THE HIPPOCAMPUS AS A LEARNING MACHINE

Thesis by

Menahem Segal

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ABSTRACT

A series of experiments were conducted with the purposes of describing a functional pathway in the rat hippocampus, characterizing some conditions necessary for activating it, and identifying critical steps in this pathway. In all experiments a classical conditioning paradigm was used and the responses of units in the hippocampus and related forebrain structures to the conditioned stimulus were measured. In the first experiment a few differences between dentate, CA-3, and CA-1, the main fields of the hippocampus, were found. Units in the dentate were first to acquire a conditioned response, CA-3 followed and CA-1 was last. This order fits with the anatomical pathway. However, dentate responses were phasic, that is, did not outlast the CS-US interval, and were not specific to the conditioned stimulus. The responses of CA-3 and CA-1 units, on the other hand, were sustained and specific. The second experiment was devoted to the analysis of conditioned response latencies, in the hippocampus as well as in septum, subiculum, cingulate, entorhinal, and related structures, all known to be input stages to the hippocampus. In this experiment unconditioned short response latencies were found in the medial septum, one of the afferents of the hippocampus. These were not changed in the process of learning. The shortest conditioned response latencies were found in area CA-3 of the hippocampus. Units in area CA-1 followed, but units in dentate did not precede those of CA-3. Units in entorhinal cortex, the

other main afferent to the hippocampus did not seem to precede hippocampal units either. The special relations between the hippocampus and the dentate were demonstrated in another part of this experiment, where dentate units lost their conditioned responses, in the process of extinction, before those of CA-3 and CA-1. It was postulated that septal input triggers CA-3 responses and these would be maintained in the presence of reinforcing dentate and entorhinal inputs.

The relations between the dentate and the hippocampus were further studied in two experiments in which aversive electric shock served as an unconditioned stimulus. In experiment 3 food and shock served as unconditioned stimuli on alternate days. In experiment 4 food and shock were presented in the same sessions as unconditioned stimuli to two different CS's. Dentate units had an excitatory conditioned response to a food signal and an inhibitory conditioned response to a shock signal in both experiments. Hippocampal units had excitatory responses to both signals. Acquisition of a conditioned response was not demonstrated within the hippocampus when the conditioned stimulus preceded shock and was slow when food or shock were applied following two different signals in the same session. However, when first trained that a signal precedes food, the conditioned response would be maintained in the hippocampus even if shock is now the US. The dentate is

probably involved in the initiation of a conditioned response in the hippocampus but not in the maintenance of it.

A sensory-sensory paradigm (experiment 5) has demonstrated the presence of unconditioned unhabituated sensory responses in two of the afferents to the hippocampus, that is, the medial septum and the cingulate cortex. It failed to show signs of conditioning in the hippocampus proper. It was proposed that in the absence of an appetitive reward and the activity of the entorhinal-dentate pathway, conditioned responses in hippocampus cannot be established.

Conditioned entorhinal responses (experiment 6) had long latency but also long time constant. Their evoked activity was maintained for periods as long as one minute. It was found that hippocampal responses were larger, if the conditioned stimulus was applied within one minute from the previous trial. Hence, a correlation between hippocampal responses and entorhinal firing rate was demonstrated. On the basis of these experiments it was proposed that septal input enters the hippocampus at the CA-3 area, is able to selectively activate these cells only in the presence of facilitation produced by entorhinal and dentate activity. The facilitatory entorhinal activity is triggered mainly by positive reward.

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A. INTRODUCTION

(i) General

The hippocampus has attracted the imagination as well as the energy of people from the whole spectrum of disciplines established over the past century in brain research. The uniqueness of this structure has led many scientists to believe that it is the "Rosetta Stone"; the key to the understanding of the mysterious gelatinous material packed en-cephalon. The basic wiring diagram of the system is now well documented, with the aid of all available anatomical methods. The physiological properties of its components are known as well; what is still a mystery is what the hippocampus does. Utilizing a variety of strategies many theories have tried to ascribe functions to the hippocampus, but upon further examination, none have been found to be satisfactory.

A similarly unsolved mystery is the basic and most unique property of the mammalian brain--plasticity. What are the neural mechanisms underlying conditioning, learning and memory? Here, again, many years of study and the utility of a variety of methods did not yield the expected results. We still do not know how the brain, or parts of it, acquires information, stores and retrieves, or forgets. The present study is addressed to these two mysteries with a modest hope of being able to contribute to the understanding of both.

Exploring cellular activity while the rat learns a simple association might give us a clue as to a mechanism of conditioning. Understanding how the hippocampus operates as a learning mechanism can give us a clue as to what the functions of the hippocampus are.

The first question is: does the hippocampus learn? Learning is (Hilgard and Bower, 1966, p. 2) "the process by which an activity originates or is changed through reacting to an encountered situation provided that the characteristics of the change in activity cannot be explained on the basis of native response tendencies, etc." Applying this definition to a search for a learning center in the brain, one would look for a neuron (or a group of neurons) which receives constant neural input and changes its responsiveness to this input. This changed responsiveness should not be attributed to temporary conditions. This part is illustrated by Eisenstein whose definition of learning (in Bullock, 1966, p. 173) "...the ability to code and retain different patterned sensitivities to the same stimulus," is complementary to Hilgard's one on a neural level.

Commonly used methods in brain research have failed to distinguish between primary loci of conditioning and loci in which a conditioned response is secondary to conditioning established elsewhere. A careful analysis of response latencies to a conditioned stimulus before and during conditioning can pinpoint cells that did not respond during pseudo-conditioning, but show

a "new" response during conditioning. Analysis can also determine which of these units, in a given system, has the briefest new response latency. The area that contains these cells has to be a primary locus affected by a conditioning procedure. It should be noted that in order to substantiate the argument that conditioning is taking place at this point, it has to be shown that, (1) this locus is getting sensory input during pseudo-conditioning, and (2) this input is not modified by the conditioning procedure, as far as latency is concerned. This is a rather easy task in the hippocampus, since one can monitor the activity of all known input elements to the system. Once a learing site is located within the hippocampus, the next step is to characterize the optimal conditions necessary to evoke and maintain the learned behavior in this site.

Secondly, a working model can be drawn and tested, and assumptions about the functions of the hippocampus presented.

The Wiring Diagram

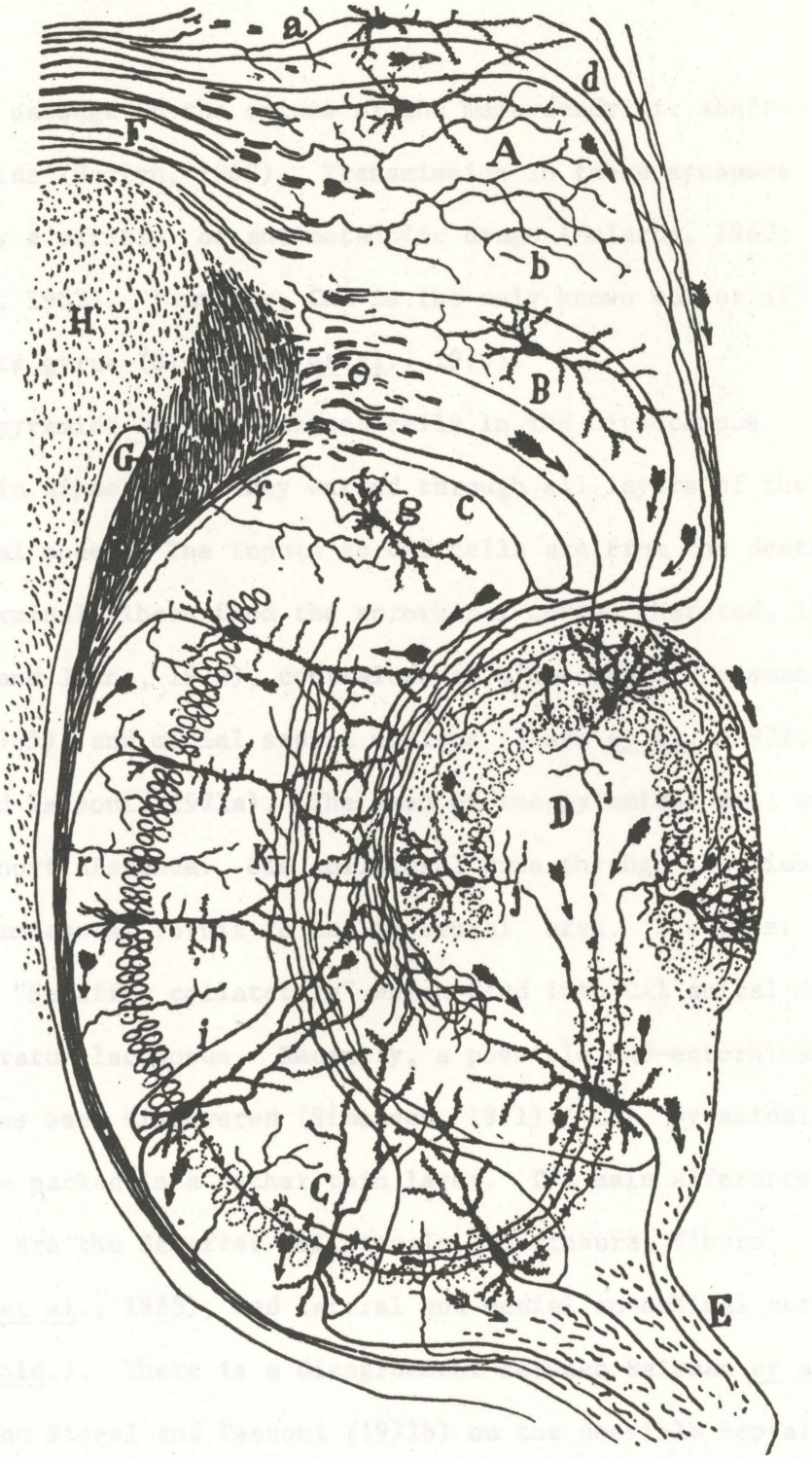
The anatomical methods used in studying the hippocampus over the past sixty years have yielded some controversial results and forced, at least twice, thorough revision of assumptions about directions of fiber flow to and from the hippocampus. As of now, the combined use of the original Nauta, the modified Nauta,

Fink-Heimer, and the electron microscopy techniques (Nauta and Ebbeson, 1970) gives us as close a picture to the truth as one can presently get.

The hippocampus is made of two sheets of paleocortex folded into each other and twisted from the anterio-medio-dorsal (septal pole) to the posterio-lateral-ventral end (posterior pole) of the telencephalon (fig. 1.1). The two subdivisions are the dentate gyrus and the Cornu Ammonis (CA). There are 6 and 4 recognizable layers in Cornu Ammonis and dentate, respectively. Although Lorente de No (1934) lists 14 different types of cells in CA and 6 in dentate, there is a tendency to recognize, by electrophysiological methods, only two types of cells. In CA - pyramidal and non-pyramidal cells (presumably Golgi type II) and in dentate -granular and Golgi type II cells. Dentate granular cells have a large dentritic field, revealed by the Golgi stains. The main inputs to the dentate are from lateral entorhinal cortex (Raisman et al., 1965; Blackstad, 1958; Simonsen and Jeune, 1972). Other inputs are commissural (Raisman et al., 1965; Cragg and Hamlyn, 1957), and septal (Ibata et al., 1971) which end on the polymorphic layer, where the axons leave the dentate gyrus (see fig. 1.2 for the laminar organization of the input pathways). The possibility of CA3 afferents to dentate has been recently proposed (Zimmer, 1971). The dentate granules send their axons, the mossy fibers, through the layers of area CA3 in hippocampus. These axons have peculiar

Figure 1.1

Horizontal section through hippocampus of adult mouse, drawn by Cajal. Fibers originating in entorhinal cortex (A) pass the subiculum (B) and end in the dentate gyrus (D). The mossy fibers (j) pass through area CA3 of the hippocampus (C). CA3 axons bifurcate, send one fiber into the fimbria (E). The other one, the Schaffer collateral, innervates the apical dendrite of pyramidal cells of area CA1 (K).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d
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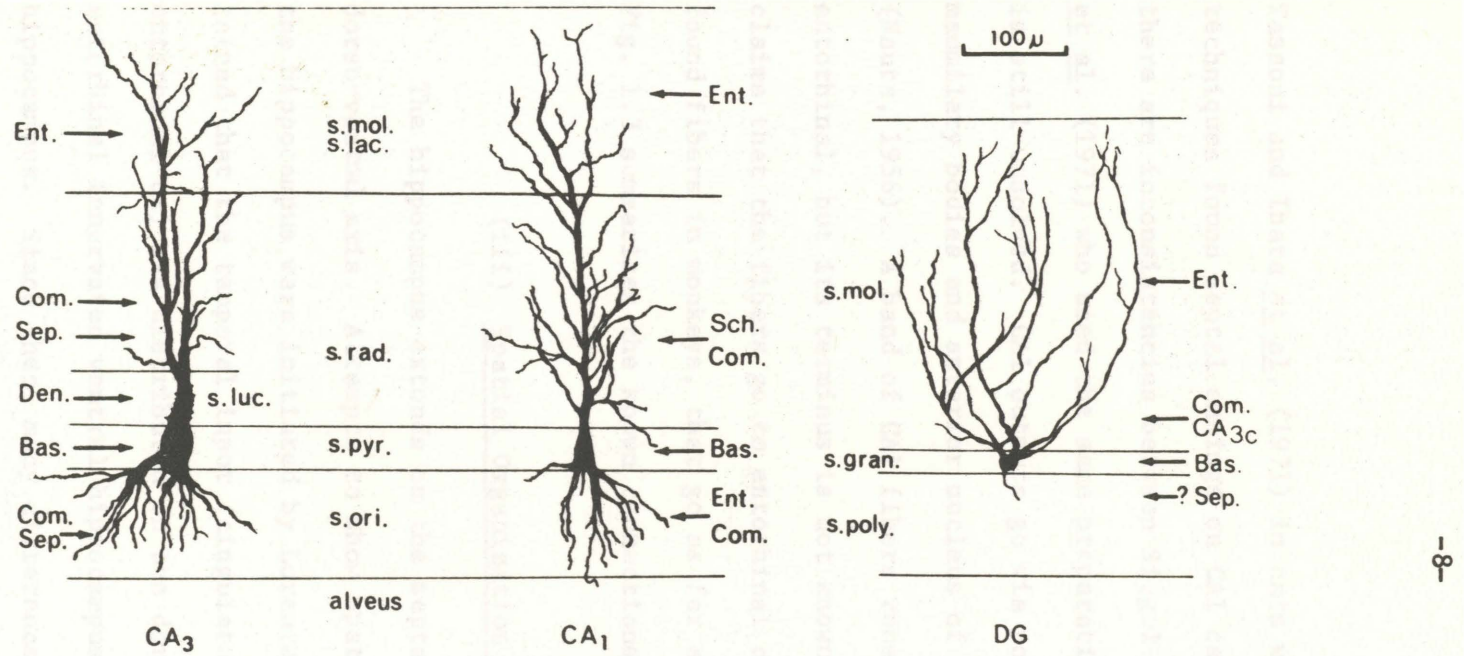
bouton en passage on the spines of the main dendritic shafts of CA3 pyramids (Hamlyn, 1962). Transmission in these synapses is blocked by a variety of antimetabolic drugs (Mclardy, 1962; von Euler, 1962). Thus far, CA3 is the only known output of the dentate gyrus (Blackstad et al., 1970).

CA3 pyramids are the largest cells in the hippocampus (20-30 μ in diameter). They extend through all layers of the hippocampal sheet. The inputs to CA3 cells are from the dentate gyrus, terminal fibers from the entorhinal cortex (Nafstad, 1967; Simonsen and Jeune, 1972), contralateral hippocampus (Raisman et al., 1965), and medial septal nucleus (Ibata et al., 1971; Siegel and Tassoni, 1971a). The axon of the pyramidal cell splits after a short distance. One collateral runs through the fimbria and precommissural fornix to end in septal area. The other forms the "Schaffer collaterals" which feed into CA1 apical dendrites in the stratum lacunosum. Recently, a possible CA3-entorhinal pathway has been discovered (Simonsen, 1971). CA1 pyramidal somata are packed in a rather thin layer. The main afferents to CA1 cells are the Schaffer collaterals, commissural fibers (Raisman et al., 1965), and lateral and medial entorhinal cortex fibers (Ibid.). There is a disagreement between Raisman et al. (1965), and Siegel and Tassoni (1971b) on the possible septal afferents to CA1 area. Raisman, who used rats with Nauta techniques could not find degenerating septal afferents to CA1. Seigel and



Camera lucida tracings of two typical pyramidal cells from fields CA_1 and CA_3 of the hippocampus, and a granule cell from the dentate gyrus (DG). The boundaries of the major strata are indicated by the horizontal lines (*s.lac.-s.mol.* = *stratum lacunosum-moleculare*; *s.rad.* = *stratum radiatum*; *s.pyr.* = *stratum pyramidale*; *s.luc.* = *stratum lucidum*; *s.ori.* = *stratum oriens*; *s.mol.* = *stratum moleculare*; *s.gran.* = *stratum granulosum*; *s.poly.* = *stratum polymorphe*). The arrows indicate the principal sources of afferents which terminate within the various strata (*Ent.* = afferents from entorhinal cortex; *Com.* = commissural afferents; *Den.* = mossy fibers from the dentate gyrus; *Sep.* = afferents from the medial septal nucleus; *Bas.* = basket endings of the basket cells; *Sch.* = Schaffer collaterals from fields CA_3 and CA_1 to field CA_1; CA_{3c} = an ipsilateral afferent pathway to the commissural zone of the dentate gyrus from subfield CA_{3c}). This figure, which is adapted from one in Raisman, Cowan, and Powell (1965), is based principally upon the work of Blackstad and his colleagues (1956, 1958) and of Raisman, *et al.* (1965).

Tassoni and Ibata et al. (1971) in cats with Fink Heimer and EM techniques found septal endings on CA1 cells. However, since there are inconsistencies between Siegel and Tassoni and Ibata et al. (1971) who used the same preparation, the issue, to my mind, is still unsolved. CA1 outputs go via post commissural fornix to mammilary bodies and anterior nucleus of thalamus and brain stem (Nauta, 1956). A band of CA1 fibers runs caudally towards entorhinal, but its terminus is not known. Adey et al. (1958) claims that the fibers go to entorhinal cortex. Votaw (1960) found fibers in monkeys, that go as far as the temporal cortex. Fig. 1.3 summarizes the known connections of the hippocampus.

(iii) Spatial Organization of the Hippocampus

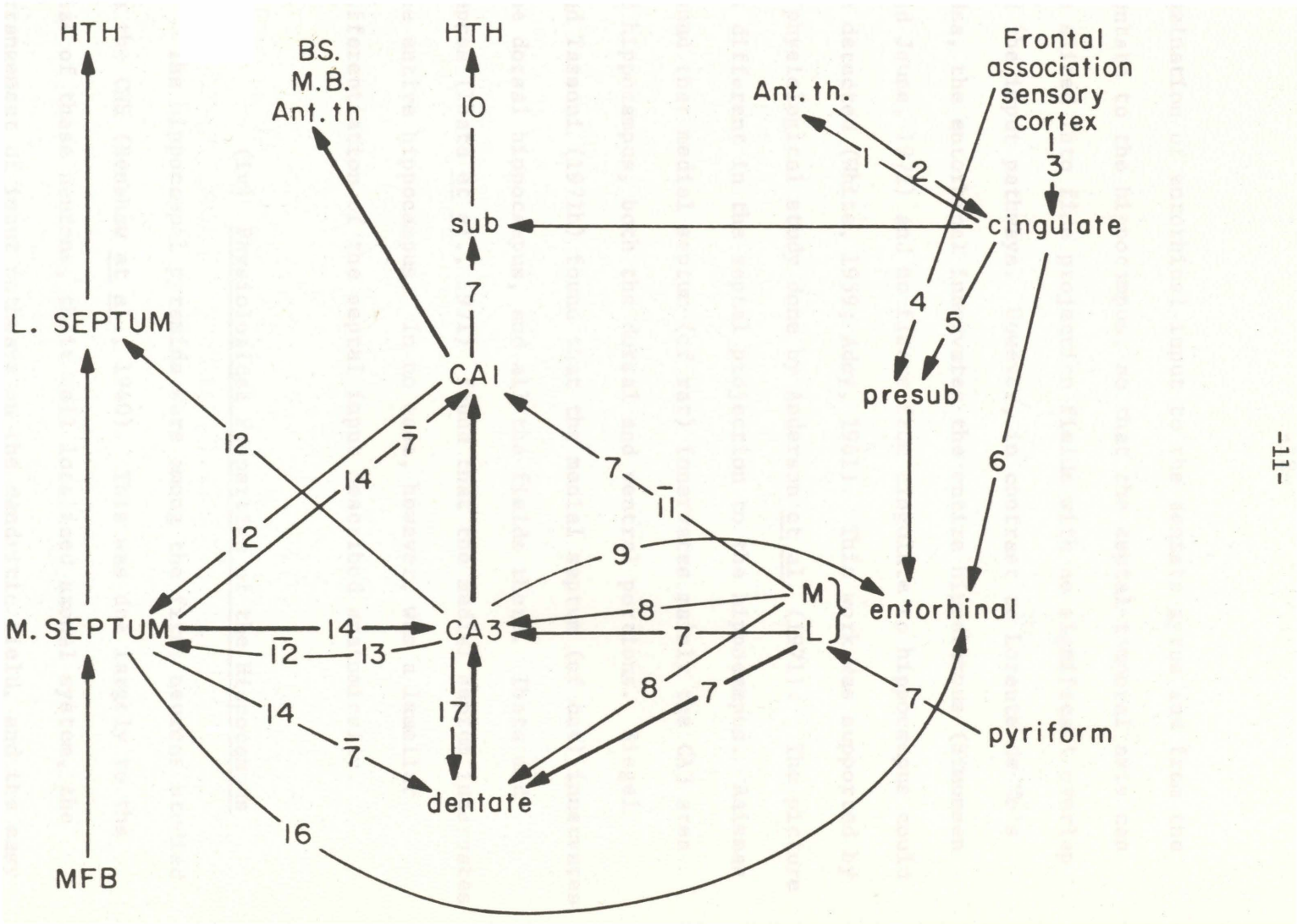
The hippocampus extends on the septal-temporal axis and the dorso-ventral axis. Attempts to show spatial differentiation of the hippocampus were initiated by Lorente de Nŏ (1934) who maintained that the temporal input (cingulate cx, gyrus lancis, entorhinal cx) was distributed on the dorso-ventral axis to that entorhinal innervated ventral hippocampus and cingulate-dorsal hippocampus. Since then, many differences in connectivity (Siegel and Tassoni, 1971a), physiological (Elul, 1964), and functional (Nadel, 1968) properties of dorsal and ventral hippocampus have been reported. Recent work in Blackstad's lab found precise

Figure 1.3

The wiring diagram of the hippocampal system. Numbers in parenthesis are references to the following listing of the major works done on the corresponding pathway. A dash ($\bar{x}$) signals a negative finding.

1. Domesick, 1969
2. Rose and Woolsey, 1948
3. Adey and Meyer, 1952
4. Cragg, 1965
5. Adey, 1951
6. White, 1959
7. Raisman et al., 1965
8. Simonsen and Jeune, 1972
9. Simonsen, 1971
10. Nauta, 1956
11. Nafstad, 1967
12. Raisman, 1966
13. Siegel and Tassoni, 1971a
14. Ibata et al., 1971
15. Defrance et al., 1971
16. Siegel and Tassoni, 1971b
17. Zimmer, 1971

Abbreviations: Ant. Th. - Anterior thalamus; BS - Brain stem; HTH - Hypothalamus; L. Septum - Lateral septum; MB - Mammilary bodies; M. entorhinal - medial entorhinal cortex; M. Septum Medial septum.


HTH
BS.
M.B.
Ant. th
HTH
10
Ant. th.
Frontal
association
sensory
cortex
1
2
3
sub
cingulate
7
4
5
L. SEPTUM
CA1
presub
12
7
14
7
11
6
12
9
M
8
entorhinal
M. SEPTUM
14
CA3
7
L
12
13
8
7
14
17
7
pyriform
7
dentate
16
MFB

lamination of entorhinal input to the dentate gyrus and from the dentate to the hippocampus, so that the septal-temporal axis can be sliced into fine projection fields with no significant overlap in the input pathways. However, in contrast to Lorente de Nǒ's idea, the entorhinal innervates the entire hippocampus (Simonsen and Jeune, 1972) and no fibers from cingulate to hippocampus could be detected (White, 1959; Adey, 1961). This work was supported by a physiological study done by Anderson et al. (1971). The picture is different in the septal projection to the hippocampus. Raisman found that medial septum (of rat) innervates mainly the CA3 area of hippocampus, both the dorsal and ventral portions. Siegel and Tassoni (1971b) found that the medial septum (of cat) innervates the dorsal hippocampus, and all the fields there. Ibata and Kappas (Ibata et al., 1971) found that the medial septum innervates the entire hippocampus. In no case, however, was a lamellar differentiation of the septal input described anatomically.

