do not lend support to the interpretation that the possible aversive or positively reinforcing properties of hippocampal stimulation had affected the behaviour of the stimulated subjects.

If hippocampal stimulation used in Experiments 3 and 4 had

any aversive effects, the stimulated subjects would have shown them by an increased latency but a decreased frequency of response on testing day, as compared to the unstimulated controls. No such tendencies were observed. Indeed, such aversive effects, if present, would have influenced posttraining performance in the same direction as any amnesic effects. Furthermore, no behavioural signs of avoidance tendencies or conditioned emotional response were observed in the stimulated subjects on testing day.

On the other hand, if hippocampal stimulation had any positively reinforcing effects, such effects would have been identified by post-training approach behaviour or enhanced performance on testing in terms of decreased latency and increased frequency of niche-exploration. No such changes in performance were found in the stimulated subjects as compared to the non-stimulated controls, indicating that at least the effects of the stimulation is not subtantially postively reinforcing.

As a matter of course, it is also possible that hippocampal stimulation has amnesic as well as rewarding properties. In such a case, the two different effects, by affecting performance in opposite directions, would balance each other out, thus resulting in a minimal change in performance in the stimulated subjects, and consequently minimal differences between this group and the non-stimulated subjects. Whereas the paradigm of the present study has not allowed for completely ruling out this possibility, there are

reasons to believe that this is not the case. (1) Hippocampal stimulation was given outside the apparatus in Experiment 3. This excluded the possible association of the niche with any rewarding consequences of the stimulation. (2) The 10- and 15-second delay between the subject's response and the application of the stimulation in Experiment 3 and 4 respectively would have minimized the response-contingent reinforcing effects of the stimulation, if present. (3) Erickson and Patel (1969) had tested for the rewarding properties of hippocampal stimulation in a bar-pressing situation. They found that hippocampal subjects (rats) pressed at a consistently lower rate than hypothalamic subjects as well as non-stimulated controls. Their data also showed that lower level (30 μa) hippocampal stimulation tended to produce a higher rate of pressing than higher level (100 μa) stimulation. The current intensity for stimulation in the present study was 115 μa which is of seizure-inducing magnitude. Indeed, in studies where moderate rewarding properties of hippocampal stimulation were found, low intensity current levels were used, as 50 μa in Ursin, Ursin, and Olds (1966), and about 50-77% of 5.3-13 v. (less than 50% of threshold level) in Shinkman and Kaufman (1972a). Also in Shinkman and Kaufman (1972a) no significant relationship between rewarding and amnesic effects of stimulation was evident.

Inferences concerning the Role of the Hippocampus in Consolidation

The present study used parameters which were found by most investigators to be effective in amnesia production, particularly in the passive-avoidance learning situation, No disruption of learning or memory was found in the present study using the one-trial appetitive learning task. However, it has also been shown that ECS treatment in the present study did result in markedly impaired retention for the same task. It seems, then, the basis for the interpretation of the present results showing the lack of effectiveness of hippocampal stimulation in amnesia production cannot be sought in the above parametric variables, or in the amenability of the learning experience to external interfernce. If it could reasonably be granted: (1) that the hippocampus was not functioning normally due to the stimulation-evoked seizure activities therein and, (2) that ECS at motoric-convulsive level successfully blocked learning and consolidation for this same task and had done so through a general disruptive effect involving most of the neural structures of the brain, then it seems plausible to conclude that the failure of hippocampal stimulation to produce any disruptive effects is an indication that the hippocampus does not play a critical role in the acquisition or consolidation of the one-trial appetitive task.

The findings of the present study that hippocampal stimulation did not disrupt consolidation of an appetitive task is consistent with early data of Correll (1957). This study showed that stimulation

of the posteroventral hippocampus of cats during learning failed to disrupt the development of an appetitively motivated conditioned instrumental response. It is recognized that there are many differences between the present study and Correll's; nevertheless, the two sets of results are in general agreement.