(iv) Physiological Properties of the Hippocampus

The hippocampal pyramids were among the first neurons studied in the CNS (Renshaw et al., 1940). This was due largely to the size of these neurons, their well localized axonal system, the arrangement of input pathways on the dendritic field, and the easy identification of the cells. Kandel and Spencer (1961) first

described the following steps in the production of an action potential in the hippocampus: (1) slow prepotentials which are not involved in initiation of a spike, are assumed to be summed EPSP's; (2) fast prepotentials, seen only in orthodromic or intracellular activation, which sometimes can be seen without a spike production, are ascribed to the dendritic spike initiated at the birfurcation of the main apical dendritic shaft (Andersen, 1966); (3) A-B break first seen in motoneurons which represents the transition of the spike from the axon hillock into the soma; (4) the spike, having the same characteristics of a motoneuron spike; (5) post spike depolarization, not seen in motoneurons, where a common hyperpolarization is observed. This post-potential depolarization is involved in production of a burst, inactivation process (Green, 1964), production of the slow wave rhythms (Kandel and Spencer, 1961), and is probably responsible for the inability of hippocampal cells to fire at a high frequency rate, as seen in motoneurons. The existence of dendritic spikes was debated for a long period. There seems to be an agreement that these may exist, although no intracellular dendritic recording has been achieved (except, maybe, in the cerebellum). Laminar analysis of field potentials (Andersen, 1960b), isolated fast prepotentials (Spencer et al., 1961) and histological arguments led to the assumption that the large apical dendritic shaft is able to transmit a dendritic spike at a slow speed and low safety factor. (This, however, may not exist in pathological conditions in which initiation of a

spike without fast prepotential was observed (Purpura, 1966a).)

Antidromic stimulation of a pyramidal cell leads to a long hyperpolarization (100 msec). This is not caused by afferent stimulation (Kandel and Spencer, 1961), but probably by activation of a local circuit (Andersen, et al., 1963) in a Renshaw loop. Support for the negative feedback Renshaw loop hypothesis was demonstrated by recording a high frequency train of spikes in hippocampal cells, while other cells demonstrated prolonged IPSP. Andersen assumes that these are Golgi type II neurons which synapse on the pryamidal cell bodies. Recently, a positive feedback local loop has been proposed (Lebovitz et al., 1971). The nature of these mechanisms is, as yet, not clear.

The connections between the hippocampal areas as well as the nature of hippocampal afferents have been thoroughly studied over the past decade. All hippocampal afferents (septal, entorhinal, and commissural) as well as intrinsic pathways (mossy fibers and Schaffer collaterals) were found to be excitatory. However, although stimulation of septal and commissural afferents can initiate time-locked spikes, the main effect of entorhinal input is initiation of long lasting EPS's. A lasting effect of stimulation of perforant path was demonstrated by Andersen (Andersen et al., 1967) and Lomo (1971): stimulation of 10-15 sec potentiate later dentate response to single pulses of perforant path. This specific frequency potentiation can last for hours (Lomo, 1971), but may not exist to

this extent in an awake, unanesthetized animal. Potentiation of hippocampal response to septal stimulation after application of a conditioning shock to entorhinal cortex was already demonstrated by Adey (Adey et al., 1958) and replicated in a preliminary fashion in our lab. The reverse, i.e., potentiation of hippocampal response to entorhinal stimulation following conditioning shock to septum, could not be demonstrated.

The Hippocampus as a Tissue

The precise arrangement of the pyramids such that all cell bodies are packed in a single thin layer, with the dendrites perpendicular to this layer, has made this structure a favorite one for studies of brain potentials. A slow wave rhythm, "Theta rhythm" (4-12 cps, depending on the animal in study) can be recorded from most parts of the hippocampus (Green, 1964). Although three different mechanisms were proposed for this rhythm (Stumpf, 1965), there are some accepted facts about its origin: (1) it is paced by cells in medial septal nucleus (Stumpf et al., 1962), (2) it is accompanied by intracellular waves recorded in pyramidal cells (Fujita and Sato, 1964), (3) it is affected by cholinergic drugs (Stumpf, 1965), and (4) it is correlated with a variety of behavioral states. The basis issue in this field of research is whether theta rhythm is correlated with the sensory, recording apparatus (Green and Arduini, 1954), or with the motor, performing apparatus

(Vanderwolf, 1971). An orthogonal issue is whether or not it is related to learning, memory, and retrieval mechanisms. Again, some findings are accepted by all people involved in these issues: (1) amount and frequency distribution of theta rhythm is different for different animals; rabbits have almost continuous theta rhythm whereas monkeys and humans seldom exhibit theta. Rats and dogs have intermittent rhythms. (2) some behavioral states are almost always accompanied by this rhythm, the most notable of which is REM sleep, when the brain is awake and the body asleep. A theta burst is evoked by a conditioned stimulus in at least some phases of a classical conditioning experiment, regardless of the animal's current behavior (Yokota and Fujimari, 1964; Konorski et al., 1968). It seems too, that theta rhythm accompanies attentional behaviors, when both orienting movements and recording of novel stimuli occur. A word of caution: the correlation between hippocampal theta and various behaviors is not complete and in the normal preparation it is not known which leads and which follows. (There are some exceptions to this statement; Komisaruk, 1970.) Moreover, in most cases, hippocampal theta is correlated with neocortical desynchronization (Stumpf, 1965). Although these two electrical phenomena seem to be independent, the possibility that neocortical desynchronization is an intermediate variable between behavior and theta has not been dissected thoroughly.

The Hippocampus and Learning

The involvement of the hippocampus in various learning tasks has been documented by a variety of strategies. There are three main ways one can go about finding out the role of a brain area in learning: (1) correlate its activity with corresponding behavioral changes, (2) manipulate its activity and observe behavioral changes, and (3) ablate it and look for effects on behavior. A fourth way, commonly used, is to look at the structure and speculate what the functions might be; one cannot avoid it when facing a structure as organized as the hippocampus.

On the biochemical level, both the correlative and the manipulative approaches have been intensively tried during the past decade. Only recently, with the development of regional and miniature neurochemistry, has it been possible to demonstrate that most of the observed changes in the brain, following training, are taking place in the hippocampus and related structures (Beach et al., 1969; Kottler et al., 1972; Yanagihara and Hyden, 1971; Kahan et al., 1970). The changes observed were mainly increased incorporation of labeled molecules into RNA and protein. Hyden (Hyden and Lange, 1970) claims that there is an increase in synthesis of S-100 (specific acidic brain protein) which is specific to learning, but this has still to be replicated in other laboratories.

Disruption of RNA or protein synthesis by the use of antibiotic drugs has been another common biochemical approach. Although the validity of this approach has been recently questioned (Nakajima,

1972), and the precise localization of the drug effect is not completely established, it is still interesting to note that inhibition of protein synthesis in hippocampal areas disrupts memory (Flexner, 1968).

The correlation of gross electrical activity of the hippocampus (the theta rhythm) with various phases of learning has been demonstrated in many laboratories. A series of experiments by Adey and his collaborators showed that in an alert cat there is theta in hippocampus, but fast, low voltage activity in dentate and subiculum (Radulovacki and Adey, 1965). During early stages of conditioning hippocampal theta leads similar activity recorded in entorhinal cortex. At later stages of learning this phase relationship reverses (Holmes and Adey, 1960). At that time theta rhythm can be recorded from dentate as well. Marked frequency shifts during performance of single trials were detected in further studies (Elazar and Adey, 1967). The generality of these results was questioned by many research groups (Grastyan et al., 1966; Bennett et al., 1971; Vanderwolf, 1971).

A manipulative approach to the role of hippocampal activity in learning was proposed by Grey (Grey, 1970), who was able to modify resistance to extinction by driving hippocampal theta at certain frequencies during learning in rats. These effects were modulated by blockade of the theta system. Another series of studies by McGaugh and his collaborators (Landfield et al., 1972) correlate the amount of theta rhythm in rats with the amount of

retention. The correlation of theta with strength of the memory trace was criticized recently by Klemm (1972). It should be remembered that theta rhythm is not a product of hippocampal activity but rather a rhythm imposed upon the hippocampus via its septal afferents. In fact, there is a rather poor correlation between hippocampal unit activity and theta rhythm (Noda et al., 1969). It certainly would be interesting to know how theta rhythm affects hippocampal functions, but identification of theta rhythm with hippocampal activity is, to say the least, not accurate.

Hippocampal lesions have been the favorite tool of psychologists in studying hippocampal functions. A vast and confusing literature has accumulated during the past fifteen years. An excellent review by Douglas (1967) helped to clarify the issue a bit. As it turns out, large hippocampal lesions (see Jackson, 1968) disrupt discriminative reversal, passive avoidance learning, extinction, and, to a smaller degree, maze learning. The lesion method has some inherent disadvantages. It is hard to distinguish between deficits that are the consequence of the lesion, the irritation of the remaining tissue and adjacent brain areas, or the disruption of the afferent pathways. Only one study was found (Entingh, 1971) that observed entorhinal lesioned cats during reversal learing. In that case, learning was also disrupted. As the quantitative and qualitative differences between entorhinal, cingulate, septal

and hippocampal lesions have never been assessed, it might well be that the functions ascribed to the hippocampus are, in fact, projected into it via the entorhinal afferents. A more profound criticism is the recent discovery of the tremendous capacities of parts of the limbic system, as well as the visual system, to modify connections upon partial de-afferentiation (Linch et al., 1972; Raisman, 1970). It seems therefore, that in addition to lack of a hippocampus, these rats may have formed new unknown connections, probably with new functions assigned to these, and this factor just obscures possibly interpretation of lesion data. In the light of this point, it is not surprising to find inconsistency between lesion experiments and those that temporarily knock out hippocampal activity (Hirano, 1965).

The best approximation of what the hippocampus does is what its elements are doing. Although recently developed, and used in only a few laboratories, the method of unit recording in awake freely moving animals promises what other methods have failed to achieve. By recording from the hippocampus and its afferents one can derive what the hippocampus is doing to information projected upon it by its afferents. A combined use of stimulation, lesions, and recording methods can verify these derivations. By comparing hippocampal unit activity to the activity recorded in other brain areas, one can detect any uniqueness in the hippocampus. This is a tedious task, but short-cuts have proved to be misleading.

B. GENERAL METHODS*

Subjects

All subjects in this study were albino male rats from the Holtzmann Company. They were 12-15 weeks of age and weighed between 250-350 grams.

Surgery

Each rat was anesthetized with sodium pentobarbital (Nembutal, 38 mg/kg) and introduced into the stereotaxic apparatus. Preliminary preparation included drilling holes (0.5 mm in diameter each) in the skull, puncturing the dura and implantation of an indifferent electrode ("ground") into the anterior forebrain. Then the implantation of the recording electrodes were started. The electrodes were made of 62.5 μ nichrome (80% nickel, 20% chrome) wires. The impedance of these electrodes was approximately 100 K. With the factory enamel insulation, they were 67.5 μ in diameter. Preparation of the electrodes from the wire consisted of cutting the wire with scissors and inserting the blunt tip of the wire into the brain. The coordinate system used consisted of the bregma as the zero point for the anterio-posterior coordinates, the midline suture as the zero line for medio-lateral coordinates, and the flat skull

*(of Olds, 1965)

for dorso-ventral coordinates.

The wires were moved down into the brain to about 0.5 mm from the target area. Then with the use of a micromanipulator, CRT, and an audio display, the electrode was moved down, until clear spikes of at least 100 μv (i.e., 4:1 signal:noise) could be monitored. For the most part, the knowledge of the characteristic unitary activity of a given area helped to zero in on it. Therefore, there were relatively few misses (i.e., recording from units in areas irrelevant to the study). There were 8 electrodes implanted in a rat, never more than 4 electrodies in one hemisphere. The electrodes were first "tacked" to the skull with dental cement and later assembled into a placque which was similarly affixed to the skull. Throughout the surgery (2-3 hrs) rats were artificially respirated.

Apparatus

A 12' circular plastic cage was housed in a sound attenuating chamber. It contained a food tray from which the rat was hand trained to retrieve 45 mg food pellets delivered by a noisy feeder. It took approximately 0.5 sec between the delivery of the pellet and the availability of the pellet to the rat. A special device dropped the pellet out of the tray if it was not retrieved within 4-5 sec. A photocell detected retrieval behavior. At the

end of every experiment, the number of unretrieved pellets were counted and this number served as one of the criteria for the exclusion of a rat from the experiment. Water was supplied ad libitum. A loudspeaker placed in the enclosure 1.5 feet from the rat, provided two tones, 1 Khz and 10 Khz.

The rat's placque was connected to a 10 contact plug which led through a low noise (Microdot) cable and a slip-ring commutator to a panel, into which field effect transistor preamplifiers were directly plugged. The weight of the plug, cord commutators and preamplifiers was counterbalanced. Under these conditions the rat was relatively free moving. No noise could be detected in the system that was caused by the rat's moving about the cage. A high noise open-ended wire was attached to the cable, and movements of the rat generated voltage in this wire, which was amplified and served as a movement detector. The signals from the preamp were fed into amplifiers with a frequency range of 500 to 10,000 hz. The gain of these amplifiers was variable and set to produce a noise level of 0.5 to 1 V, and accordingly the signal was 2-3 V in amplitude. The output of the amplifier was fed into a unit "window" discriminator. This discriminator used the amplitude and the fall time of a spike as criteria for selection. It rejected, upon appropriate setting, spikes of different amplitude or duration, as well as various electrical noises. To assure exclusion of these events, a continuous sample of the discriminated spikes

was taken and plotted throughout the whole experiment (Figure 1.4).

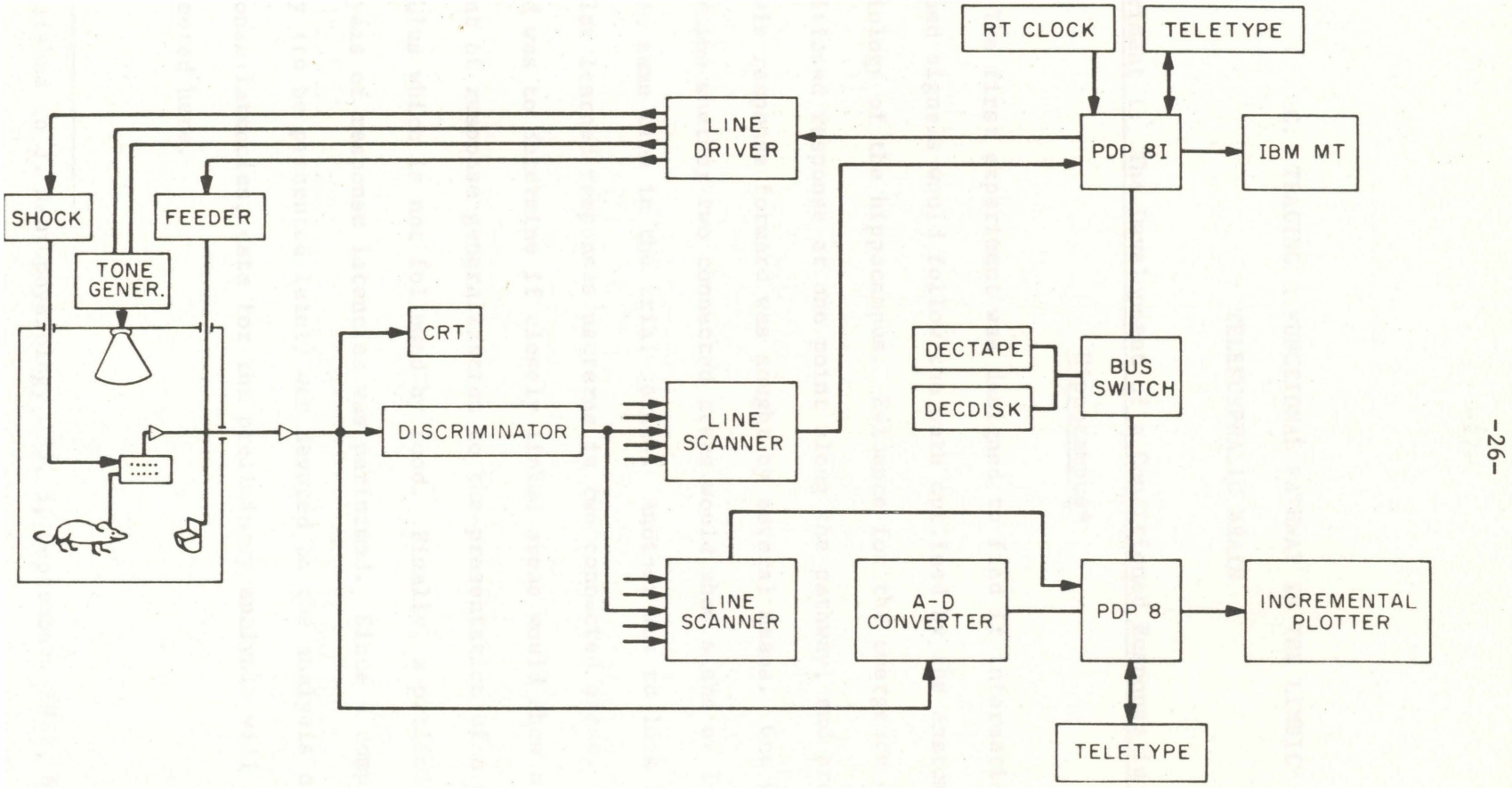
The discriminator, upon detection of a spike, produced a 0.1 msec binary pulse which was recorded on a IBM type by a PDP 8I computer which controlled the experiment and accumulated the data. Analysis of the data was done with the aid of a 370/155 IBM computer.

Histology

At the end of an experiment rats were sacrificed by means of terminal anesthesia. Location of the recorded probes was marked by passing 10 μa positive current for 15 sec through the tip of the electrodes. The brains were perfused with physiological saline followed by 10% formalin. At least two days later, the brains were sliced into 60 μ slices on a freezing stage, and stained with cresyl violet and Weil stains for alternate sections. These procedures helped to locate accurately the recorded units by means of a camera lucida and microscope examination.

Figure 1.4

Block diagram of the experimental setup.


RT CLOCK
TELETYPE
LINE DRIVER
PDP 8I
IBM MT
SHOCK
FEEDER
TONE GENER.
CRT
DECTAPE
DECDISK
BUS SWITCH
DISCRIMINATOR
LINE SCANNER
LINE SCANNER
A-D CONVERTER
PDP 8
INCREMENTAL PLOTTER
TELETYPE

C. TRACING A FUNCTIONAL PATHWAY IN THE LIMBIC TELENCEPHALIC BRAIN

Experiment 1: The Development of a Conditioned Response in the Hippocampus*

The first experiment was designed to find if information from learned signals would follow the path outlined by the anatomy and physiology of the hippocampus. Evidence for the emergence of a conditioned response at one point along the pathway, and projection of this response forward was sought by several means. One was to determine whether two connected areas would show signs of learning at the same time in the trial series. Another was to look for similar learned responses patterns in two connected areas. A third was to determine if closely linked areas would show a similar amount of response generalization to the presentation of a neutral stimulus which is not followed by food. Finally, a preliminary analysis of response latencies was performed. Since a complete study (to be presented later) was devoted to the analysis of response latencies, data for the preliminary analysis will not be presented here.

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(i) Methods

Sixty Holtzman rats 12-15 weeks of age and weighing between 250-300 gm served as subjects. During a 3-4 day recovery period after surgery the food supply was limited so that body weight was reduced to 75% of the preoperative level. Following this each rat was introduced to the training box.

The experiment was conducted on two successive days. On the first day (pseudoconditioning) three events--delivery of the food pellet and two tones--were presented randomly with an inter-stimulus interval of 1 min; each was presented about 300 times in a 16-hour period. This was followed by an 8 hour wait and then a second day's experiment. The second day was divided into two parts. First, 50 of each stimulus were presented as above (pseudoconditioning). The second and main part of the day (conditioning) the food pellet (US) was delivered 1 sec after the onset of one of the tones (randomly selected and assigned as CS+). The other tone was presented randomly (and assigned as CS-). Each of the tones was presented 300 times during this phase.

Data Collection and Reduction

The collection of data was made for 3 sec periods including the 1 sec pre=CS (background sample) and 1 sec CS-US interval and 1 sec after application of the US (see Figure 2.1). Each second was divided into 100 bins, 10 msec each, and a binary record was made indicating the presence or absence of unit detections for every bin and every trial. An average probability histogram was prepare for successive 300 bins of 300 CS+ trials and 300 CS- trials during a pseudo-conditioning and the same during conditioning.

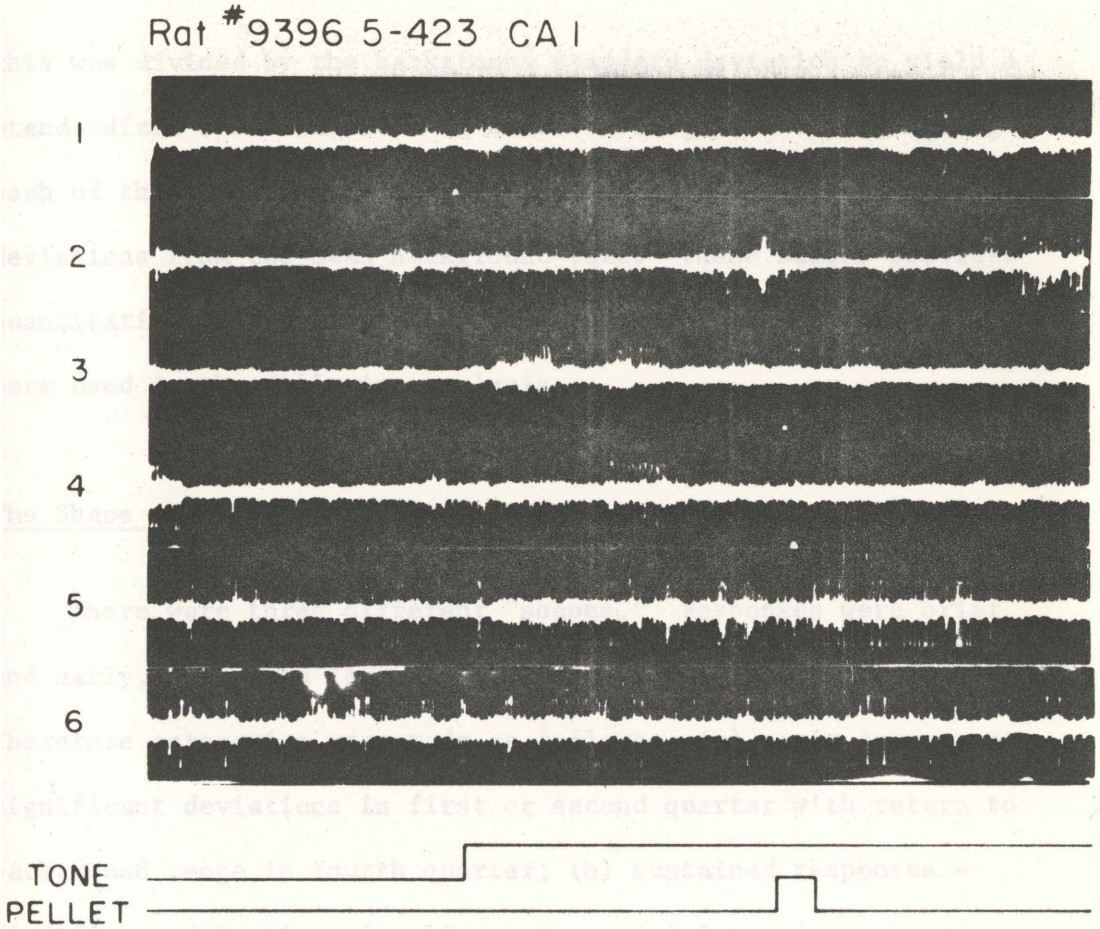
Prior to analysis a number of cases were excluded from the final sample for the following reasons: (1) failure to isolate unit activity or inclusion of "noise" in the records (detected by inspecting plotted samples of the identified units); (2) insufficient data (i.e., the unit disappeared or change height during training, causing the mean frequency in the 1 sec pre-CS to be $\leq$ 1 response in 5 sec); (3) inability to localize accurately the recorded site in the brain. Based on these criteria 25 units were excluded from the final sample.

The CS-US Interval

The 1 sec CS-US interval was divided into 4 quarters, each being 25 successive bins. For each of these the average firing rate was converted into a deviation from the background rate, and

Figure 2.1

Single traces of a unit recorded over a period of 3 sec in the four periods of the experiment. Form top to bottom: beginning of pseudoconditioning; end of pseudoconditioning; beginning of conditioning-response to CS+; beginning of conditioning--response to CS-; end of conditioning--response to CS+; end of conditioning--response to CS-.



1 Beginning of Pseudoconditioning
2 End of Pseudoconditioning
3 Beginning of Conditioning CS+
4 Beginning of Conditioning CS-
5 End of Training CS+
6 End of Training CS-

this was divided by the background standard deviation to yield a standardized score called a "Q score." It gave the rate during each of these post-stimulus quartiles as a number of standard deviations from the mean background rate. These scores provided quantitative representation of the averaged response pattern and were used in the following analysis.

The Shape Analysis

There were three different "shapes." Responses were brief and early, brief and late, or early and late (i.e., enduring). Therefore categories were made as follows: (a) early responses = significant deviations in first or second quarter with return to background range in fourth quarter; (b) sustained responses = significant deivations in all quarters; (c) late responses = no significant deviations in the first and second quarters, followed by significant deviation in the third and still larger responses in the fourth quarter; (d) no response = no significant deviations in any quarters. The cut-off point for significant deviations was 0.9 standard deviations from the background mean.

The Generalization Ratio

It was impossible to make an accurate comparison of response change to CS+ with response change to CS- because responses to the CS- were not measured during pseudoconditioning. The unresponsiveness of units on the first day made it relatively unnecessary. Because such a large proportion of the responses on the second day were "learned," a reasonably accurate way to test for "generalization" to the CS- was to compare second day responses to the 2 stimuli. A "generalization ratio" was computed which stated response to the CS- as a percentage of the response to the CS+ for the quarterly interval yielding the largest CS+ score. Statements about generalization derived from this measure were in accord with observations made on the combined "learning curves" described below.

The Learning Curve

For each 10 trials the amount of response to the CS+ was calculated by subtracting the average frequency in the pre-CS period from the average frequency in the CS-US interval and these responses were plotted for successive groups of 10 trial to form a learning curve. The individual learning curves of all the units in a given area were averaged to give a representation of the change over trials in this area.

(ii) Results

Description

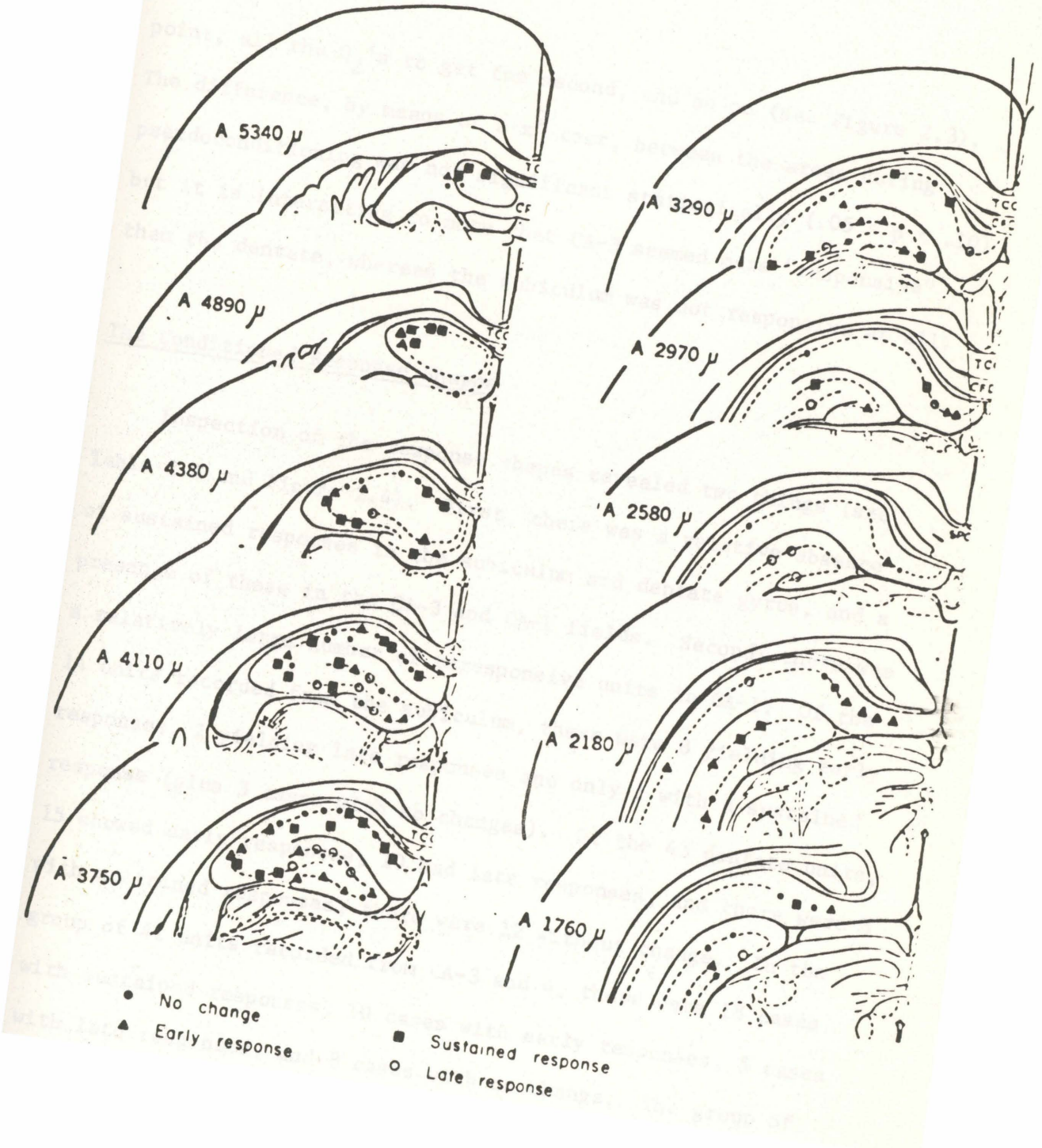
140 units were identified, recorded and passed the various quality criteria (Figure 2.2). These units were divided into 4 main groups by areas: the dentate gyrus (43 units), CA-3,4 (41 units), CA-1 (42 units), and subiculum (14 units). The boundaries of the areas are in accord with Lorente de Nŏ (1934) and Raisman *et al.* (1965). No attempt was made to differentiate anterior-posterior levels. Almost all the units were located in the pyramidal layer of the Ammon's horn or the granular layer of the dentate gyrus.

The Unit Activity during Pseudoconditioning

Most of the units did not respond to the tones during pseudo-conditioning. The units that did respond showed an intermittent short period of change from the background level of activity. It should be noted that the method of averaging 300 trials was relatively insensitive to changes in unit activity if the response to the novel stimuli habituated in a small number of trials. However, 33% of the CA-3 units showed a significant response in at least one of the quarterly intervals; 20% of the GD units and 16% of those in the CA-1, and none of those in the subiculum. On the basis of the Q-scores, grand averages were computed for each brain area; i.e., all the Q_1 scores were averaged to get the first

Figure 2.2

Distribution of the points in the hippocampus, marked on sagittal sections of the rat brain. Sections are redrawn from Köning and Klippel's Stereotaxic Atlas of the Rat Brain.


A 5340 μ
A 4890 μ
A 4380 μ
A 4110 μ
A 3750 μ
A 3290 μ
A 2970 μ
A 2580 μ
A 2180 μ
A 1760 μ
No change
Early response
Sustained response
Late response

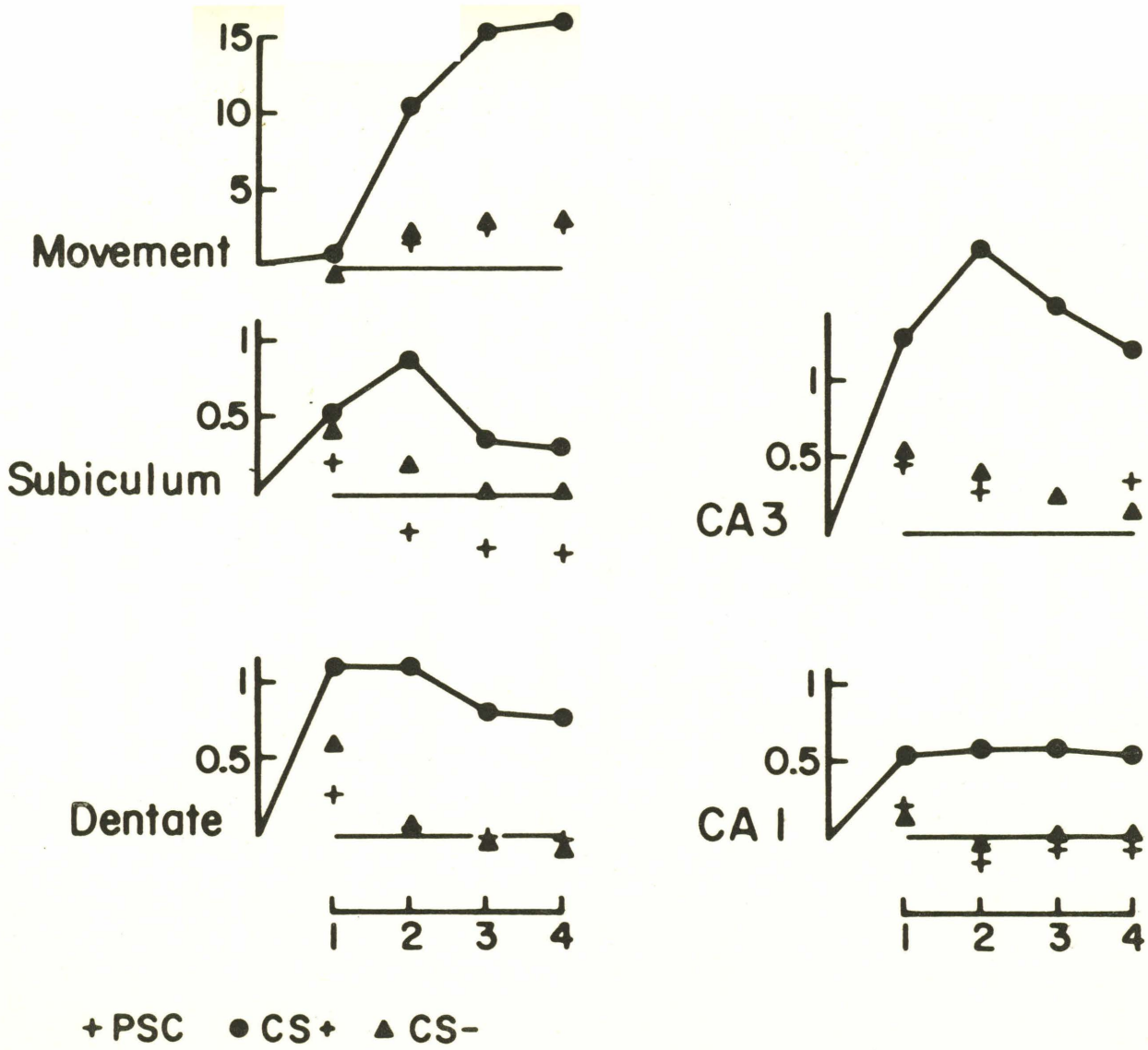
point, all the Q_2's to get the second, and so on (see Figure 2.3). The difference, by means of a x^2 test, between the areas during pseudoconditioning was not significant statistically ($.05 < p < .10$) but it is interesting to note that CA-3 seemed more "responsive" than the dentate, whereas the subiculum was not responsive at all.

The Conditioned Response Shapes

Inspection of the response shapes revealed two things (see Table 2.1 and Figure 2.4). First, there was a relative absence of sustained responses in the subiculum and dentate gyrus, and a presence of these in the CA-3 and CA-1 fields. Second, there was a relatively large number of unresponsive units in CA-1. Of the 14 units recorded from the subiculum, there were 8 yielding early responses, 2 yielding late responses and only 1 with a sustained response (plus 3 cases with no changes). Of the 43 dentate units, 15 showed early responses, 10 had late responses, and there were 6 with sustained responses; there were 12 without change. In the group of 41 units recorded from CA-3 and 4, there were 18 cases with sustained responses, 10 cases with early responses, 5 cases with late responses, and 8 cases with no change. The group of 42 CA-1 units had 16 cases with sustained responses, 4 cases with early ones, and 1 case with late responses. Besides this, there

Figure 2.3

Averaged Q-scores for all units in the various areas during pseudoconditioning expressed as deviation from mean background rate (PSC) in response to CS+ and to CS-; abscissa: value of Q's in standardized scores; ordinate: 4 periods of the CS-US interval, each 250 msec in duration.


15
10
5
Movement
1
0.5
Subiculum
1
0.5
CA3
1
0.5
Dentate
1
0.5
CA1
1 2 3 4
1 2 3 4
+PSC ●CS+ ▲CS-

were 21 cases without change in the CA-1 area; this is a significantly larger number without change than appeared in any of the other fields. Although not considered as responding, 15 of these units had a (-) sign to their largest Q score.

Generalization of the Conditioned Response

The amount of "generalization" was different for units in the various areas. The subiculum and dentate units that showed mainly an early transient response to the CS+, generalized it to the CS-. However, units that had late transient responses to the CS+ did not respond to the CS- (see Figure 2.4).

The other hippocampal areas were less variable, and had relatively small responses to CS-.

A comparison of averaged generalization ratios, by means of a one-way analysis of variance, yielded a significant difference among the areas ($F = 3.66$ $p < .05$). There was less generalization in CA-3 averages than in the dentate ones (Figure 2.5). CA-1 was not different from CA-3; subiculum was not different from the dentate. A possible linear trend ($t' = 6.22$ $p < .10$) was found in subiculum, dentate, CA-3 and CA-1 averages (taken in this order).

Figure 2.4

Post-stimulus time histograms averaged over 300 trials each for units in various hippocampal areas. For every unit 3 traces represent the responses to a tone during pseudoconditioning (PSV), to CS+ and CS- during conditioning. Scale - sec. Bar to the left of every triad of traces represents a probability of firing = 0.10 per bin.

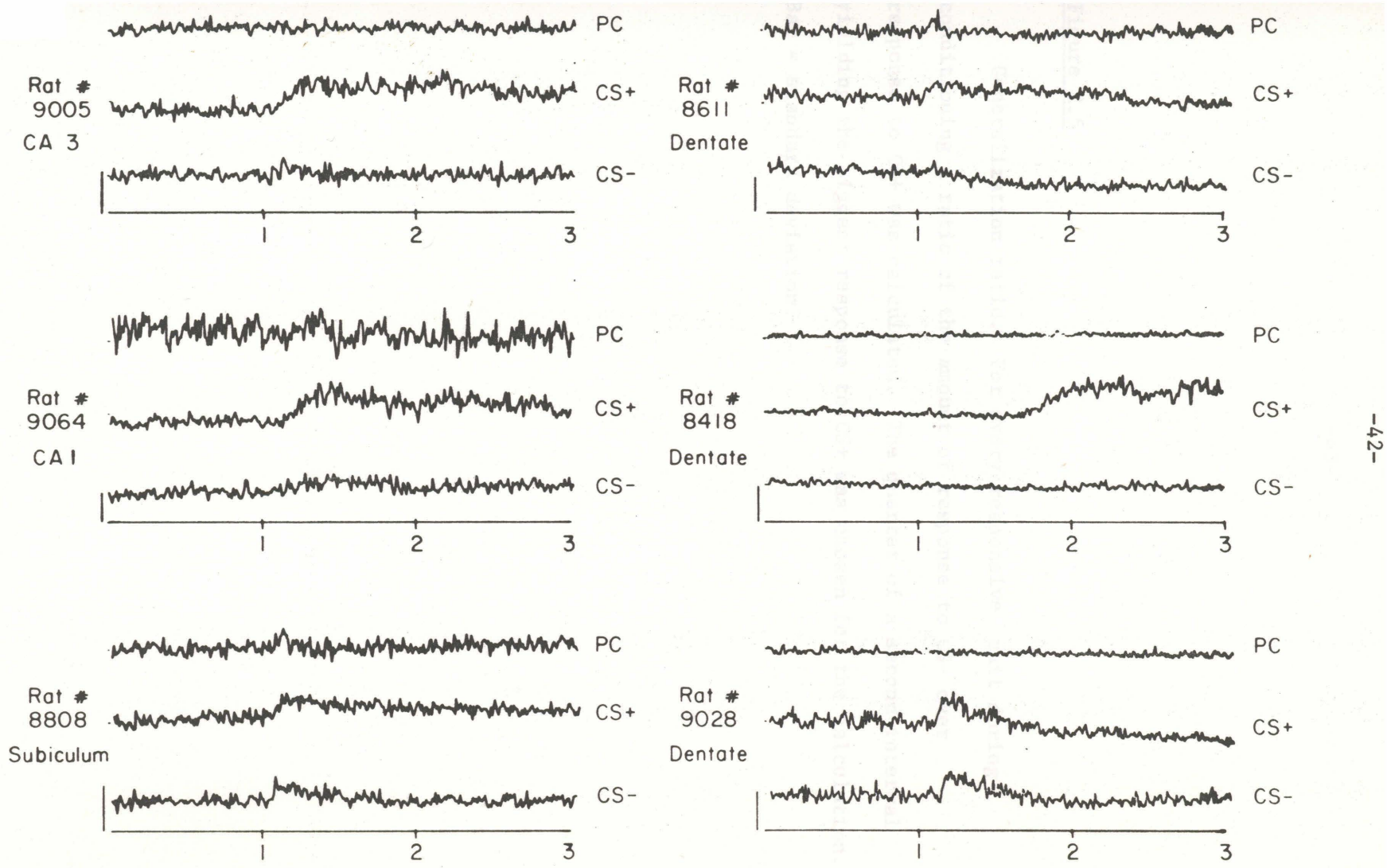
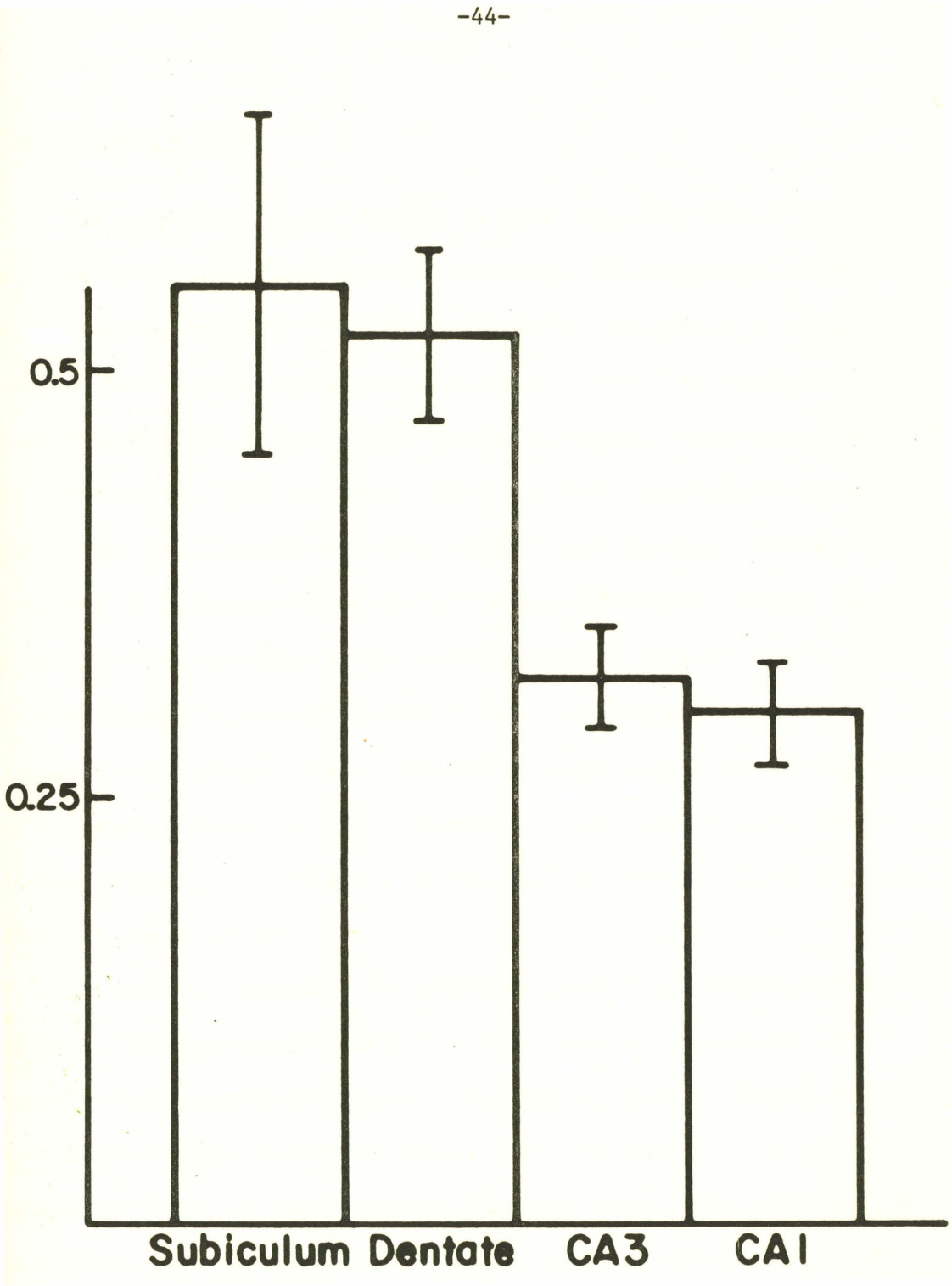

PC
Rat # 9005
CS+
CA 3
CS-
1
2
3
PC
Rat # 8611
CS+
Dentate
CS-
1
2
3
PC
Rat # 9064
CS+
CA 1
CS-
1
2
3
PC
Rat # 8418
CS+
Dentate
CS-
1
2
3
PC
Rat # 8808
CS+
Subiculum
CS-
1
2
3
PC
Rat # 9028
CS+
Dentate
CS-
1
2
3

Figure 2.5

Generalization ratio. For every responsive unit during conditioning a ratio of the amount of response to CS- over response to CS+ was calculated. The quarter of a second interval yielding the biggest response to CS+ was chosen for the calculation. Bar = standard deviation.


0.5
0.25
Subiculum
Dentate
CA3
CA1

The Learning Process

The responses (i.e., changes in rate caused by CS+ or CS-) of all the units in a given area were averaged in successive blocks of 10 trials, starting from 30 trials before the point at which the CS+ became correlated with the pellet. Based on these averages (see Figure 2.6), analyses were performed on the learning curves of the dentate, CA-3 and CA-1. The subiculum was analyzed separately due to the small sample of units taken from this area. A 3-way analysis of variance showed a significant difference between the areas in their response to the correlated tone ($F = 69.84$ $p < .01$). No significant interactions were found. Further analysis showed a significant linear trend, indicating improvement from the beginning to the end of training. This appeared in the dentate group where it was largest, in CA-3 where it was smaller, and in the CA-1 group where it was smallest.

In order to determine the point at which the learning curves start to deviate significantly from the averaged response to the tone in the uncorrelated state (the first 3 points in Figure 2.6), a Dunnett multiple t-test (Edwards, 1968) was performed on the successive means of each averaged learning curve. The first points to deviate from the mean of the uncorrelated trials with a $p < .01$ were found to be the third, fifth, and seventh in the dentate, CA-3 and CA-1, respectively. The results of this analysis are represented by stars in Figure 2.6. It is interesting to note that

Figure 2.6

Averaged learning curve for the various hippocampal areas. Ordinate = number of trials; abscissa = probability of firing per 10 msec bin. Arrow represents the point of beginning of the conditioning.