Where disruption of learning and memory was observed in appetitive learning situations (Stein & Chorover, 1968; Gilman, 1970; Zornetzer & Chronister, 1973), unambiguous retrograde disruptive effects have not been demonstrated, with the exception of the last study. Both Stein and Chorover (1968) and Gilman (1970) used large numbers of pretraining threshold-gauging trials and massed stimulation training trials with short intertrial intervals. In Gilman (1970) stimulation was almost constantly on before, during and after learning. Stein and Chorover (1968) used a short training-test interval of 6 hours. In these cases, the possible confounding influence of kindling (e.g., Goddard, 1967; Shinkman & Kaufman, 1972a where seizure threshold was found to have been lowered from repeated stimulation) as well an anterograde effects (e.g., Flynn & Wasman, 1960, where ADs were found to last up to 90 seconds after stimulation; Lidsky et al., 1970) are difficult to dismiss. Zornetzer and Chronister (1973) is the only study demonstrating a clear-cut disruptive effect of posttrial hippocampal stimulation in a one-trial appetitive situation. The source of discrepancy between the present data and those of Zornetzer and Chronister's cannot be

accurately ascertained. A possible explanation may lie in the difference between the two studies in terms of the site of stimulation (dorsal vs ventral hippocampus), different species of experimental subjects (rats vs mice) and task characteristics (open-field vs maze).

While the findings of the present study are far from definitive concerning the effects of hippocampal stimulation on consolidation, they are consistent with a number of studies showing the ineffectiveness of hippocampal stimulation in producing amnesia in aversive learning situations (Flynn & Wasman, 1960; Erickson & Patel, 1969; Zornetzer & Chronister, 1973; Bresnahan & Routtenberg, 1972; Shinkman & Kaufman, 1972b).

In Flynn and Wasman (1960), it was demonstrated that learning occurred as the result of training undertaken during hippocampal afterdischarges elicited by stimulation. Cats given conditioned active-avoidance trials during hippocampal ADs showed a significantly higher rate of response over controls after ADs were discontinued. Such retention indicated that learning had already occurred during training trials under the condition of ADs. Erickson and Patel (1969) showed that at high intensity (200 μa, for 3 sec.) dorsal hippocampal stimulation did not have any disruptive effect on the acquisition of a bar-pressing response to avoid footshock. Though it was not reported in their study whether the stimulation produced seizure or not, it seems likely that at such high intensity, seizure

These studies comprise a variety of learning situations (appetitive tasks, active-avoidance, passive- or inhibitory-avoidance, CER), current parameters (seizure-producing and sub-seizure currents, concurrent and posttrial stimulations, bilateral and unilateral stimulations), sites of stimulation (ventral and dorsal hippocampus or both simultaneously), and species of experimental subjects (rats, mice, cats). There appears to be no commonality underlying these studies that will provide a satisfactory explanation for the lack of disruptive effects of hippocampal stimulation on learning and memory.

The study of the interference effects of localized stimulation on memory has contributed to the understanding of the underlying mechanisms involved in the permanent storage of memory. However, it is clear that much information is still lacking, and many inconsistencies have yet to be resolved. More research is needed to clarify the degree of involvement of different neural structures in various learning situations, and to increase our knowledge of the relationship between electrophysiological events in these structures in the brain and the interference effects of stimulation.