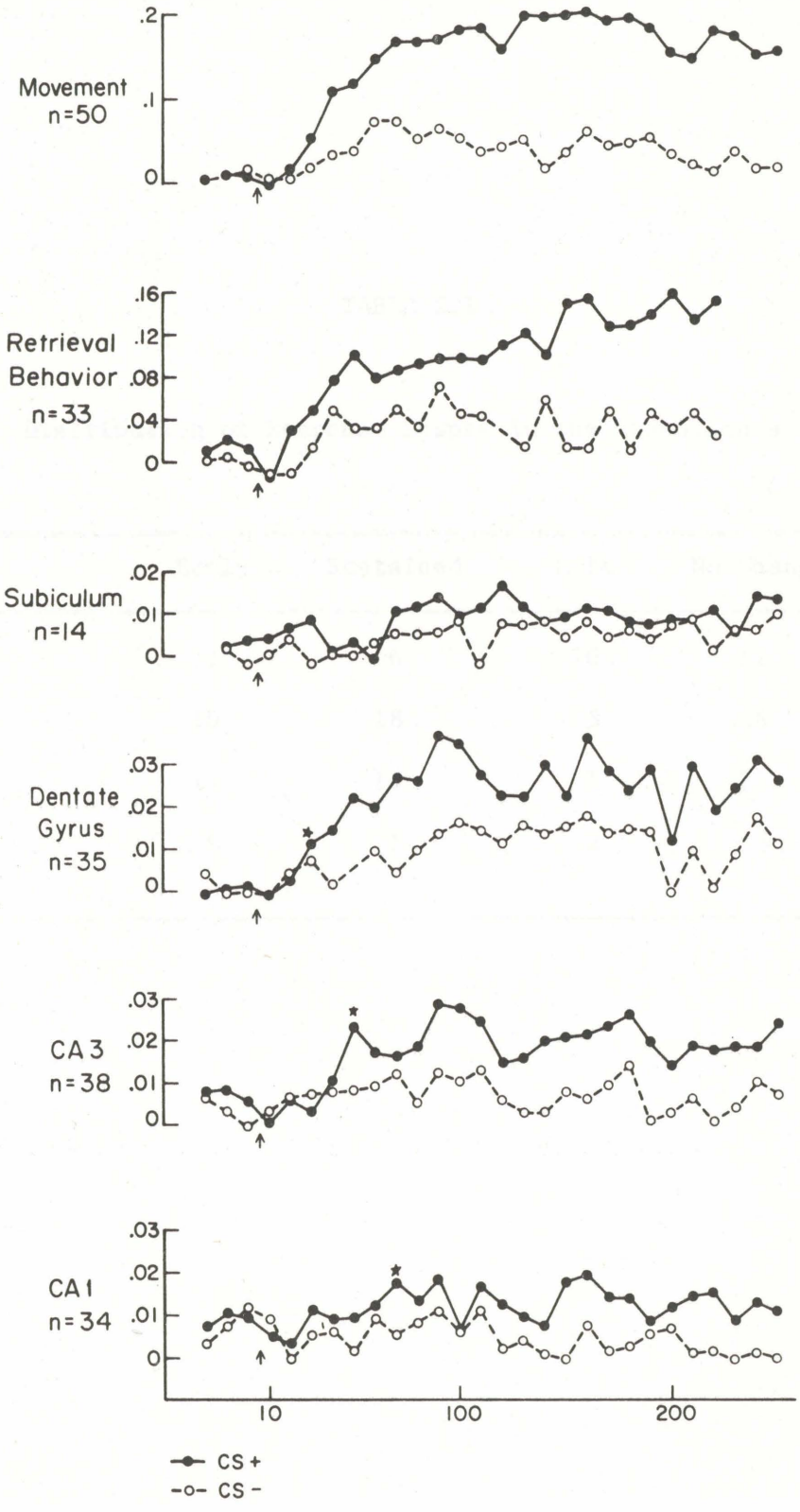

Movement
n=50
.2
.1
0
Retrieval
Behavior
n=33
.16
.12
.08
.04
0
Subiculum
n=14
.02
.01
0
Dentate
Gyrus
n=35
.03
.02
.01
0
CA3
n=38
.03
.02
.01
0
CA1
n=34
.03
.02
.01
0
10
100
200
CS+
CS-

TABLE 2.1

Distribution of Response Shapes in the Hippocampus

	Early	Sustained	Late	No Change
Dentate	15	6	10	12
CA-3	10	18	5	8
CA-1	4	16	1	21
Sub	8	1	2	3

during the 20-30 trial period (3rd point), the dentate group response was significantly above that of the CA-3 group; but during the 40-50 trial period (5th point) they were about equal. During the 40-50 trial period, both of these were significantly larger than responses in the CA-1. During the 60-70 trial period (7th point), CA-1 had responses as large as those in CA-3 (but not as high as those in the dentate group).

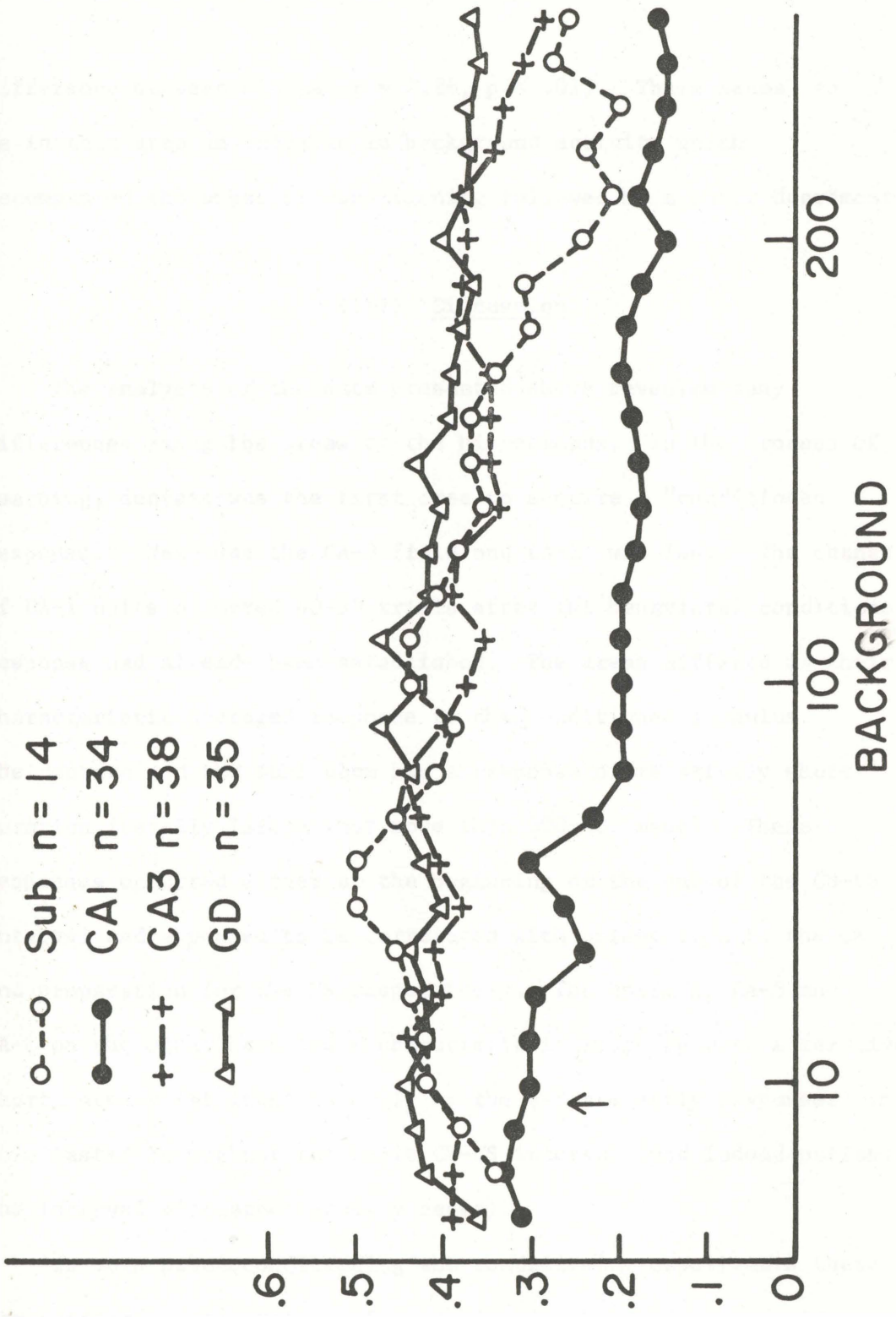
The subiculum was analyzed separately by means of a one-way analysis of variance. This analysis did not yield a statistically significant change in the responses during the sequence of blocks of trials ($F = 1.37$, $p > .05$).

Background unit activity was sampled in the 1 sec pre-CS periods. It was plotted and averaged (Figure 2.7) in a way similar to that for the "response" curves. A two way analysis of variance was performed to compare the amount of background activity in dentate gyrus, CA-3 and CA-1, and to study the changes in these background rates from trial to trial. No trends indicating changes from trial to trial were found. The difference between areas ($F = 37.95$ $p < .01$) was significant. CA-1 had the lowest background rate of activity in comparison to dentate and CA-3.

As mentioned earlier, subiculum was analyzed separately. Surprisingly, a one-way analysis of variance to detect changes across blocks of trials in background activity yielded a significant

Figure 2.7

Background activity recorded during 1 sec pre-CS period. Ordinate = number of trials; abscissa = spikes per 0.1 sec.


Sub n=14
CA1 n=34
CA3 n=38
GD n=35
.6
.5
.4
.3
.2
.1
0
10
100
200
BACKGROUND

difference between blocks ($F = 2.86$, $p < .01$). There seems to be in that area an increase in background activity which accompanied the onset of conditioning followed by a later decrement.

(iii) Discussion

The analysis of the data presented above revealed many differences among the areas of the hippocampus. In the process of learning, dentate was the first area to acquire a "conditioned response." Next was the CA-3 field and CA-1 was last. The change of CA-1 units occurred 40-50 trials after the behavioral conditioned response had already been established. The areas differed in their characteristic averaged response to the conditioned stimulus. The dentate and the subiculum had a response of relatively short duration (usually lasting not more than 300-400 msec). These responses occurred either at the beginning or the end of the CS-US interval and appeared to be correlated with orientation to the CS and preparation for the US respectively. The units of CA-3 and CA-1 on the other hand had a characteristic response with a relatively short latency (at least as early as the dentate early responses) and this lasted throughout the whole CS-US interval (and indeed outlasted the interval altogether in many cases).

In both pseudoconditioning and conditioning experiments there were different proportions of the units in the different fields

showing responses. CA-3 field yielded more responses during pseudo-conditioning; these were brief (similar to those which characterized the dentate units after conditioning). CA-1 was the only field with a high percentage of unresponsive units after conditioning.

There were also marked differences in the degree of generalization. The dentate early responses were more generalized (showing almost no differences between CS+ and CS-). The CA-3 and CA-1 responses were by contrast well discriminated (showing clear difference between responses to CS+ and CS- in almost all cases). Best discriminated of all however were the late responses in dentate which were in all cases clearly different for CS+ and CS- (as were the behavioral responses to the two stimuli).

There were also some differences in background change during the course of learning. The spontaneous activity rates (observed in the recording periods just prior to CS application) were relatively constant during, before, and after learning in dentate and in CA-3 and CA-1, but there were increases in background activity in subiculum which occurred during the period of rapid behavioral improvement and then returned to normal as the gross conditioned response became stabilized.

In addition to these findings, other important differences among hippocampal areas were observed in this experiment. These were reported separately (Olds et al., 1972) and will be summarized briefly. CA-3 units sometimes had very early latencies of conditioned

responses (in the 10-20 msec range). CA-1 units and dentate units had somewhat longer latencies of their earliest conditioned responses (the earliest observed were in the 20-40 msec range). Subiculum units were not observed in large enough numbers to yield a clear answer.

What conclusions can be derived concerning the nature of the activity in the hippocampus circuit during conditioning?

Several different findings seemed compatible with the possibility that a "conditioned response" emerged in the CA-3 field (or in its entorhinal or septal afferent systems) and that the response was projected from there to the CA-1 field. The first supporting evidence was that the CA-1 response did not appear in the course of training until after there was already a response in the CA-3 field. Second, there were the shorter latencies observed in the CA-3 field. Third, there was the similar character of the responses in the two areas (responses were relatively early in onset and were sutained through and after the CS-US interval). Fourth, there was the difference in responses between CS+ and CS- (which was the same in both areas). These four similarities lent credence to the view that these two areas are linked.

Other evidence exists that the projection was not simple and that there were quite likely secondary synaptic changes between the two fields. First, the CA-1 responses did not appear concurrently with those in CA-3 but rather lagged behind by a number of trials.

Second, there was the relatively smaller number of units which developed conditioned responses in CA-1 (it appeared that most of the CA-3 units were less selective in their readiness to participate in a learning process). The projection from CA-3 into CA-1 may therefore involve secondary changes occurring at the linkage between the two fields. This would most likely be in the synapses between the Schaeffer collateral axons and the CA-1 apical dendrites.

All of the tests which indicated this possible relation between CA-3 and CA-1 were also applied to the relation between the dentate gyrus and the CA-3 field. Only one of them seemed compatible with a dentate to CA-3 projection: dentate units showed learning changes earlier in the course of training than did the CA-3 units. Several other findings, however, militated against a projection of the conditioned response from dentate to CA-3. First, there was a difference in response pattern between the sustained responses of CA-3 and the transient ones that appeared in the dentate gyrus. Second, there was a difference in degree of generalization between CS+ and CS- , there was a large degree of generalization in the early dentate unit responses but almost none in the early CA-3 responses. Because of these findings against projection of a conditioned response, the possibility was considered that some change in background activity of dentate units might

program the new responses in CA-3 which would then be triggered or released by a different afferent system. However, there was no observed change in the background activity of dentate gyrus units during the course of conditioning. For all of these reasons it was scarcely conceivable that there was any significant direct dependence of the CA-3 responses on their being triggered by dentate afferents.

Nevertheless, CA-3 neurons represent the main projection field of the dentate neurons and therefore some indirect relation of a non-"triggering" nature must be presumed. The special relations between dentate units and the overt behavior seemed to help clarify one possible meaning of this indirect relation. Several facts tied dentate units to behavior. The early and late dentate responses were closely tied in time to the orienting response on the one hand or to the food-preparatory response on the other. Also, in behavior and dentate units, the early response was poorly discriminated between CS+ and CS-, and the late response was well discriminated. This suggested that they were fed back from behavioral performance in some way. The possibility must be considered, therefore, that dentate served to reflect information about ongoing behavior into CA-3. This might serve in one way or another to modulate or reinforce the activity of the hippocampal fields. The re-afferent information might help to prolong CA-3 activity after conditioning,

thereby accounting for the difference between the brief CA-3 response during pseudoconditioning and the relatively enduring CA-3 actitivy after conditioning had occurred.

The relatively late appearance of the hippocampal responses in the course of the training rules out the possibility of its involvement in the early phases of classical conditioning. It leaves, however, the possibility of its involvement in the establishment of a long term memory. This possibility was indicated by many stimulation (9), and lesion (13) studies. An alternative interpretation of the later appearance of conditioned responses in these areas is suggested by the fact that the animals continued to improve in performance of the retrieval behavior long after the first signs of learning appeared, and this leaves open the possibility of involvement of the hippocampus in elaboration of skilled instrumental behavior (15).

One objection to the view that the conditioned responses observed in hippocampus were generated by changes in synaptic connection between afferents from septal area or entorhinal cortex and the CA-3 elements lies in the possibility that the responses were generated in one of those afferent systems (or earlier) and projected to this point. Prior conditioned responses in other brain areas are rendered somewhat unlikely because the conditioned response latencies of some of the CA-3 units were among the shortest observed in a study (Olds et al., 1972) which made, in

at least a preliminary fashion, a fair sample of the brain. It is possible nevertheless that conditioned activity in some other area preset the CA-3 units to respond with a latency this short. One candidate would be the subiculum which showed interesting changes in background activity of the sort that would need to be involved in any presetting mechanism (see Figure 2.7). The difficulty is that the time course of background changes (which consisted of a transient increment during the early part of the learning series) did not have the same form as the learning curve for CA-3 units (which consisted of an increment somewhat later which was not transient). If there was a "presetting" afferent system therefore it was more likely the entorhinal area or other similarly placed sources of the perforant pathway, the latter being perhaps the largest system of afferents projecting to CA-3. It is therefore possible either that the entorhinal cortex elements set the CA-3 dendrites, via the perforant path, to respond with a short latency to some other input--septum perhaps, or that there was actually a local synaptic change in the connections between axons from entorhinal cortex, or septum and the CA-3 dendrites. Further experiments probing these two afferent areas are required to resolve the issue. Both interpretations, however, agree that CA-3 may have been the junction point upon which information converged, and in which decisions (such as differentiation) were made.

Experiment 2: Analysis of Response Latency in Hippocampus and Related Structures

The previous experiment demonstrated the progression of a conditioned response through the hippocampus. A few points needed further clarification: (1) Do dentate unit responses have longer latencies than units in area CA-3, to which the dentate is projecting? (2) What are the relations between the latencies of hippocampal units' responses and its afferents; what is driving the hippocampus? (3) Are the conditioned responses observed in hippocampus a mere reflection of conditioning established elsewhere? Several considerations led to the design of the present experiment. Since the main variable of interest was the response latency, the finest grain of separation within the limits of the instruments was used. In order to optimize learning, a shorter CS-US interval and shorter pseudoconditioning session were used. As another control and as a consequence of the assumed involvement of the hippcampus in extinction processes, an extinction session was introduced. These procedures would reduce variability and give a more coherent picture of response latency in the examined areas. On the other hand, variables other than response latency having already been examined, were not considered.

(i) Methods

Subjects

155 rats were used in this experiment.

Procedure

The experiment was conducted on two successive days. Each day was divided into two equal sessions. First there were pseudo-conditioning; two tones, each 400 msec in duration, and food pellets were presented in random order, with random inter-trial interval (mean = 1 min), 150 times each. Following was a conditioning session, i.e., a food pellet was present 300 msec after the onset of one of the tones. 150 trials of food reinforced-tone (CS+) and non-reinforced tone (CS-) were presented in this session, distributed over equal amount of time as in the pseudo-conditioning session. The first day was followed by an 8 hour wait and then a second day's experiment began. This was identical to the first day, with the order reversed: first conditioning, and then pseudoconditioning extinction session.

Data Collection and Analysis

Unit activity was sampled during 150 msec prior to application of the stimuli and for 300 msec from the onset of the tones. The data was gathered in successive 1.5 msec bins. There were 100 bins

for the pre-CS period and 200 bins for the CS-US interval. Ten successive trials were grouped (see Figure 3.1) and stored on an IBM tape for further analysis. Two types of analysis were done.

1. The Unit Response Latency. For every unit, an averaged firing histogram was prepared for every session, each including 130 trials (excluding the first 20 trial group of a session) (see Figure 3.2). The mean of the 100 bin background firing rate was calculated as well as the standard deviation of the mean. The averaged firing rate in four successive bins (6 msec) during the CS-US interval was then converted to standard scores by subtracting their mean firing rate from the mean of the background and dividing by the standard deviation of the background. The firing rate at a given point (6 msec interval) was considered as a response to the tone if its probability of occurrence by chance (i.e., in the absence of stimulation) was less than 1 percent (two-tail test). Since this test was made on successive points, a more stringent criterion was used for determining a response latency: the first point in the CS-US interval to deviate significantly from background firing mean was considered as the response latency only when it was followed by a similar significant point in at least one of the next three successive points. The probability of two out of four adjacent points with a significant value occurring by chance is very low and in fact, this occurred only when a response started, that is, when most of the successive points had a significant value. For

Figure 3.1

Unit responses recorded throughout an experiment. Each line represents the summed unitary activity during successive ten trials. Trial periods are successive, from bottom to top. Lines 3-15 = pseudoconditioning; lines 16-30 = conditioning. The unit activity is recorded during 150 msec (pre-CS period) and 300 msec (tone period). In the conditioning session food pellets are applied at the end of the recording period (300 msec CS-US interval).

UNIT IN CA_3

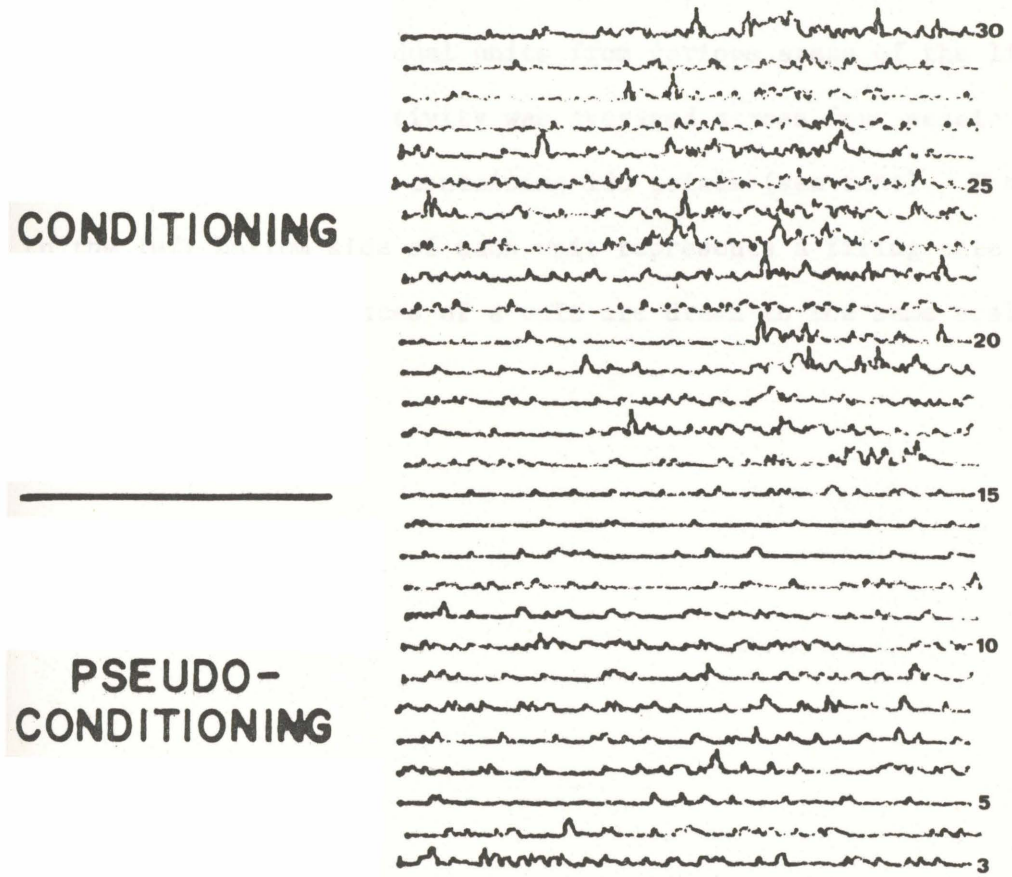
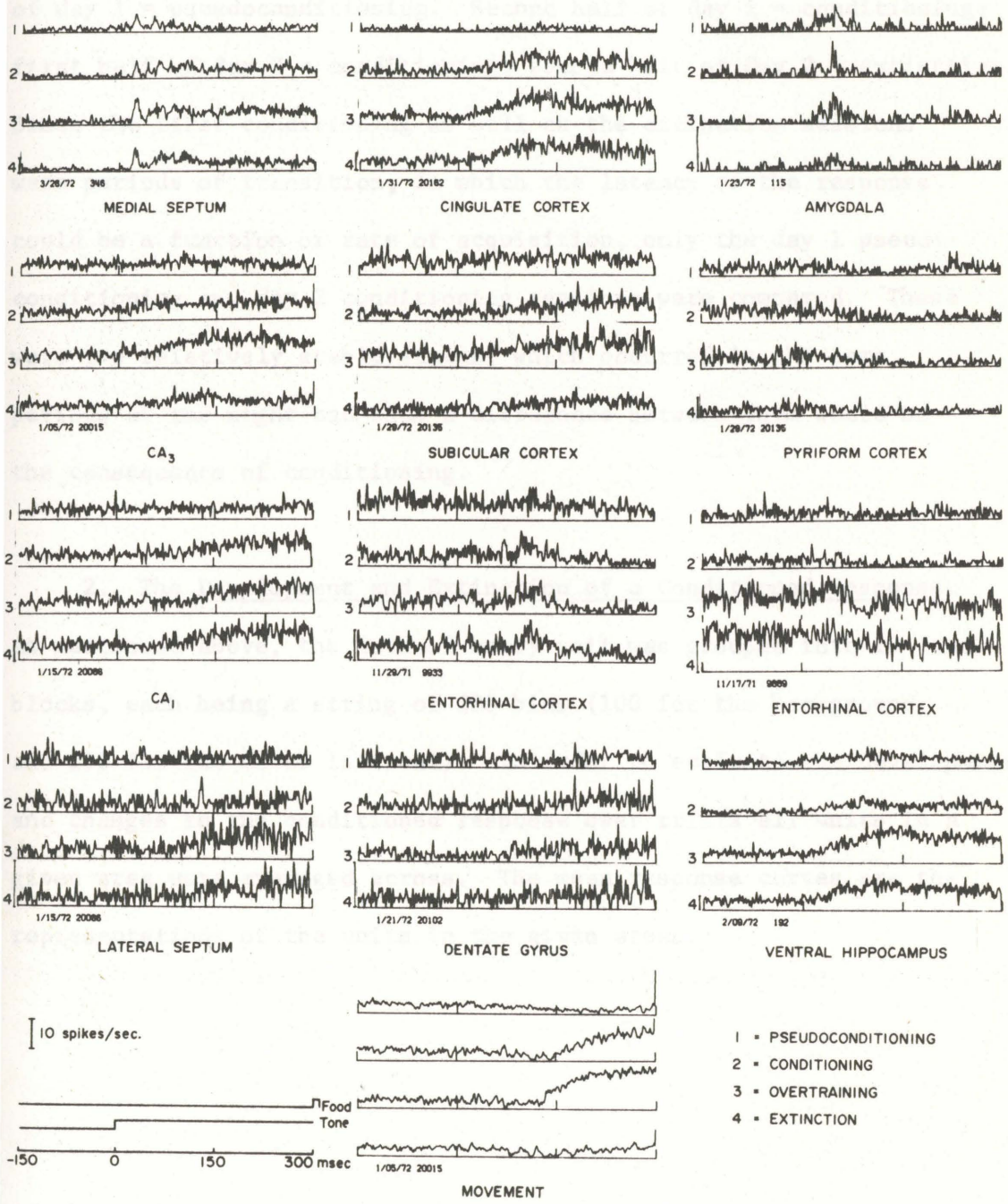


Figure 3.2

Examples of individual units from various areas of the limbic system. The unitary activity was averaged across four sessions of the experiment, each containing 130 trials (see text). The bar to the left bottom side of each unit represents a firing rate of 10/sec . All four traces of a unit are drawn to the same scale.

EXAMPLES OF UNIT RESPONSES TO THE CONDITIONED STIMULUS



every unit four response latencies were determined: First half of day 1 = psuedoconditioning. Second half of day 1 = conditioning; first half of day 2 = conditioning; second half of day 2 = extinction. Since the first conditioning as well as the extinction sessions were periods of transition, in which the latency of the response could be a function of rate of acquisition, only the day 1 pseudo-conditioning and day 2 conditioning sessions were compared. These were two relatively stable states, which occurred in the same periods of the night and thus a difference between them would be the consequence of conditioning.

2. The Development and Extinction of a Conditioned Response. As mentioned above, the data of every unit was grouped into 10 trial blocks, each being a string of 300 bins (100 for the background, and 200 for the CS-US interval). In order to evaluate the development and changes in the conditioned response over trials all units in a given area were averaged across. The mean response curves are the representations of the units in the given areas.

(ii) Results

General

Out of 155 rats participating in the experiment, the data of 15 rats had to be excluded because of experimental failures (i.e., rats that did not learn) or mechanical failures (such as no collection of data, feeder jammed). 56 additional units had to be excluded because of: (1) inclusion of noise in the records (detected by inspecting plotted samples of the identified spikes), (2) insufficient data (i.e., disappearance of the unitary activity during a session and (3) units located outside the areas of interest in the brain. 473 units passed the various criteria and are included in this study.

The distribution of the units is to the following areas: medial septal nucleus, 36; area CA-3 of hippocampus, 46; CA-2 (units that could not be accurately localized in area CA-3 or CA-1), 27; CA-1, 68; dentate, 59; posterior subicular cortex (including subiculum and presubiculum, 43; entorhinal cortex (including lateral and medial entorhinal), 35; posterior cigulate cortex (including retrosplenial area), 43; pyriform cortex, 15; amygdala (posterio lateral and basal groups), 14; lateral septal nucleus,42; ventral hippocampus, 45 units. The distribution of the units in the brain is presented in Figure 3.3. Since the boundaries between some areas are not distinct, a careful microscopic examination had to be made. Considerable help was achieved by comparisons with

schemes drawn by Lorente de Nŏ (1934), Raisman et al. (1965), Rose and Woolsey (1948), and Adey (1961). In some cases (like area CA2) where no clear agreement exists between anatomists, a special category had to be made. In others, when no clear differences between subgroups of units could be detected and there was a small number of units in each subgroup, these were lumped together (i.e., medial and lateral entorhinal).

The Response Latency

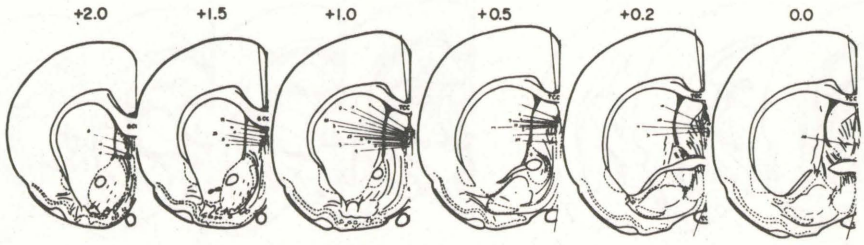
1. Responses During Pseudoconditioning. The majority of units in the areas studied did not show a significant response during pseudoconditioning. The percentage of responsive units was as follows: 28% in area CA3, 31% in CA1, 35% in subiculum, 33% in ventral hippocampus, 30% in dentate, 31% in entorhinal cortex, and 33.2% in lateral septum. In sharp contrast to these areas, there were the medial septal nucleus, 64% and the posterior cingulate cortex, 56%. In the medial septal nucleus, 25% of the units had a response latency of 36 msec or less and 50% of the units responded within 96 msec of CS onset. In posterior cingulate cortex, 25% of the units responded within 84 msec, and more than 50% of the units responded within 180 msec. In amygdala (mainly the lateral posterior group), 4 units (29%) responded within 60 msec (Figure 3.4). It seems, therefore, that while the main body of the hippocampus was relatively inactive during pseudoconditioning,

Figure 3.3

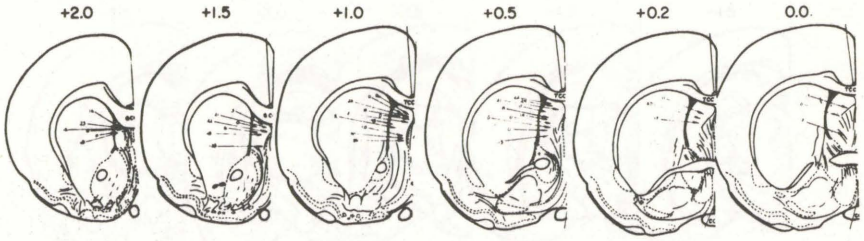
Distribution of the recorded units in the various areas of the rat brain. Each arrow represents a single unit. Brain sections are taken from König and Klippel. Numbers on the top of each section are anterior-posterior coordinates. Bregma (on the skull) is the zero line.

DISTRIBUTION OF RECORDED UNITS

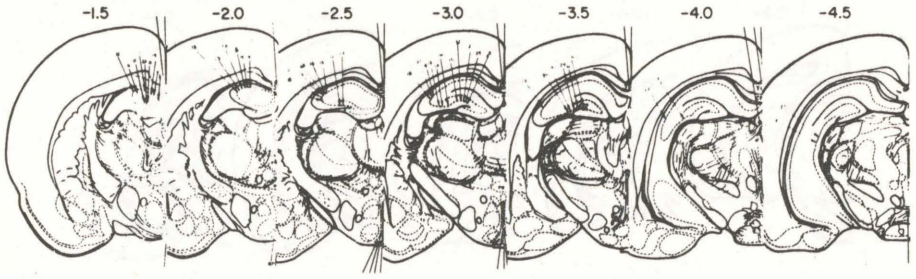
MEDIAL SEPTUM



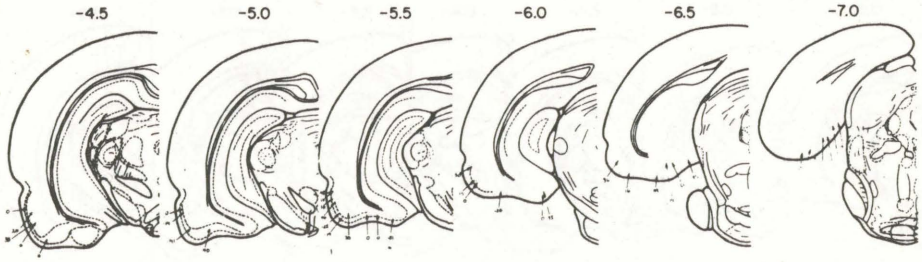
LATERAL SEPTUM



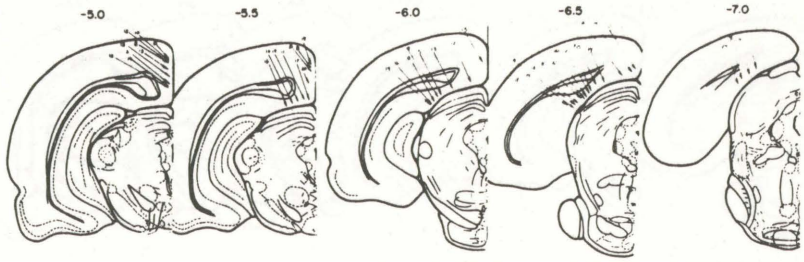
CA_3



ENTORHINAL CORTEX



POSTERIOR CINGULATE CORTEX



DISTRIBUTION OF RECORDED UNITS

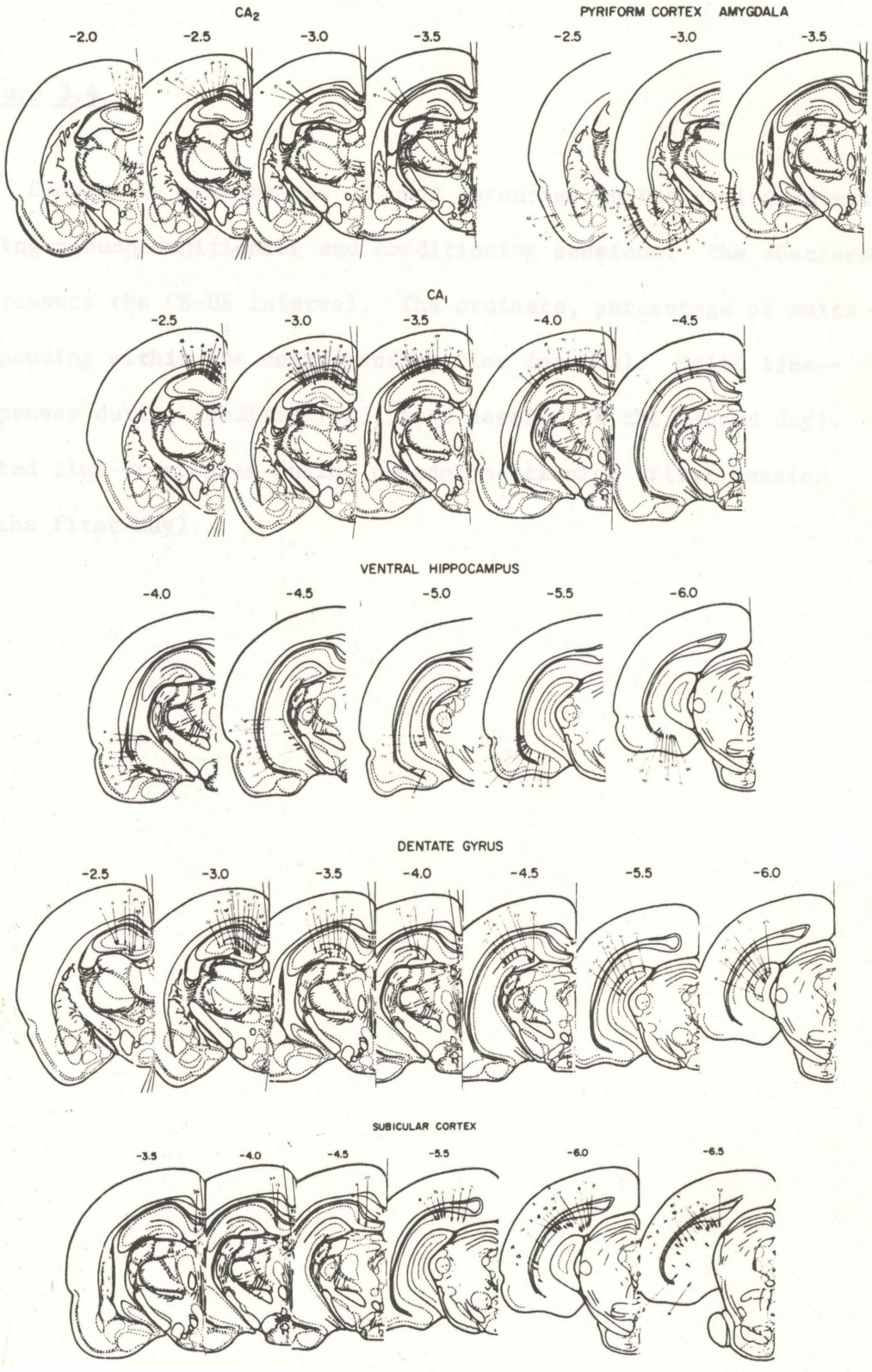
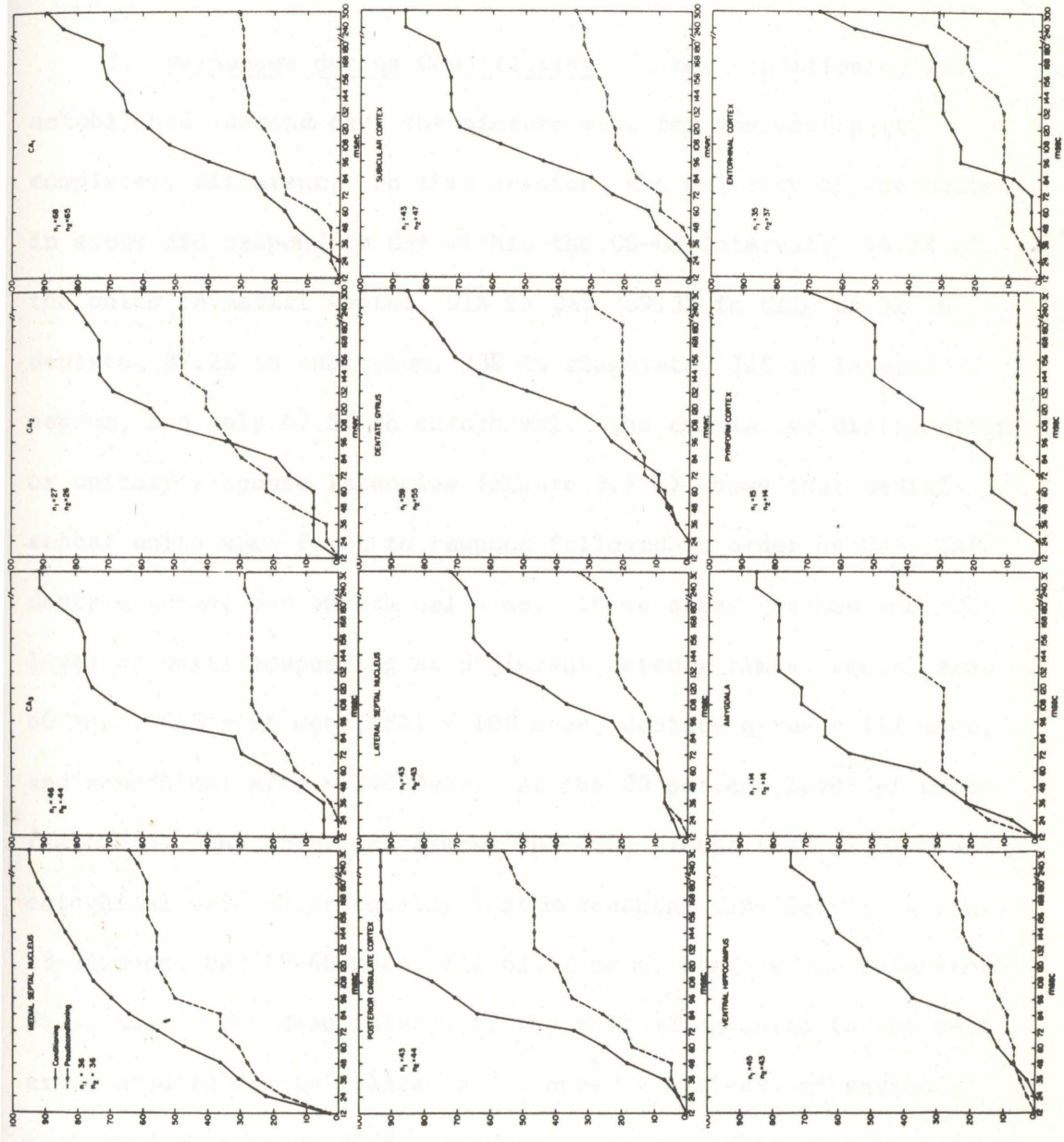


Figure 3.4

Cumulative percentages of unit latencies in the various areas during pseudoconditioning and conditioning sessions. The abscissa represents the CS-US interval. The ordinate, percentage of units responding within the corresponding time interval. Solid line--responses during conditioning (first session of the second day). Dotted line--responses during pseudoconditioning (first session of the first day).


MEDIAL SEPTAL NUCLEUS
Conditioning
Pseudoconditioning
CA1
CA2
CA3
SUBICULAR CORTEX
DENTATE GYRUS
LATERAL SEPTAL NUCLEUS
ENTORHINAL CORTEX
PYRIFORM CORTEX
AMYGDALA
POSTERIOR CINGULATE CORTEX
VENTRAL HIPPOCAMPUS
msec

larger numbers of unit responses in its afferent fields could be detected.

2. Responses during Conditioning. When conditioning was established (second day) the picture was, for the most part, completely different. In this session, the majority of the units in study did respond to CS+ within the CS-US interval: 94.5% of the units in medial septum, 91% in CA3, 89.3% in CA1, 85.5% in dentate, 87.2% in subiculum, 93% in cingulate, 72% in lateral septum, and only 67.5% in entorhinal. The cumulative distribution or unitary response latencies (Figure 3.5 b) shows that medial septal units were first to respond followed in order by CA3, CA1, dentate gyrus, and entorhinal area. These areas reached the 50% level of units responding at different latency times: septal area = 60 msec, CA3 = 96 msec, CA1 = 108 msec, dentate gyrus = 132 msec, and entorhinal area = 240 msec. At the 20 percent level of units responding, the order was almost the same except that dentate and entorhinal were about equally fast in reaching this level: septal 13-24 msec, CA3 49-60 msec, CA1 61.72 msec, dentate and entorhinal 85-96 msec. The mean latency of the responding units in the main areas studied was calculated and a one-way analysis of variance performed to compare differences among areas. This test yielded a significant difference among the areas studied ($F(8:343) = 9.77$, $p < .01$). Further individual comparisons by the Newman-Keuls procedure (Table 3.1) showed no differences in mean response latency

Figure 3.5a

Cumulative percentages of unit responses latency in the main hippocampal areas and the afferents to the hippocampus during pseudoconditioning. Scale is the same as in Figure 3.4.

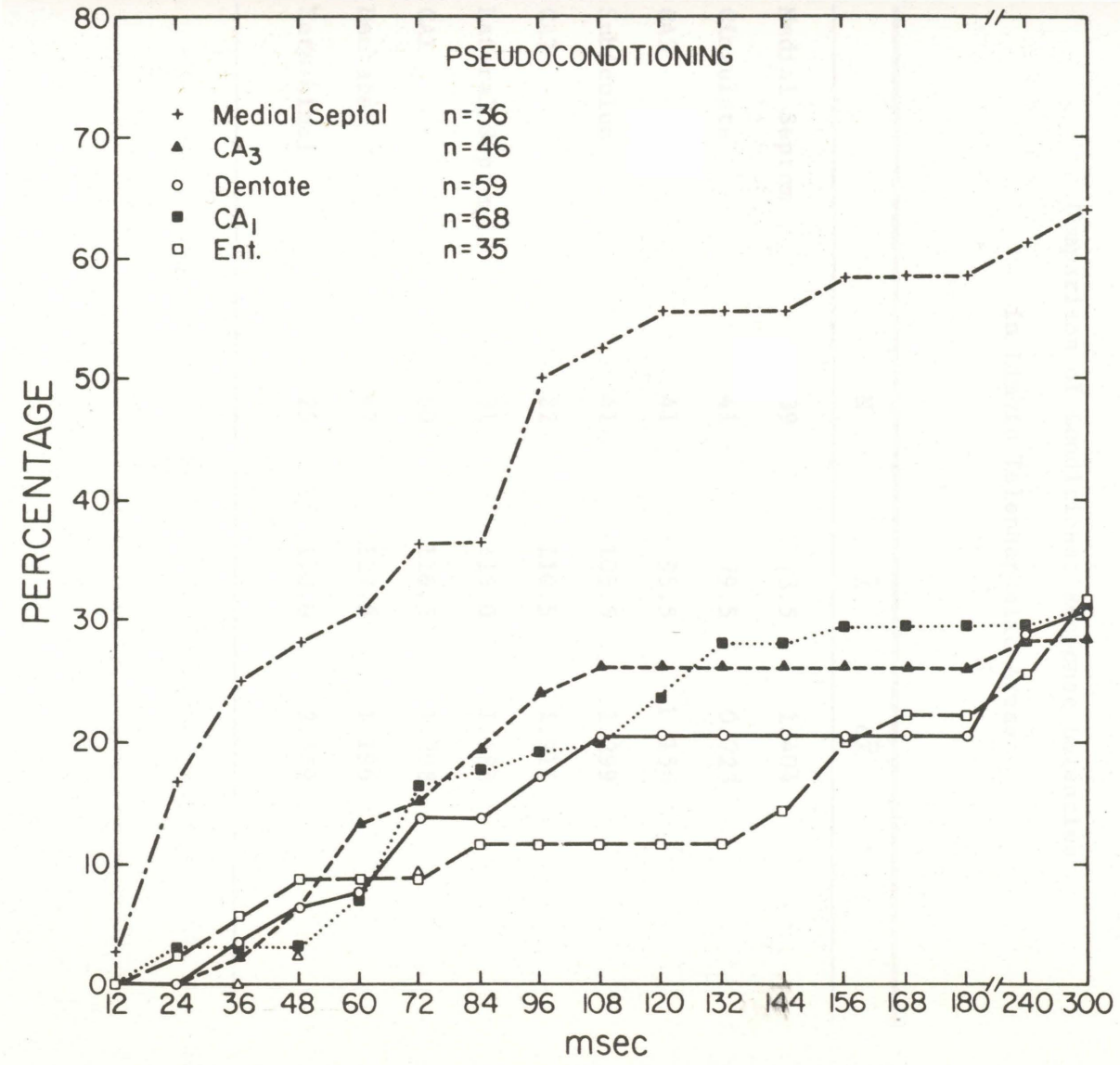

PSEUDOCONDITIONING
Medial Septal n=36
CA3 n=46
Dentate n=59
CA1 n=68
Ent. n=35
PERCENTAGE
msec
0
10
20
30
40
50
60
70
80
12
24
36
48
60
72
84
96
108
120
132
144
156
168
180
240
300

TABLE 3.1a

Comparison of Conditioned Response Latencies

in Limbic Telencephalic Areas

	N	$\bar{X}$	$\sigma\bar{X}$
Medial Septum	39	73.5	1.404
Cingulate	41	79.5	0.723
CA3	41	95.5	1.158
Subiculum	41	105.5	1.299
CA2	22	110.5	1.911
Lateral Septum	31	115.0	1.688
CA1	60	116.5	1.398
Dentate	47	127.5	1.196
Entorhinal	25	170.0	2.489

TABLE 3.1b

Newman-Keuls Comparisons

	M. septum	Cingulate	CA3	Subiculum	CA2	L. septum	CA1	Dentate	Entorhinal
Medial septum									
Cingulate	N.S.								
CA3	*	*							
Subiculum	*	*	*						
CA2	*	*	*	N.S.					
Lateral septum	*	*	*	*	N.S.				
CA1	*	*	*	*	N.S.	N.S.			
Dentate	*	*	*	*	*	*	*		
Entorhinal	*	*	*	*	*	*	*	*	*

*Significant difference at the .05 level

N.S. = not significant

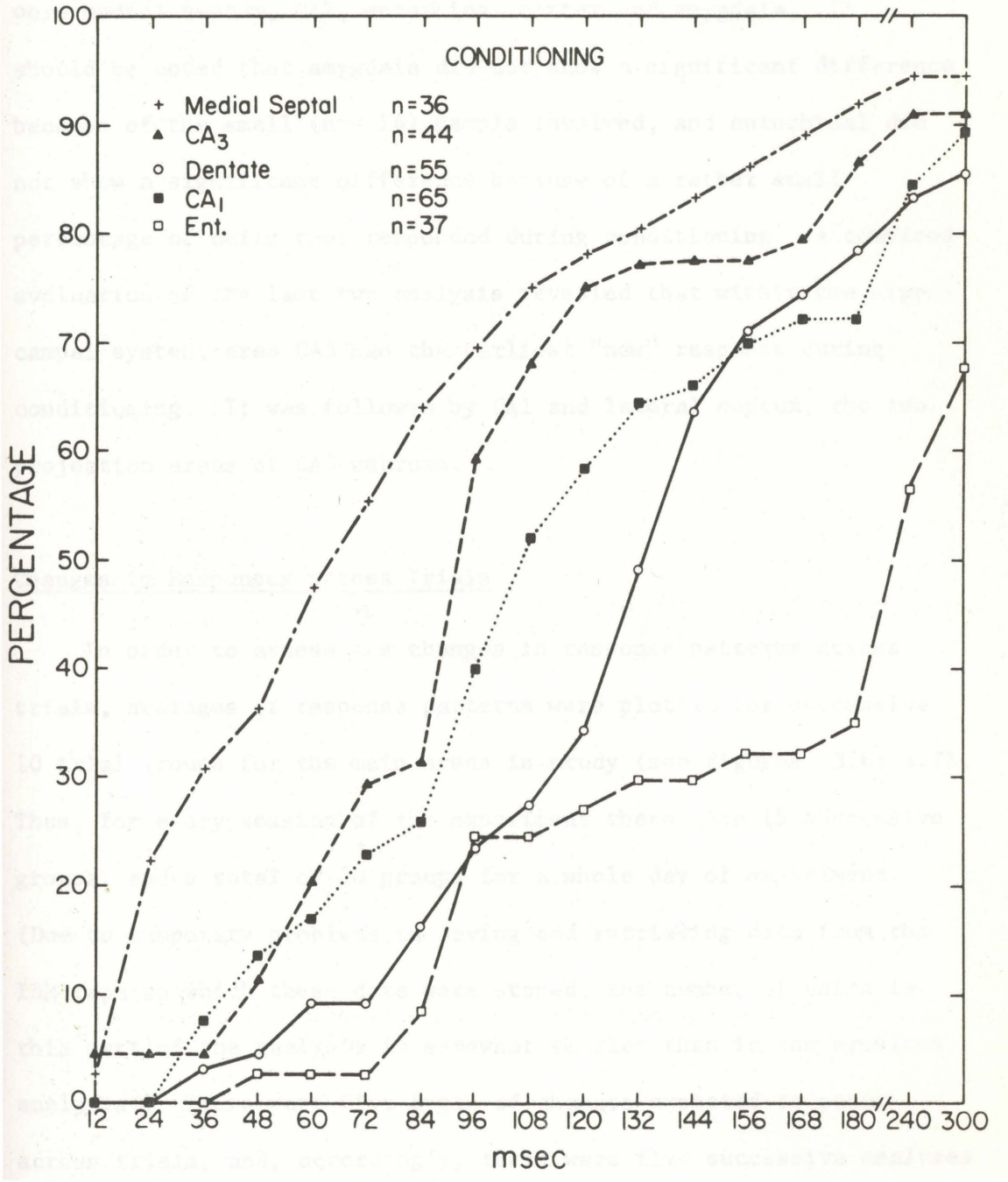
between medial septal area and posterior cingulate, between CA2, CA1, and lateral septum, and between CA2 and subiculum. All the other comparisons yielded significant differences between areas. The order of response latency (from the shortest to the longest response latencies) was the following: medial septal ($\bar{X}$ = 70 msec) and posterior cingulate (76 msec), CA3 (93 msec), subiculum (102 msec). CA2 (108 msec), CA1 (113 msec) and lateral septum (112 msec), dentate (124 msec), and last, entorhinal (167 msec). Based on the known wiring diagram there seems to be three functional pathways in this system: (1) medial septum--CA3--CA2--lateral septum, (2) entorhinal--dentate, and (3) cingulate--subiculum.