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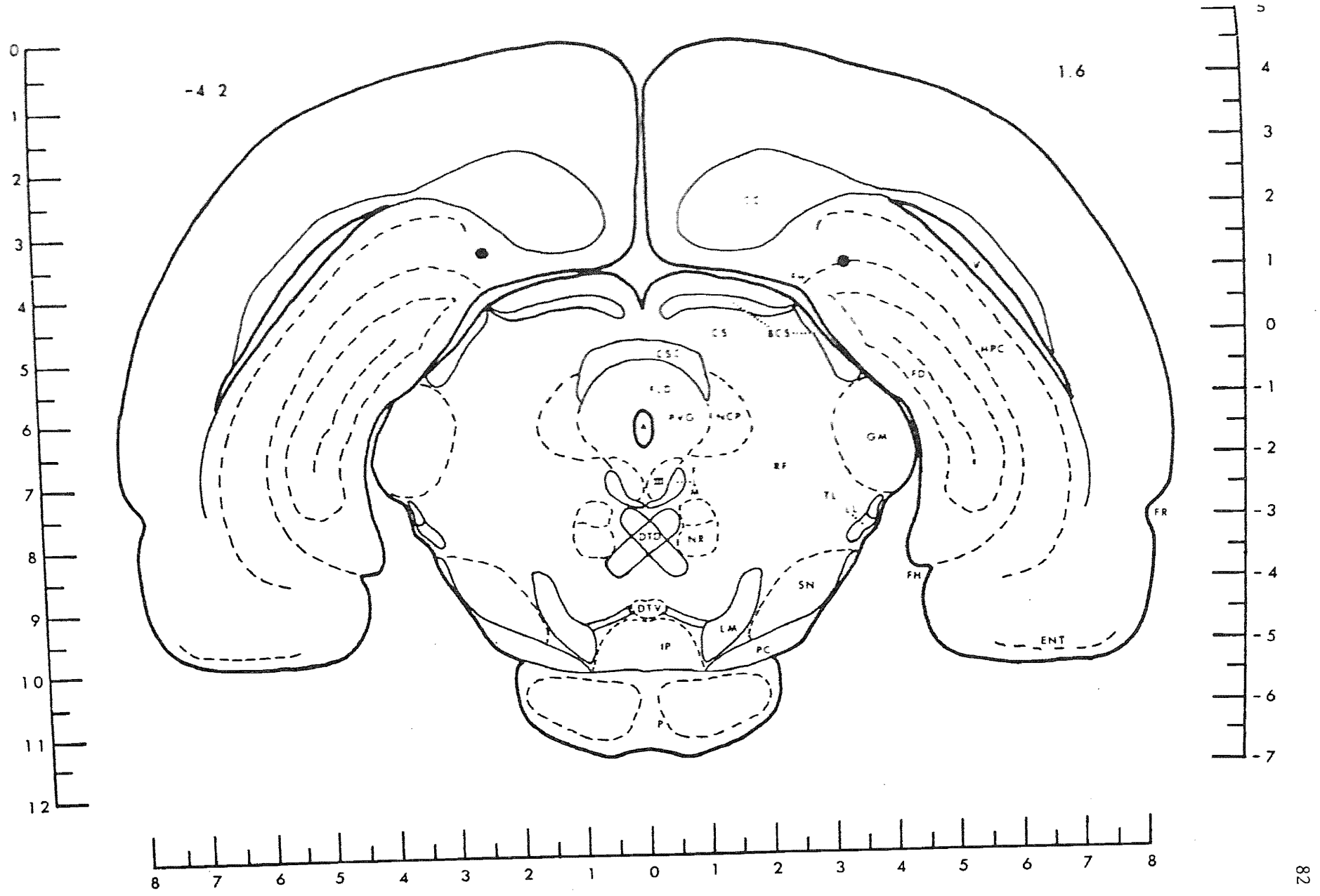
APPENDIX A