Differences between Pseudoconditioning and Conditioning Sessions

The distribution of latencies in pseudoconditioning and conditioning sessions were compared in every area in order to find which were the areas that showed a "new response" in the conditioning session. A two sample Kolomogorov-Smirnov test (Siegel, 1956) was used for this purpose. A cutoff point for significant D (difference) values was chosen to be of $p \leq .01$. The areas that showed a significant difference between pseudo-conditioning and conditioning were: CA3, CA1, dentate, subiculum, ventral hippocampus, lateral septum, pyriform cortex, and posterior cingulate. The areas that showed no difference (at the .01 level)

Figure 3.5b

Cumulative percentages of unit response latency in the main hippocampal areas and the afferents to the hippocampus during conditioning. Same scale as in Figure 3.4.


CONDITIONING
Medial Septal n=36
CA3 n=44
Dentate n=55
CA1 n=65
Ent. n=37
PERCENTAGE
msec
0
10
20
30
40
50
60
70
80
90
100
12
24
36
48
60
72
84
96
108
120
132
144
156
168
180
240
300

were medial septum, CA2, entorhinal cortex and amygdala. It should be noted that amygdala did not show a significant difference because of the small (n = 14) sample involved, and entorhinal did not show a significant difference because of a rather small percentage of units that responded during conditioning. A combined evaluation of the last two analysis revealed that within the hippocampal system, area CA3 had the earliest "new" response during conditioning. It was followed by CA1 and lateral septum, the two projection areas of CA3 neurons.

Changes in Responses across Trials

In order to assess the changes in response patterns across trials, averages of response patterns were plotted for successive 10 trial groups for the main areas in study (see Figures 3.6; 3.7). Thus, for every session of the experiment there were 15 successive groups, and a total of 30 groups for a whole day of experiment. (Due to temporary problems in saving and retrieving data from the IBM tape on which these data were stored, the number of units in this part of the analysis is somewhat smaller than in the previous analysis.) There were five types of changes expected to occur across trials, and, accordingly, there were five successive analyses done on the data of every area. First, there were changes across habituation and pseudoconditioning sessions. For this purpose, the changes within trial and across the first fifteen groups were

Figure 3.6

Averaged response curves for successive ten trial blocks throughout the first day of the experiment. Each histogram is made of 30 successive points; five from the pre-CS period and 25 of the CS-US interval. (Each point being 12 msec interval). The shaded area in each histogram is the amount of unit activity that is above the man of 100 background sample points. The distance between adjacent bars to the left of each series of histograms represents a probability of 0.1 per interval. For every area in the brain there are 30 histograms. The first 15 are the pseudoconditioning session and the last 15, the conditioning session.

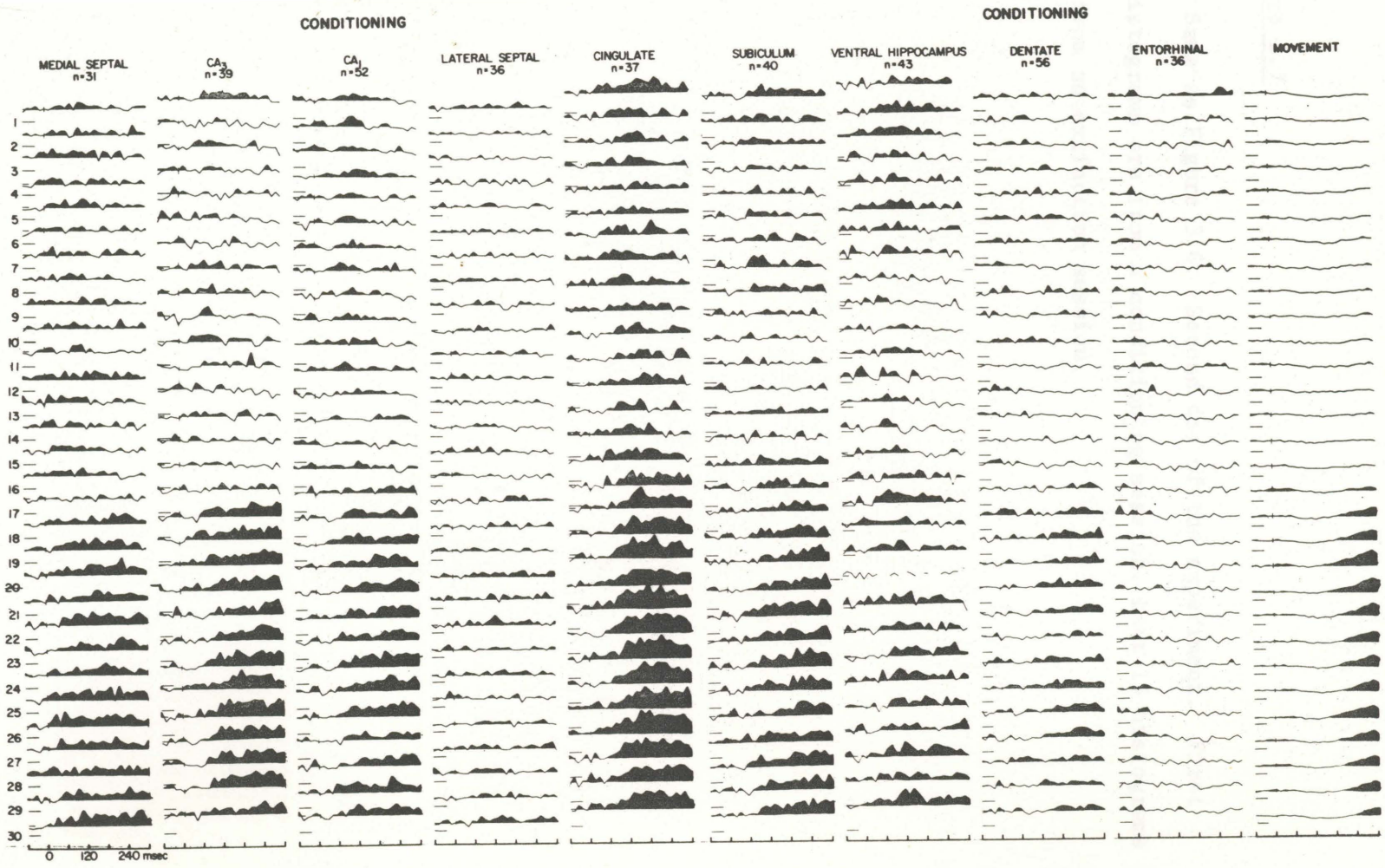
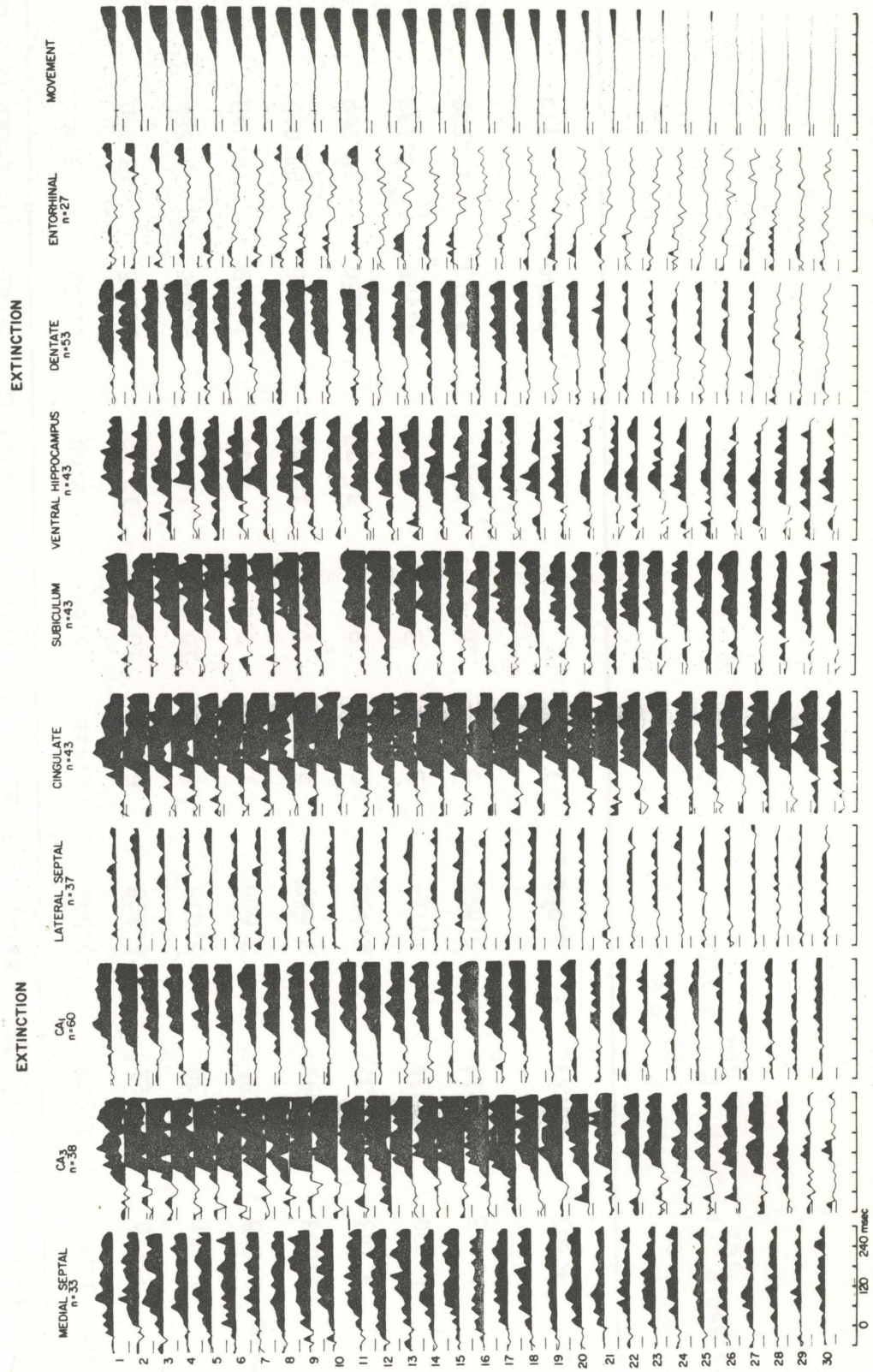

CONDITIONING
CONDITIONING
MEDIAL SEPTAL n=31
CA3 n=39
CA1 n=52
LATERAL SEPTAL n=36
CINGULATE n=37
SUBICULUM n=40
VENTRAL HIPPOCAMPUS n=43
DENTATE n=56
ENTORHINAL n=36
MOVEMENT
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
0 120 240 msec

Figure 3.7

Same as Figure 3.6. Second day of the experiment. First 15 histograms are from a conditioning session. Last 15 histograms are from an extinction session.


EXTINCTION
EXTINCTION
MEDIAL SEPTAL n=33
CA3 n=38
CA1 n=60
LATERAL SEPTAL n=37
CINGULATE n=43
SUBICULUM n=43
VENTRAL HIPPOCAMPUS n=43
DENTATE n=53
ENTORHINAL n=27
MOVEMENT
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
0
120
240 msec

Analysis of variance for trends across treatments

	Pseudoconditioning		Conditioning		Overnight		Conditioning		Extinction	
	Across trials (1)	Within trial (2)	Across trials (1)	Within trial (2)	Across trials (1)	Within trial (2)	Across trials (1)	Within trial (2)	Across trials (1)	Within trial (2
Medial septum	1.202	1.986	5.942	1.412	5.64	2.80	1.169	3.801	3.337	1.765
CA3	2.170	1.921	6.828	6.956	10.67	13.92	1.248	13.960	7.662*	4.769
CA1	1.287	4.438	5.075	8.378	10.90	16.80	2.100	19.524	12.635*	7.349
Lateral septum	2.658	1.684	3.686	1.097	2.23	2.04	1.569	2.853	1.849	2.292
Cingulate	1.083	1.649	2.689	8.648	7.31	16.82	0.936	23.808	2.820	23.399
Subiculum	3.145	2.137	2.450	9.170	9.25	16.78	1.260	18.269	3.201	10.832
Entorhinal	4.320	1.834	1.774	3.140	3.61	4.61	4.720	8.130	4.674*	4.440
Dentate	4.513	1.213	5.895	5.161	16.52	14.46	3.313	17.909	19.128*	6.068
Ventral hippocampus	3.385	2.247	2.042	1.203			0.740	6.775	1.625	4.273

(1) df = 14,359 $P_{.05}$ = 1.73 $P_{.01}$ = 2.15

(2) df = 23,359 $P_{.05}$ = 1.57 $P_{.01}$ = 1.86

compared by the use of a two-way analysis of variance. Second, there were the changes in the first conditioning session during the development of a conditioned response. For this analysis the fifteen groups of day 1 conditioning session were compared, again by the use of a two-way analysis of variance. Third, there were changes in the responses between the end of the first day of training and the beginning of the second day. For this analysis the last 5 groups of the first day were compared with the first ten groups of the second day. Changes across day 2 conditioning session were assessed by analyzing them in the same fashion. Fifth and finally, the changes in the response across extinction were assessed. For this purpose, the fifteen groups of the extinction session were analyzed. The results of these comparisons are summarized in Table 3.2.

The gross movement of the rats, an indicator of overt behavior, showed a momentary freeze. When conditioning started this freeze was followed by an increment in movement as the reinforcement time drew near. This incremental response was already noticed in trials 11-20 but it increased in size and achieved asympotote within 30-40 trials. The latency of this response was 180-240 msec. The overt conditioned response increased in intensity, to a small degree, between the end of the first day and the beginning of the second day. The response extinguished rather rapidly (within 51-60 trials)

after the extinction session started.

In the first 10 trial block of the first day of the experiment almost all areas had a response which disappeared in some cases (CA3, lateral septum, subiculum, entorhinal), or was maintained as a brief phasic response in a second set (ventral hippocampus, CA1) or was not changed much across pseudoconditioning trials in a third group (medial septum, cingulate).

During conditioning, most areas showed a conditioned response within a relatively small number of trials. A conditioned response was established within 21-30 trials in medial septal area, CA3, CA1, ventral hippocampus and dentate. Some signs of conditioned responses could be seen within 1-10 trials (subiculum and cingulate) but this was not pronounced enough to be statistically significant. Some of these areas (medial septum, posterior cingulate) conditioned response gradually. The comparison between medial septum and CA3 is pertinent. CA3 had a conditioned response that emerged de novo but was already large in trials 21-30. Septal conditioned responses emerged gradually and reached a plateau later in trial series. Cingulate conditioned responses were also gradual.

The extinction procedure produced a better differentiation among brain areas studied than did the conditioning procedure. Here three types of processes could be detected: (1) areas that did extinguish. The most susceptible area to the extinction procedure was the dentate gyrus. It extinguished at approximately the same

number of trials as the gross behavior of the animal. Next to extinguish was area CA3. (2) areas that changed the pattern of response during extinction. The most notable one was the ventral hippocampus which, following extinction procedure, maintained the early component of the response and dropped the later (240-300 msec) component. A slightly different process was performed by CA1 units. Here the large response was attenuated rather rapidly (within 51-60 trials, at the same time the dentate responses extinguished), but was maintained on this attenuated level throughout the rest of the extinction session without further change. (3) some areas did not extinguish very well within the period of the experiment. The most notable of these were the cingulate, subiculum, and medial septum. A summary of changes in amount of response across the various stages of the experiment is presented in Figure 3.9.

A possible explanation for the reductions in the amount of response during the extinction session is satiation or reduction in the amount of need and thus a reduction in the animal's interest in the experiment. In order to control for such a possibility, the unitary response to the presentation of food was plotted as well, during the extinction session (Figure 3.8). There were no marked decrements in the amount of responses to food during the extinction session except in the dentate group. Moreover, the

Figure 3.8

Averaged responses to the application of food pellets during the extinction session. The ordinates and abscissa are the same as in Figure 3.6 and 3.7. Every histogram an average of 19 trials is made of 60 msec (5 points, 12 msec each) prior to and 300 msec (25 points, 12 msec each) after the application of food pellets. Only the last 80 trials of the session (8 histograms) are presented for every sampled area.

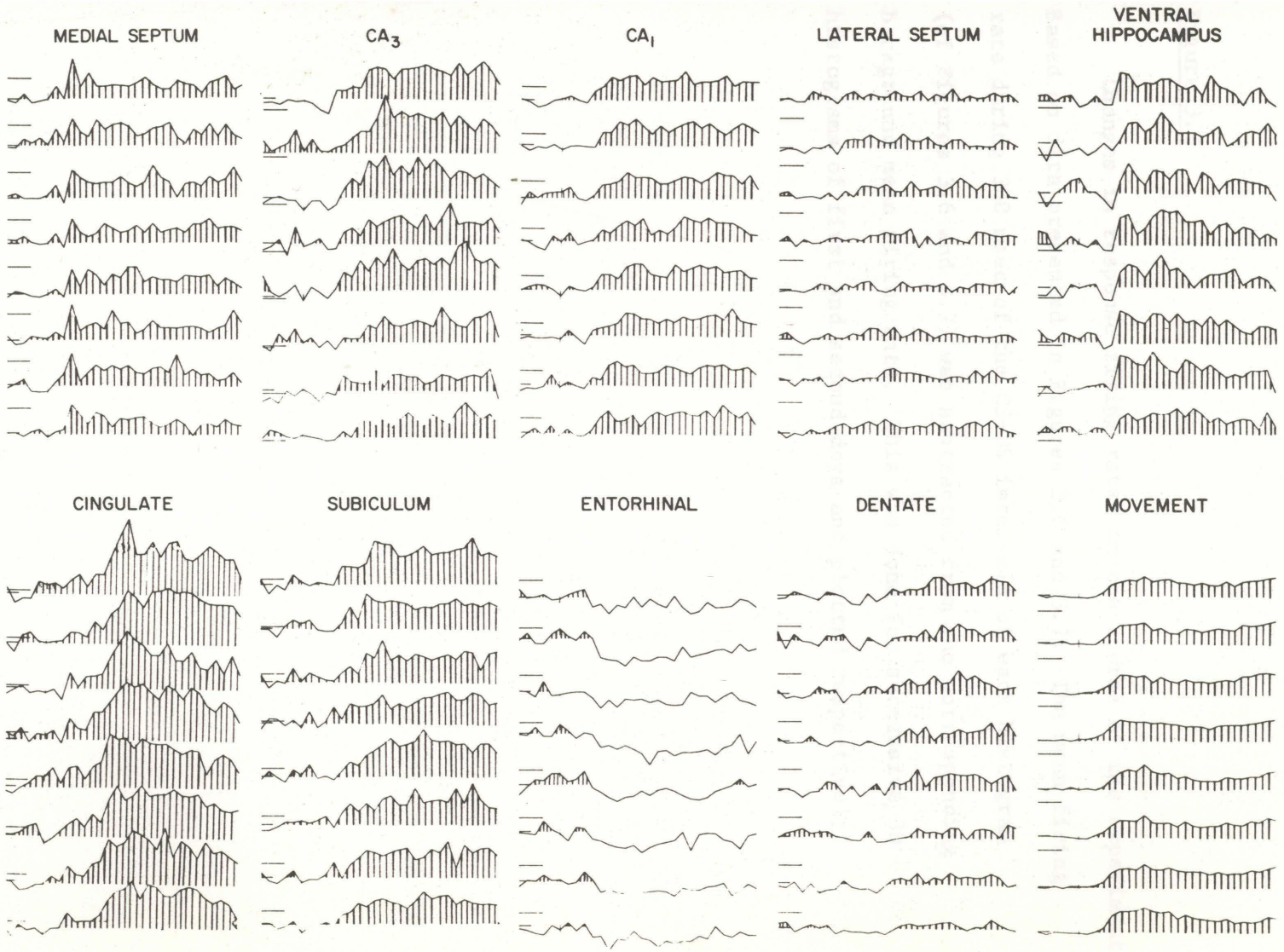
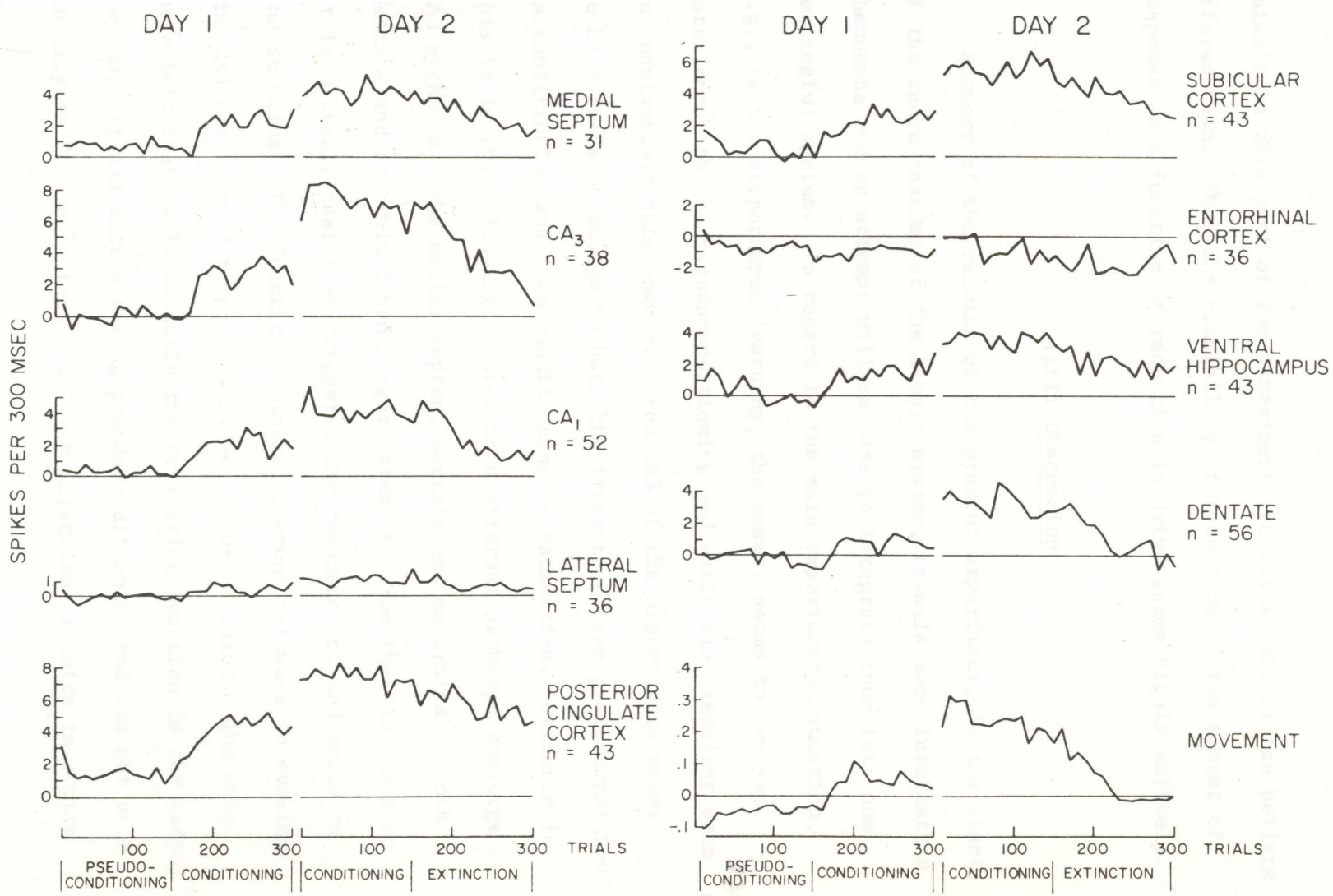

MEDIAL SEPTUM
CA3
CA1
LATERAL SEPTUM
VENTRAL HIPPOCAMPUS
CINGULATE
SUBICULUM
ENTORHINAL
DENTATE
MOVEMENT

Figure 3.9

Changes in response firing rates in the course of the experiment. Based on data presented in Figures 3.6 and 3.7. The mean firing rate during 300 msec of the CS-US intervals of each histogram (of Figures 3.6 and 3.7) was subtracted from the corresponding background mean firing rate. This was done for successive 30 histograms of first and second days and plotted respectively.