Reproductions of Representative Secetion to illustrate Placement of Electrodes in Subjects receiving Bilateral Hippocampal Stimulation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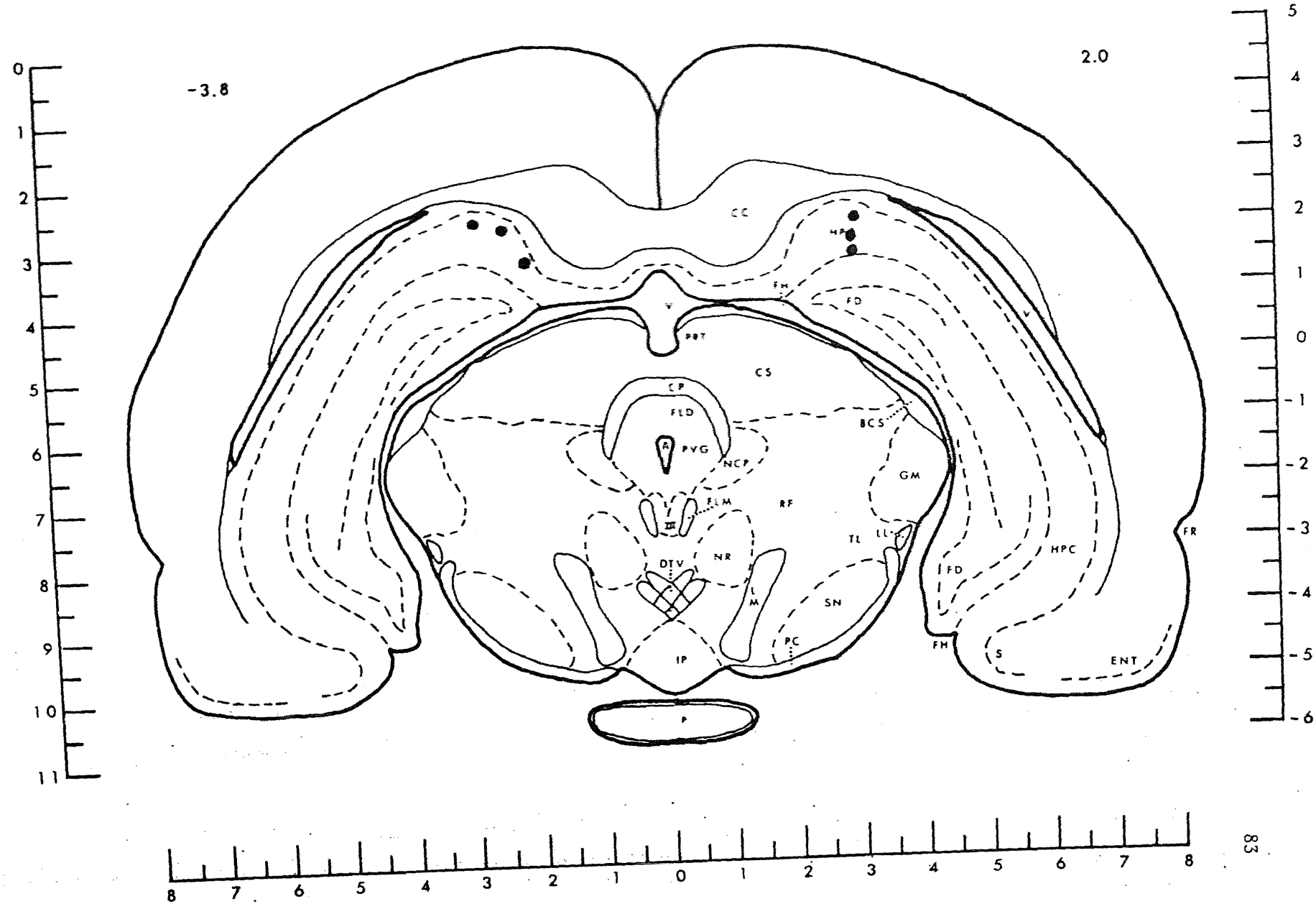
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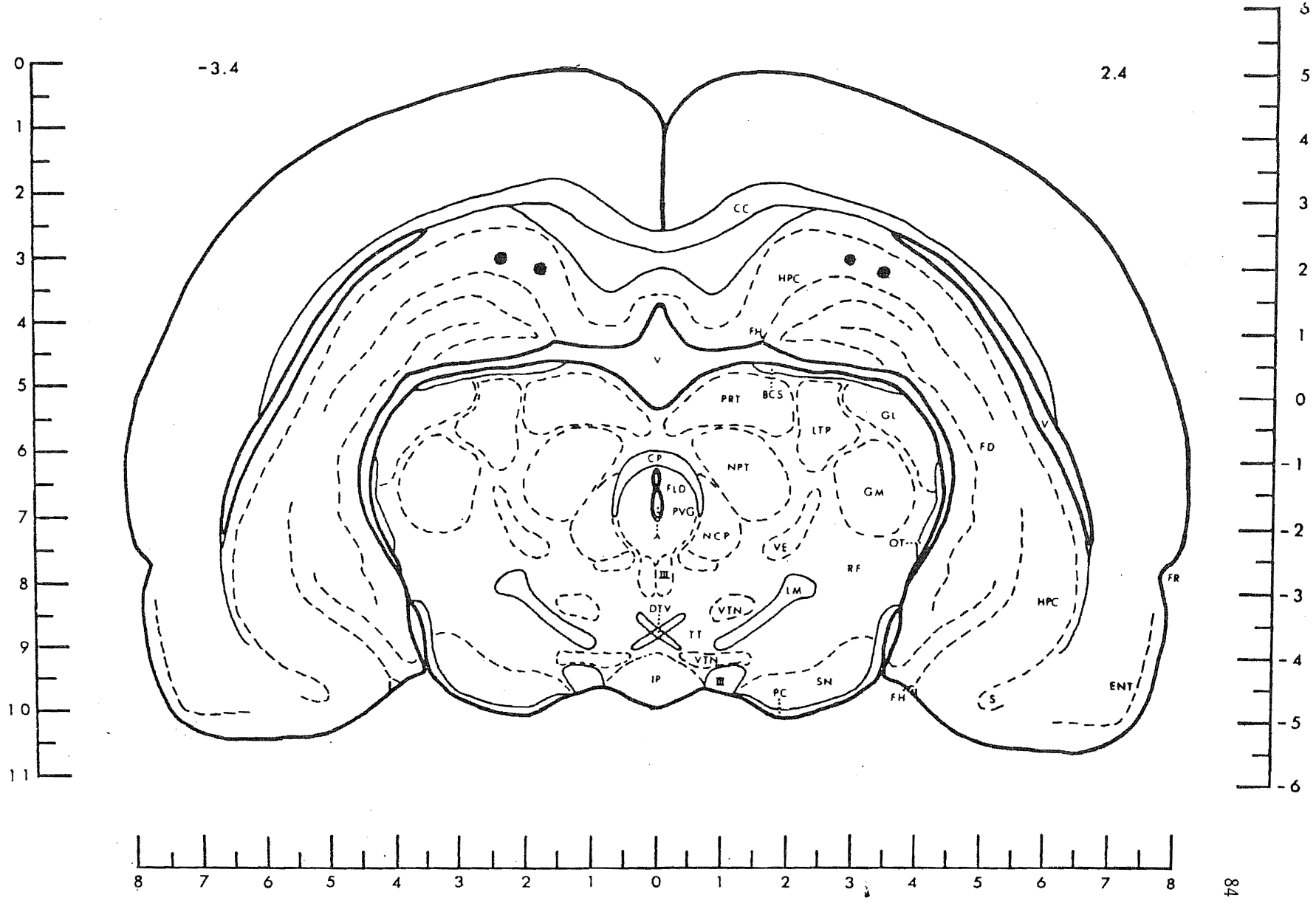
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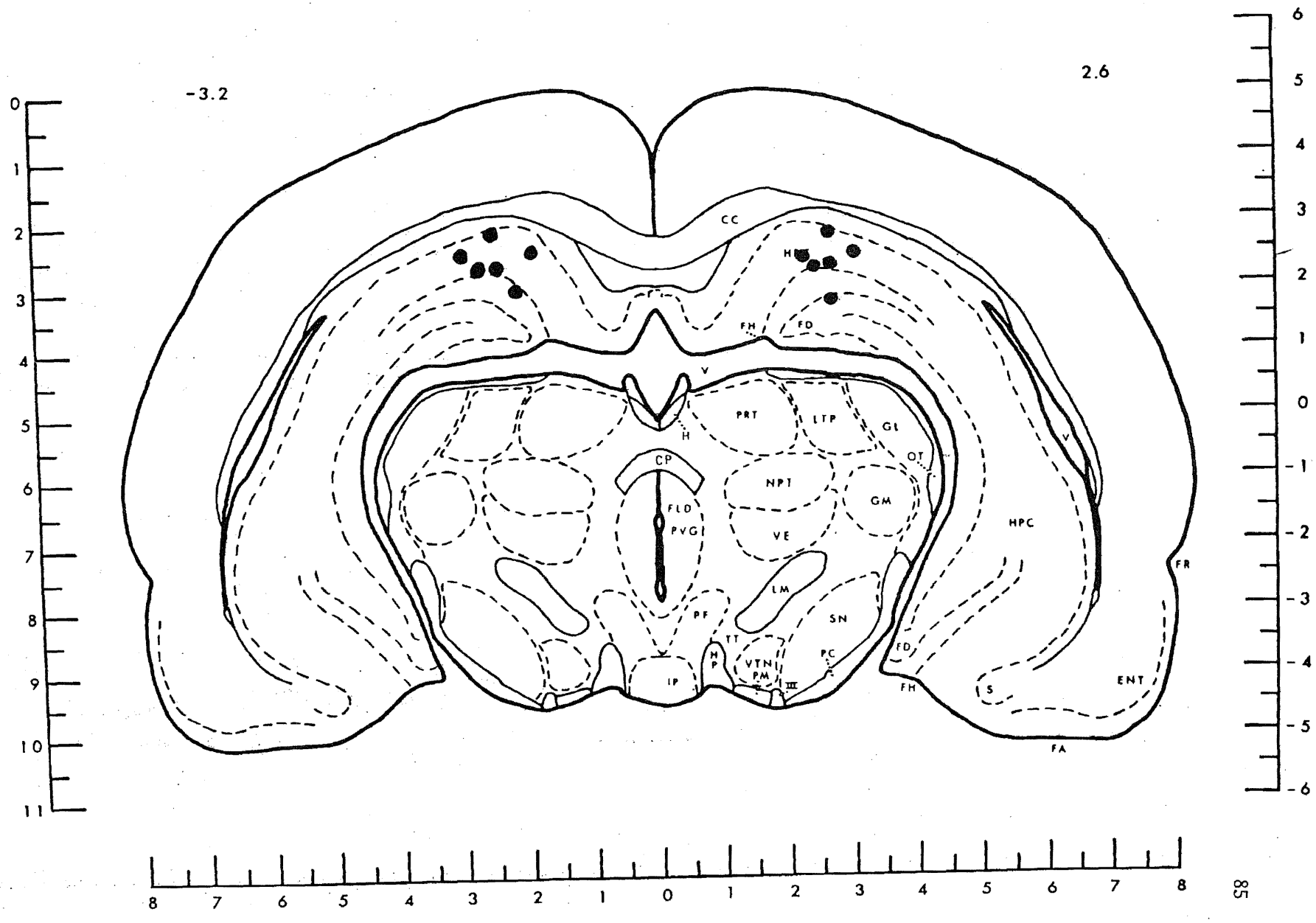
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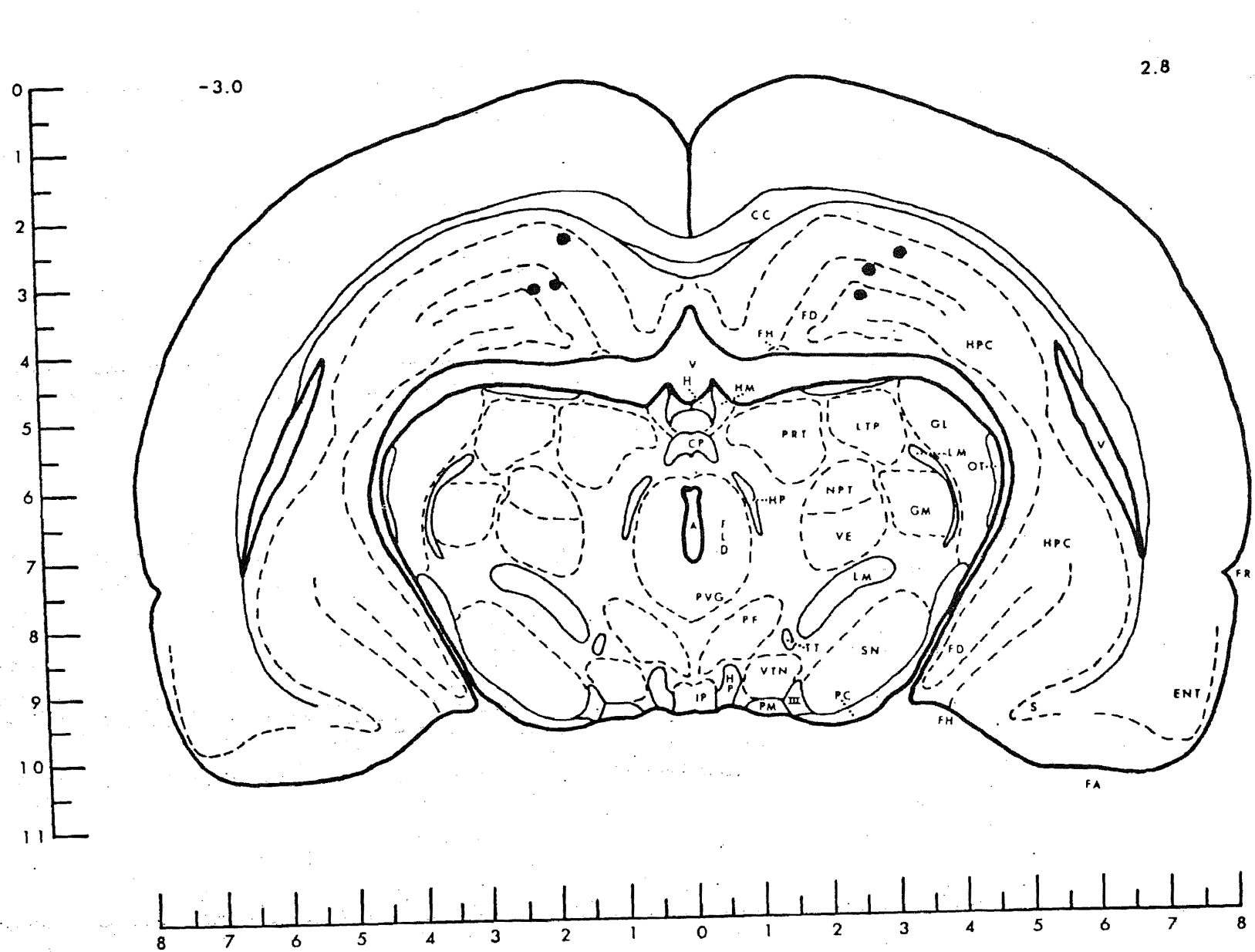