DAY 1
DAY 2
DAY 1
DAY 2
SPIKES PER 300 MSEC
MEDIAL SEPTUM n = 31
CA3 n = 38
CA1 n = 52
LATERAL SEPTUM n = 36
POSTERIOR CINGULATE CORTEX n = 43
SUBICULAR CORTEX n = 43
ENTORHINAL CORTEX n = 36
VENTRAL HIPPOCAMPUS n = 43
DENTATE n = 56
MOVEMENT
100 200 300 TRIALS
PSEUDO-CONDITIONING
CONDITIONING
CONDITIONING
EXTINCTION

animals in this part of the experiment ate almost all of the pellets offered them. Thus the possibility of reduction of the amount of response as a function of reduction in drive seems highly unlikely.

(iii) Discussion

Summary of the results of the present experiment, in the light of the known anatomy of the limbic system, reveals many interesting phenomena and an attempt will be made to integrate them into one meaningful system. In regard to the main experimental question, e.g., is the hippocampus learning, the answer seems to be--yes. Data from both the pseudoconditioning and extinction sessions seem to substantiate this answer. Area CA3 of the hippocampus seems to be a focus of change in both the transition from pseudoconditioning to conditioning and from conditioning to extinction. Evidence for this is in the following: three main afferent pathways converge on CA3 pyramids: the medial septum, dentate and entorhinal. Both dentate and entorhinal had longer latencies than the CA3 units so it is unlikely that they triggered the responses of CA3 units to the stimulus. The function of these afferent pathways in evoking the CA3 conditioned response will be discussed later. The only area left that could maintain the triggering function is the medial septum. It was active during pseudoconditioning and was not able to trigger CA3 units then. In fact latencies of units in septum

did not change between the two sessions of the experiment. The main change observed in septum was prolongation of the response during conditioning. This prolongation could be the consequence and not the cause of CA3 responses during conditioning since it took place after CA3 responses were initiated, both within trials (latency-wise) and across blocks of trials. Moreover, during extinction there was a substantial reduction in CA3 responses without any comparable change in the septum. Finally, the latency difference between septum and CA3 is longer than needed for this monosynaptic path. It seems therefore, that the hippocampal responses are to some degree independent of those seen in medial septum, which merely served as a triggering mechanism without control on the triggerability of its efferent field--the CA3 area of the hippocampus.

Our analysis indicates three different subsystems in the limbic telencephalic brain. Each of these systems has an input stage and a projection field. The three subsystems may serve different functions, but they seem to funnel into the main body of the hippocampus where initiation of a limbic conditioned response is probably taking place. The first system starts in the medial septal nucleus, which gets its sensory input via the medial forebrain bundle and the hypothalamus which exhibited similar short latency sensory responses (Linseman, 1972) and maybe from the amygdala. There are bidirectional pathways from amygdala to septum, but since the amygdala sample was rather small in the present study it was not clear which leads and which lags.

The septum feeds into the hippocampus which when activated, evokes responses in lateral septum (via CA3 afferents) and medial septum (via CA1 afferents or CA3-lateral septum--medial septum pathway). The function of the septal input, as suggested by previous studies is to evoke an arousal state in the hippocampus (Green, 1964). It is tempting to speculate that this time locked and precise input is searching the hippocampus to find the locations where "information" loaded via the other input pathways is stored. As discussed above, the medial septum cannot be regarded as solely responsible for CA3 responses; therefore one has to look for the effect of the other subsystems on the main chain of the hippocampus, the CA3-CA1 fields.

The second subsystem is the entorhinal dentate pathway. This subsystem seems to be very peculiar in the telencephalic limbic brain. The entorhinal cortex did not seem to do much within the CS-US interval and if anything, has a very late response latency. In view of the fact that the entorhinal projects the main input pathway to the hippocampus--the perforant path--and that this pathway is highly laminated and has special physiological features (i.e., frequency potentiation), it seems strange that it did not participate in the initiation of the hippocampal responses. Further experiments (see Exp. 6) showed that the entorhinal cortex did indeed play an important role in the establishment of a conditioned response.

The dentate gyrus, the main projection area of the entorhinal, seems also to have a special role in the constellation of responses

in the hippocampus. Its response latency was longer than that of CA3, the only known area to which it projects, thus it did not seem to be responsible for the triggering of CA3 neurons. However, additional evidence for the role suggested previously, namely, that dentate activity reflects information about ongoing behavior into the hippocampus and thus serves to modulate or reinforce the activity of the hippocampus, can be seen in the present experiment. First, dentate units did not respond during pseudoconditioning and without it the CA3 response, present in the first pseudoconditioning group, could not be maintained. Second, when conditioning started, dentate seemed to respond first and this was followed by CA3 response. Finally, and the most striking result of the extinction data, dentate was the first to extinguish. This happened when the animal stopped exhibiting the conditioned response, and CA3 extinction followed. Since no comparable extinction could be observed in septal units, dentate seems to be the main afferent system responsible for the extinction in CA3 units. The mechanism of entorhinal and dentate activity will be discussed later.

The third system is the cingulate-subicular. Anatomically, the main projection of the cingulate cortex and the dingulum bundle is to the subicular cortex (including presubiculum). No connections have been reported to exist between the cingulate cortex (including area retrospleneum) and the main body of the hippocampus (Adey, 1961; White, 1959; Raisman et al., 1965). Curiously enough, the

subicular cortex and its connections, although they occupy a large part of the telencephalon, have been poorly studied. Inputs other than the cingular and the cingulum carrying fibers from frontal cortex and anterior cingulate cortex (Cragg, 1965) are not known. The efferents of the subiculum are poorly known as well, except for the corticohypothalamic pathway (Nauta, 1956; Raisman et al., 1966). With these reservations in mind, the cingulate and the subiculum seem to be closely linked. The cingulate as an input stage has a substantial response during pseudoconditioning. Both the cingulate and the subiculum gain the conditioned response with a rather small number of trials. The response in cingulate units precedes those in subicular cortex. Finally and most convincing--both areas did not extinguish readily. These results bear upon some issues raised in the past. The question was whether the subiculum is an output stage of the hippocampus. The present results mitigate this possibility. It seems that the subicular cortex is an independent entity. A similar question was raised about the cingulate cortex: since it is heavily innervated by the anterior thalamus (Rose and Woolsey, 1948), which is one of the main output areas of the hippocampus (Nauta, 1956), can one conceive of the cingulate gyrus as an output stage of the hippocampus? The present results mitigate this, too. It seems that the cingulate gyrus is a major sensory association area of the limbic cortex, a view already proposed by Maclean and his associates (Cuenod et al., 1965).

In short, it is proposed that there is a convergence of arousal, sensory and reinforcing information on the hippocampus. This convergence with the right timing and proportions would activate the hippocampal cells. The neurophysiological mechanisms involved in this will be discussed later.

D. DETERMINANTS OF CONDITIONING IN THE HIPPOCAMPUS

The previous two experiments demonstrated some differences among the areas studied, suggesting different roles for the various components of the hippocampal system. It was proposed that the entorhinal-dentate-CA3 pathway is a "reinforcement" path, the septum-CA3 is an "arousal" path and the cingulate-subiculum is a "sensory" path. The next series of experiments is aimed at examining the necessary components involved in the initiation and maintenance of a conditioned response in the hippocampus. This was done by manipulating levels of reinforcement, arousal and sensory inputs. One basic question is: will the hippocampus develop responses under conditions different from those utilized in the previous experiments? If so, will the relations between latencies in the various hippocampal areas remain the same or might they differ, indicating different pathways of information flow.

Experiment 3: Hippocampal Unit Activity During Classical Aversive and Appetitive Conditioning*

The dentate gyrus was assumed to reflect into the hippocampus information about the tendencies to react, thus serving as a reafferent, reinforcing mechanism to the main body of the hippocampus. According to this hypothesis, dentate activity is required for initiation and maintenance of a conditioned response in the hippocampus. A way to test this hypothesis is to set up a situation where two clearly distinct behaviors are performed, namely food retrieval versus freezing, as a response to a stimulus which serves as a signal for different reinforcers on different sessions of training. If the behavior involves freezing, would dentate units react to the conditioned stimulus? If they would not, how would the main body of the hippocampus react? Once a hippocampal response was established, how would it react to an absence of dentate input? The following experiment attempts to answer these question.

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(i) Methods

Subjects

Twelve rats with six to eight electrodes implanted in the dentate gyrus and CA3 and CA1 fields of the hippocampus served as subjects.

Procedure

Six of the rats were trained in the following paradigm. Day 1 consisted of pseudoconditioning: one of three stimuli (two tones and a 45-mg food pellet), randomly selected, was presented once every minute for 16 hours. On days 2 and 3, one of the tones, the positive conditioned stimulus (CS+), was correlated with a food pellet, the unconditioned stimulus (US), with a CS-US interval of 1 second. The CS+ was randomly alternated with the second tone (CS-), which was presented without reinforcement. On days 4 and 5, the CS+ was correlated with an electric shock (US). The shock was a continuous 1/4-second, 50-hz pulse, 1.5 to 2.5 ma, applied through two wires 4 to 5 cm apart, implanted under the shoulder skin. On day 6, food was served again as the US. The other six rats were trained in the same paradigm except that on day 1 pseudoconditioning consisted of two tones and 1/4-second shock presented randomly. On days 2, 3, and 6 the US was shock, and on days 4 and 5 it was food. Between every switch from one US to another there were 2 to 3

hours of extinction (=pseudoconditioning)--the two tones and the forthcoming US presented randomly. For the daily sessions (involving 300 trials of each CS) an average of the unit activity in the 1-second pre-CS and the 1-second CS-US intervals was plotted. For the averages (pre- and poststimulus histograms) a 10-msec bin width was used, that is, each point indicated the average firing during a particular 10 msec interval. A similar average was prepared on the basis of the gross movement of the whole animal, this being the measure of the overt conditioned responses. Examples of average response curves for single units are shown in Figure 4.1.

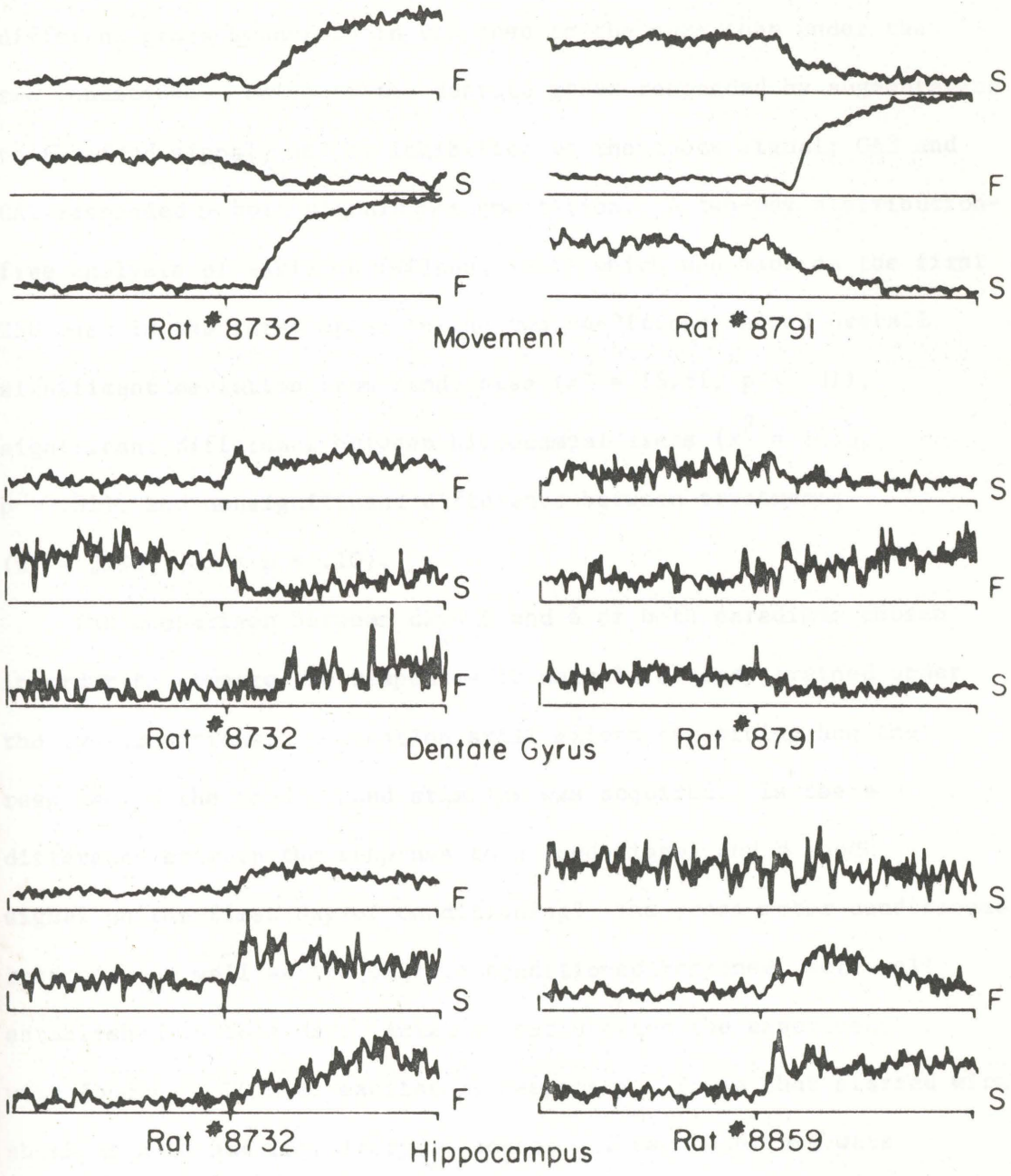
(ii) Results

A total of 33 units were recorded from the various hippocampal areas, divided as follows: 13 from the dentate gyrus, 9 from CA3 and 11 from CA1 areas of the hippocampus proper.

For analysis, the 1 second CS-US interval was divided into four successive 250 msec periods. The mean activity in these periods was presented as a deviation from the mean of the 1 second pre-CS period in standard scores. Grand averages, that is, averages of the standard scores for all of the units in each of the various hippocampal areas for the fifth and sixth days of training under the two conditions, are presented in Figure 4.2. It shows clearly

Figure 4.1

Histograms of averaged response probability of three units and the overt behavior of corresponding animals recorded during the third day of the experiment. Each histogram is composed of 3 successive seconds: 1 sec pre-CS, 1 sec CS-US interval, and 1 sec following application of the US. In the left column the US is food, in the right column, shock. The bar to the left of every pair of histograms represents 5 spikes per second.

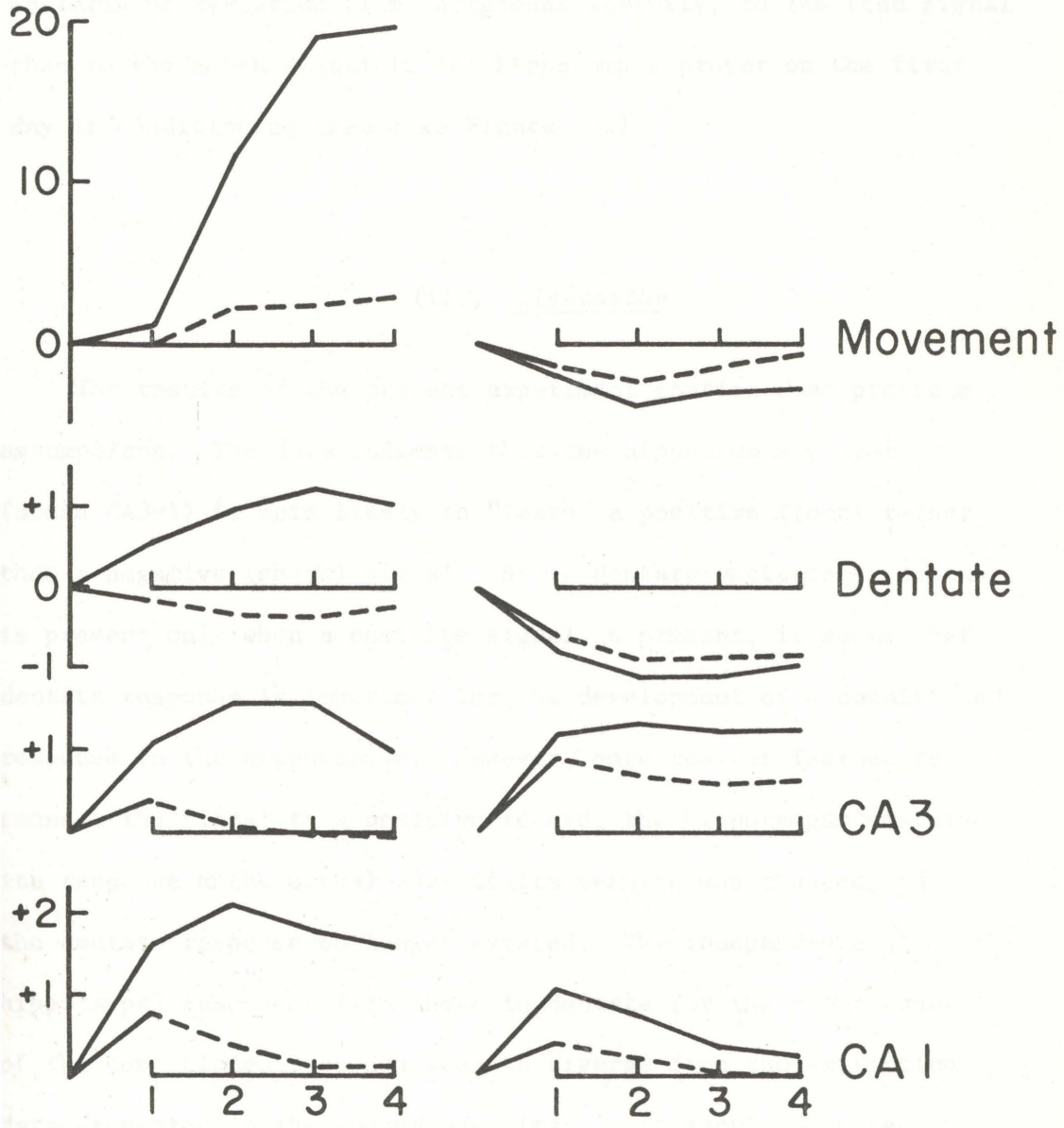

F
S
F
S
F
S
Rat #8732
Movement
Rat #8791
F
S
F
S
F
S
Rat #8732
Dentate Gyrus
Rat #8791
F
S
F
S
F
S
Rat #8732
Hippocampus
Rat #8859

different gross behaviors in response to the same tone under the two conditions. Units of the dentate gyrus responded by augmentation to the food signal, and by inhibition to the shock signal; CA3 and CA1 responded to both signals by augmentation. A two-way distribution-free analysis of variance (Wilson, 1956) which was made on the first 250 msec in the three areas in the two conditions showed overall significant deviation from randomness ($x^2 = 15.81$, $p < .01$), significant difference between hippocampal areas ($x^2 = 10.3$, $p < .01$), and nonsignificant difference between treatments ($x^2 = 2.96$, $.05 < p < .10$).

The comparison between days 5 and 6 of both paradigms chosen in order to compare the responses in animals already trained under the two conditions. A question still exists concerning how the response to the conditioned stimulus was acquired. Is there a difference between the response to a food signal and a shock signal on the first day of conditioning? The gross motor conditioned response, as well as the dentate conditioned response, were well established in this day. Animals that started the experiment with food as a US show excitatory responses. Those that started with shock as a US had inhibitory responses. These behaviors were reflected in dentate units. However, a comparison (Siegel, 1956) between units in the hippocampus proper in rats that started the experiment with food as US and those that started with shock as a US showed that there was a more intense response ($U = 19$, $p < .05$),

Figure 4.2

Averaged response curves in the various hippocampal areas. The data of the kind presented in Figure 4.1 were reduced to four periods of 250 msec each, for the 1 second CS-US interval. The amount of activity in each of these periods is presented in terms of deviation from a background level. The averages are separate for the two experimental conditions. Solid line = response to CS+; dashed line = response to CS-. The ordinate scale is of standard scores. The sample sizes are: dentate, 13; CA3, 9; CA1, 11; gross movement, 12.



Responses in Days 5&6

in terms of deviation from background activity, to the food signal than to the shock signal in the hippocampus proper on the first day of conditioning (see also Figure 4.1).

(iii) Discussion

The results of the present experiment confirm some previous assumptions. The data suggests that the hippocampus proper (areas CA3-1) is more likely to "learn" a positive (food) rather than a negative (shock) signal. Since dentate excitatory response is present only when a positive signal is present, it seems that dentate response is important for the development of a conditioned response in the hippocampus. However, once the rat learned to connect the signal to a positive reward, the hippocampus sustained its response to the signal even if its meaning was changed, and the dentate response no longer existed. The independence of hippocampal responses from those in dentate for the maintenance of the conditioned response seem to diverge from the extinction data presented in the second experiment. It should be noted, however, that the two situations are completely different. In the present experiment the conditioned signal is still meaningful and the rat is probably highly aroused, unlike the situation during extinction.

The dentate gyrus was found to be different from the hippocampus proper in several respects. It responded by augmentation to the food signal and by inhibition to the shock signal, and seemed to reflect the gross behavior. Unlike behavior though, the dentate responses to the shock signal could be predicted by the response of some dentate units to a "no food" signal (Experiment 1, Figure 2.2). This fits Amsel's (Amsel, 1962) theory which equates positive and non-negative signals, and negative and non-positive signals, although in the present experiment "no shock" signals did not produce similar response to those produced by a "food" signal. Finally, the response for dentate units, as well as those in the hippocampus proper, to the "shock" CS needs further analysis. Are these true conditioned responses or do they merely represent sensitized responses evoked in a highly emotional state. The next experiment is designed, partly, to answer this question.