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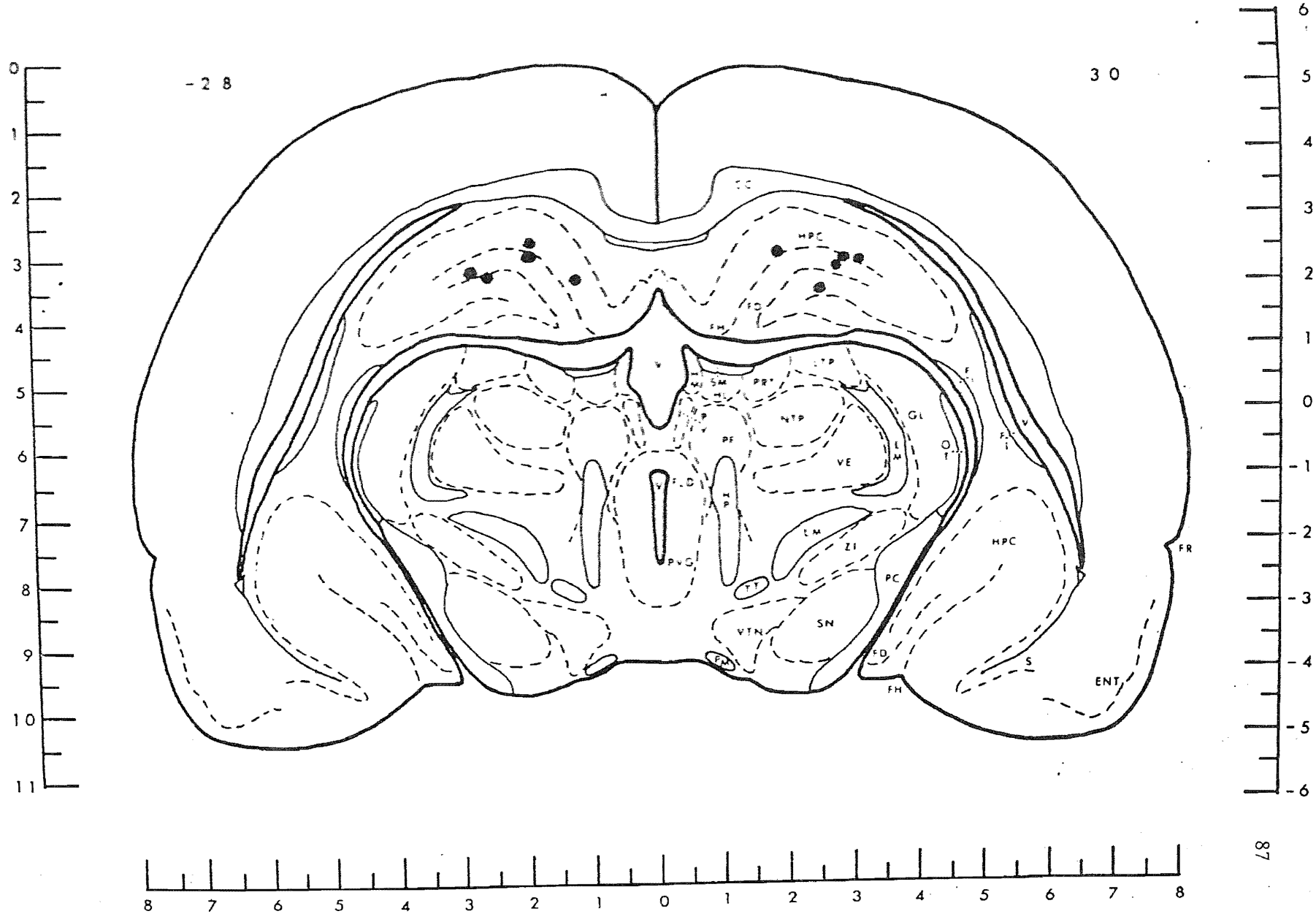
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