Experiment 4: The Activity of Units in the Hippocampal Circuit of the Rat during Differential Classical Conditioning*

The present experiment addresses itself to the following questions: (1) are the inhibitory responses exhibited by dentate units true conditioned responses or are they due to sensitization? If they are indeed true conditioned responses then one would expect that in animals conditioned to two stimuli, dentate units would show excitatory responses to positive CS and inhibitory responses to a negative one. If they represent sensitization then these units would respond in the same way to every stimulus in the situation regardless of its incentive value. (2) In one earlier study (Olds, Mink and Best, 1969) CA3 units appeared to respond differentially to two possitive conditioned stimuli (one for food and the other for water) presented alternately in the same test sequences. In Experiment 3, presented above, CA3 and CA1 units could not distinguish between the positive and negative significance of a stimulus which alternated its significance from day to day. The question arose whether the day to-day alternation, or the use of a negative reinforcer accounted for this difference. It was

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interesting to see, therefore, if, in the same session these units would differentiate between the two stimuli, one correlated with reward and the other with punishment.

To answer these questions an experiment was conducted in which food and aversive electric shock were applied in the same sessions as unconditioned stimuli following two different conditional auditory stimuli. Within this paradigm, a further question was asked: will the latencies of hippocampal unit responses to the conditional stimuli in this highly emotional situation be different from those in a situation in which there is only one positive US? In case of deviation from results presented earlier, will the relations between latencies in the various hippocampal areas remain constant or might they differ, indicating a different pathway of information flow.

(i) Method

Subjects

Seventeen male albino rats were used.

Surgery

Besides the recording electrodes, implantation of which were described above, two large electrodes were placed in contact with the skull and the dura to be used for reference electrodes and for application of aversive stimuli; these were wires attached to

uninsulated screws which were lowered through the skull to touch the dura mater (the screws were at least 10 mm apart).

Procedure

Prior to the experiment the intensity of electric shock applied to the skull and the dura was adjusted (0.1-0.3 ma 60 cps applied continuously for 1/4 sec) at an intensity which caused obvious aversive reactions. These constituted freezing and squeaking. No behavioral convulsions or disruption of electrical activity in the hippocampus were detected.

The experiment was conducted over a period of three successive nights, with sessions of 16 hours each. The first nightly session was divided into two parts of 8 hours each. First, the four events (i.e., the two tones, electric shocks and the delivery of the food pellets) were presented in random order, one of them every one minute. A total of about 100 of each of the stimuli was applied throughout this pseudoconditioning period.

On the second half of the first session and throughout the second and third sessions, a conditioning paradigm was used: a food pellet was applied one second after the start of one of the 2 sec tones (assigned as CS+). The 1 sec interval between the onset of the CS and the onset of the US is called the "CS-US" interval; actually the CS preceded and overlapped the US. The other 2 sec tone, assigned as CS-, was correlated with a skull shock,

with the same CS-US interval (Figure 5.1). Each of the conditioned stimuli was applied about 120 times in the first session and 300 times in each of the following sessions.

Data and Collection and Reduction

A record of neural activity was taken during a 2 sec period, including 1 sec prior to application of the CS and 1 sec CS-US interval. Each second was divided into 100 10 msec bins and a binary record was made indicating the presence or absence of unit detections for every bin and every trial. An average frequency histogram was prepared for successive bins over a whole period (first and second halves of first session, whole second and third sessions) and was calculated separately for CS+ and CS- trials. Examples of such averaged probability strings are presented in Figure 5.1.

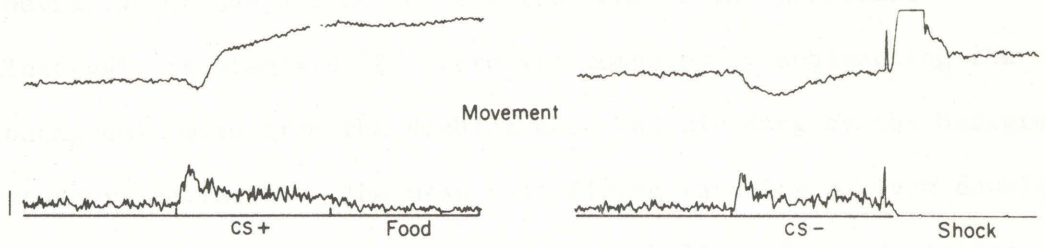
Based on these data, attention was focused on two characteristics of the unit activity during the CS-US interval: (1) the response latency; (2) the pattern of the averaged response for the whole 1 sec CS period.

The Response Latency

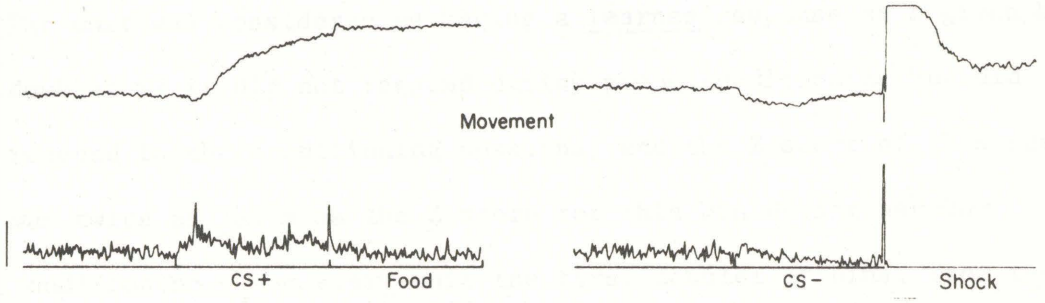
For every unit the first 8 bins after stimulus onset were considered in groups of two (1 and 2, 3, and 4, etc.). The mean scores for these doublets were compared with the mean and standard

Figure 5.1

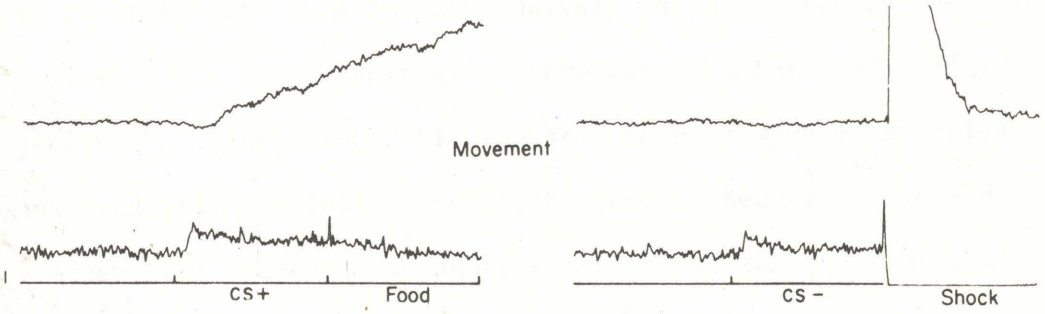
Computer-averaged response histograms for units and behavior. Every histogram is an average of 300 successive trials. The histogram is divided in halves: the first half is combined of 100 points in the pre-CS period; the second half is combined of 100 points of the CS-US interval. The US is either food (F) or shock (S). The bar to the left of each histogram represents an average firing probability for a 10 msec interval which is equal to 0.02 (equivalent to two spikes per second). Every set of three histograms represents the same unit, or gross movement of the same animal, under three successive treatments applied in day 2 (first day of conditioning) and days 5 and 6 (days 4 and 5 of conditioning); the left column is taken from a situation in which the unconditioned stimulus is food-shock-food. The right column is shock-food-shock.



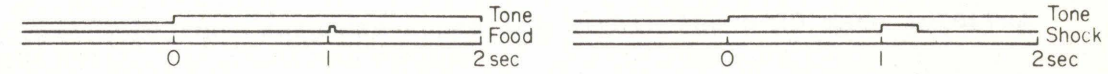
4/04/71 9085 CA1



4/13/71 9156 Dentate



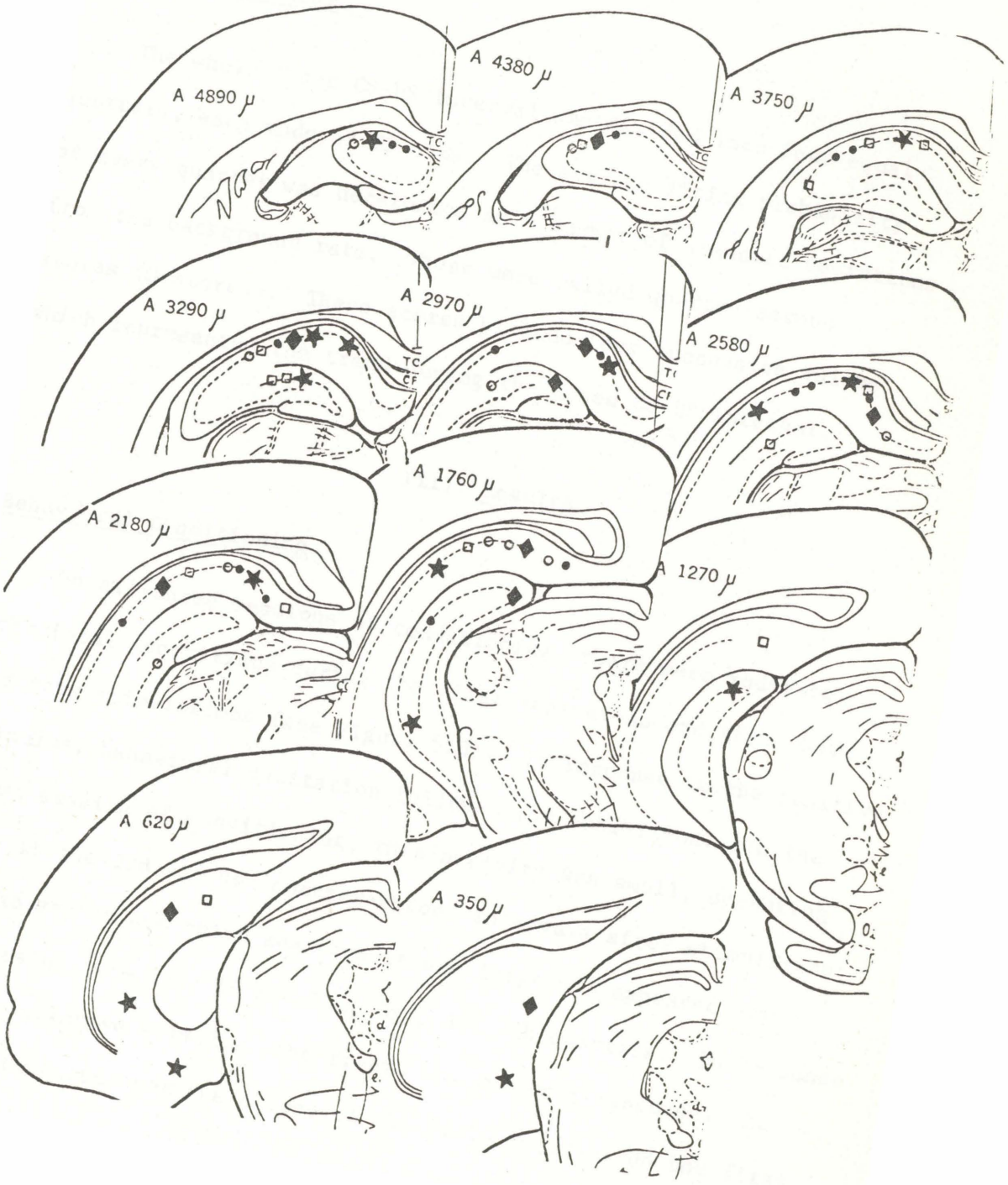
4/30/71 9211 CA3



deviation of the scores for the 100 bins of the prestimulus interval. A standard "Z" score was computed by subtracting the background mean from the doublet mean and dividing by the background standard deviation. The mean unit firing rate for a given doublet was considered as a respone if the probability of its deviation from the background mean occurring by chance was less than 1%. The unit was considered as having a learned response at a given bin doublet if it did not respond during pseudoconditioning but did respond in the conditioning sessions, and the Z score of this response was twice as large as the Z score for this bin during pseudo-conditioning. For every unit the first doublet of bins, from the application of the tone on, to show a learned response, was considered as its latency. This latency analysis was performed on the first 8 bins of the CS-US interval. Therefore, the intervals utilized were 0-20, 21-40, 41-60, 61-80 msec. These response latencies were measured from the initiation of the tones. Because there was a 3-4 msec lag between this and the arrival of the sound at the ear of the rat, the part of the latency accounted for by receptor and neural events can be calculated by subtracting about 4 msec. Thus a learned response appearing within the 0-20 msec interval would have a latency $\leqq$ 16 msec from arrival of the signal at the ear.

Figure 5.2

Location of units recorded during the experiment and their shortest latency detected in at least one of the experimental sessions. Cross sections of the rat brain in the appropriate anterior-posterior levels are retraced from König and Klippel (1963). The latency code is the following: Star = 20 msec response latency; diamond = 40 msec; square = 60 msec; open circle = 80 msec; dot = more than 80 msec.


A 4890 μ
A 4380 μ
A 3750 μ
A 3290 μ
A 2970 μ
A 2580 μ
A 2180 μ
A 1760 μ
A 1270 μ
A 620 μ
A 350 μ

The Response Pattern

The whole 1 sec CS-US interval was divided into four equal quarters, each made of 25 bins. The average firing probability of every quarter was determined as a number of standard deviations from the background rate. These were called quarter-second scores (Q scores). These scores yielded four successive values which represented the trend during the 1 sec CS-US interval.

(ii) Results

Behavioral Conditioning

On all three sessions of conditioning, there were moderate behavioral inhibitions during the first quarter-second after both auditory stimulations (see Figure 5.3). In response to the positive stimulus, behavioral excitation followed the inhibition. On the first session of conditioning, this activity was small, appearing only in the 3rd and 4th quarter-second intervals after stimulation; on the second and third sessions it was large and appeared with increasing intensity in the 2nd, 3rd, and 4th quarters. In response to the negative stimulus, the freeze was the only response. It was attenuated over the course of the CS-US interval on the first 2 sessions of conditioning, but on the third session it was sustained during the whole interval. Thus, the head movement behavior showed clear and differential conditioned responses to

the alimentary and aversive conditioned stimuli even though it was not a specific measure of food-related or aversive reactions. There was a biphasic, but mainly increased movement response to the CS+, and a monophasic slowing to the CS-. The same behavioral changes were exhibited in all animals. Sixteen rats completed three days of training and showed clearly differentiated overt conditioned responses by the third day.

Unit Conditioning

Data were collected from a total of 67 units (see Figure 5.2). Due to occasional disappearance of unitary activity over time, recording from some electrodes was stopped and other electrodes from which unit activity could be detected were substituted. Other units were recorded throughout the training periods. For this reason no analysis of trends of single units across the training periods was done, but instead all units recorded on a given session were grouped, regardless of their recordability on other sessions.

On the third session of training when all subjects had mastered the differential task, a record was taken from all eight units in dentate gyrus of seven subjects, six in the CA3 area of the hippocampus of six subjects, 30 in CA1 in thirteen subjects, and nine in the subiculum of seven subjects. No clear interanimal differences that could account for the differences among the brain areas under study were found.

Comparisons for response latency were made between units recorded in the third session (n = 53) when learning and differentiation were established. A high percentage of CA1 units had short latencies: three units responded within 20 msec, seven within 40 msec eleven within 60 msec. One unit of CA3 area responded within 20 msec. (Due to the small number of CA3 units, they were added to CA1 units for this and the following latency comparisons.) In contrast, none of the dentate units responded within 40 msec, only one responded within 60 msec, and two responded within 80 msec from the application of the CS (see Table 5.1). The difference between the hippocampus proper (CA3 + CA1), and the dentate was statistically significant in a comparison of the proportion of short latency (within 40 msec) units in both areas (Fisher exact probability < .01). Thus there was a clear lag of at least 20 msec between the earliest CA3-CA1 units and those sampled in the dentate gyrus.

A given unit responded to both stimuli but with slightly different latencies. For the most part the responses evoked by positive conditioned stimuli had shorter latencies than those evoked by the negative stimuli but this bias was not significant statistically.

In order to detect changes in the response latency over days of training and to determine if these were a function of conditioning, the latencies of learned unit responses that had been recorded throughout the whole experiment were tabulated (Table 5.1A) over

Figure 5.3

Averaged response curves in the various hippocampal areas. The data of the kind presented in Figure 1 were reduced to four periods, 250 msec each, for the 1 second CS-US interval. The amount of activity in each of these periods is presented in terms of deviation from a background level.

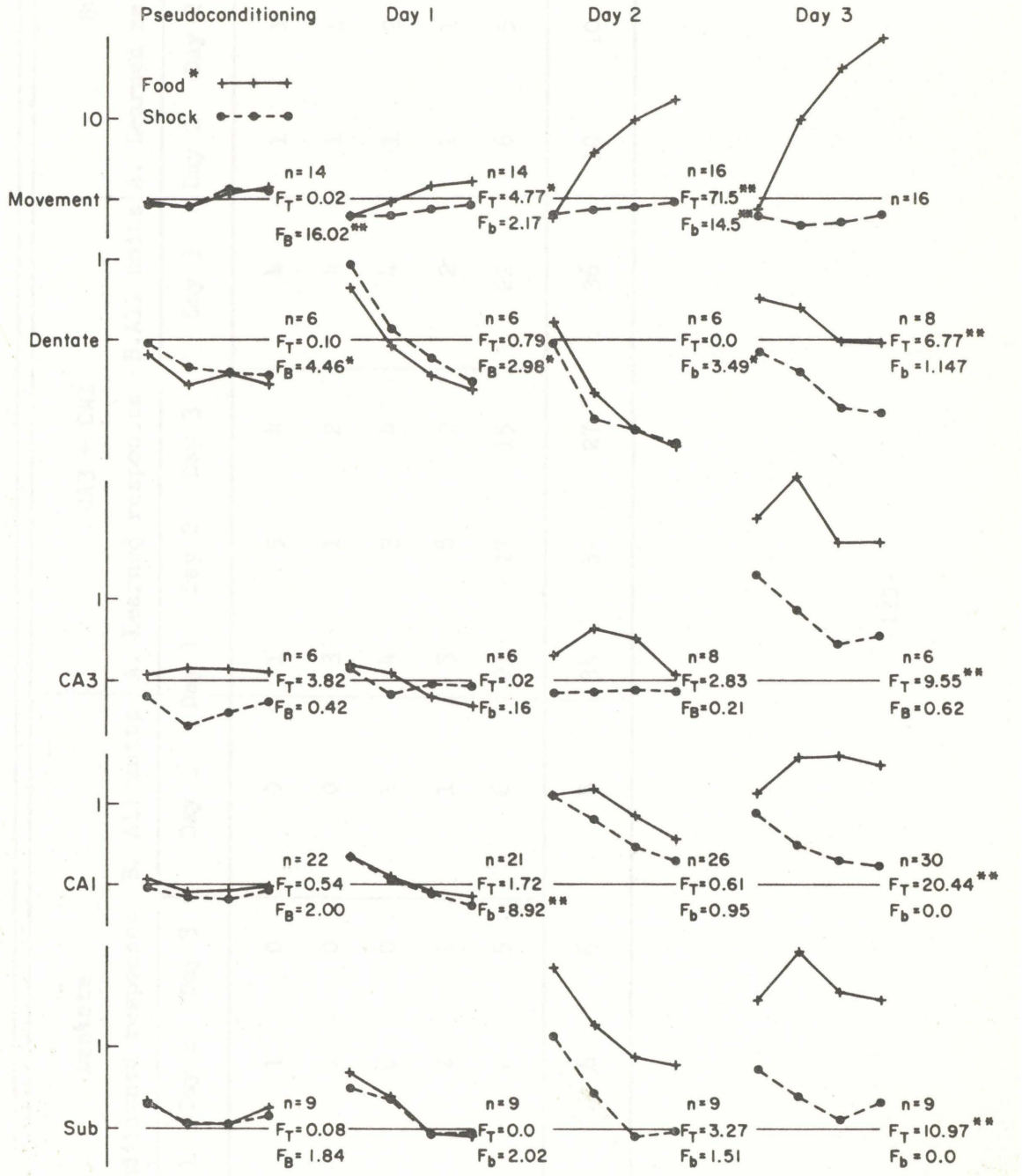

Pseudoconditioning
Day 1
Day 2
Day 3
Food*
Shock
10
Movement
n=14
F_T=0.02
F_B=16.02**
n=14
F_T=4.77*
F_b=2.17
n=16
F_T=71.5**
F_b=14.5**
n=16
1
Dentate
n=6
F_T=0.10
F_B=4.46*
n=6
F_T=0.79
F_B=2.98*
n=6
F_T=0.0
F_b=3.49*
n=8
F_T=6.77**
F_b=1.147
1
CA3
n=6
F_T=3.82
F_B=0.42
n=6
F_T=.02
F_b=.16
n=8
F_T=2.83
F_B=0.21
n=6
F_T=9.55**
F_B=0.62
1
CA1
n=22
F_T=0.54
F_B=2.00
n=21
F_T=1.72
F_b=8.92**
n=26
F_T=0.61
F_b=0.95
n=30
F_T=20.44**
F_b=0.0
1
Sub
n=9
F_T=0.08
F_B=1.84
n=9
F_T=0.0
F_b=2.02
n=9
F_T=3.27
F_b=1.51
n=9
F_T=10.97**
F_b=0.0

TABLE 5.1

Response Latencies

Response latency (msec.)	Dentate				CA3 + CA1				Subiculum			
	A. Conditioned responses			B. All units	A. Learned responses			B.All units	A. Learned responses			B.All units
	Day 1	Day 2	Day 3	Day 3	Day 1	Day 2	Day 3	Day 3	Day 1	Day 2	Day 3	Day 3
0-20	0	1	0	0	1	5	4	4	1	1	2	2
21-40	0	1	0	0	3	1	2	4	1	1	0	0
41-60	2	0	0	1	4	3	4	4	1	2	0	2
61-80	1	2	1	1	5	8	2	2	1	1	4	4
>80	3	2	5	6	21	17	15	22	6	5	3	1
Total	6	6	6	8	34	34	27	36	10	10	9	9

days of conditioning. The percentage of short latency (0-80 msec) units increased from 40% in day 1 to 52% on the second day of conditioning and then dropped back to 40% on the third day. These changes were not significant statistically (in a x^2 test). Finally, the shortest response latency of a given unit, exhibited in at least one conditioning session, was determined for all units participating in the experiment (n = 67). No significant difference between the hippocampal areas was found, i.e., there were short latency responses in every area in at least one of the three sessions of the experiment (Figure 5.2). In short, although no clear differences between response latency of units in the areas studied could be detected in the process of learning (sessions 1 and 2), a difference was found when learning and differentiation were established; i.e., a clear lag of at least 20 msec between the earliest CA3-CA1 units and those sampled in dentate gyrus.

The Averaged Response Pattern

The response during the CS-US interval had two components. The first one, developed early in training, was given by a unit response to both stimuli, and lasted for 250-500 msec. Later in training a differentiation was shown in the last 500 msec of the response. This was well established by the third day. The quarter-second scores (Q scores) were averaged for all the units recorded in a given area in a given session. These averages were calculated

separately for CS+ and CS- trials. A two-way analysis of variance (Edwards, 1968) was performed for every group in order to detect differences between treatments (CS+ vs. CS- trials) and between successive Q scores. The averages and the results of the analysis are presented in Figure 5.3.

Dentate units were inhibited during pseudoconditioning. This inhibition was pronounced in the last half of the CS-US interval ($F_{block} = 4.46$, $p < .05$). During conditioning sessions they had a phasic excitatory response followed by inhibition ($F_b = 2.98$, $p < .05$; $F_b = 3.49$, $p < .05$ for first and second conditioning sessions, respectively). This biphasic response was generated to both signals indiscriminately, and only by the third day did a difference between the two conditioned responses appear: a phasic excitation to the CS+ and inhibition to the CS- ($F_{treatment} = 6.77$, $p < .01$).

The responses from other areas of the hippocampus were different from the dentate in several respects. They did not have statistically significant responses to the tones during pseudoconditioning. Their responses to the conditioned positive stimulus were sustained throughout the CS-US interval. Except for the CA1 group in the first conditioning session, there were no statistically significant changes across the four averaged Q scores of the CS-US interval.) They had excitatory responses to the CS- as well. However, this response had a smaller magnitude than the response to the positive conditioned stimulus and it was not sustained throughout the

interval. In the third conditioning session the differences between treatments were: CA3, $F_t = 9.55$, $p < .01$; CA1, $F_t = 20.44$, $p < .01$; subiculum, $F_t = 10.97$, $p < .01$.

(iii) Discussion

Dentate units exhibited differential conditioned responses, accelerating for a positive stimulus and decelerating for a negative one. Other areas of hippocampus yielded accelerating responses to both stimuli although responses were substantially larger and more sustained to positive stimuli. The differential responses in dentate gyrus occurred only after overtraining. During pseudoconditioning and on the first sessions of conditioning, inhibitory, generalized responses were dominant. The day 1 conditioned response in the dentate could not be regarded entirely as a sensitized response, since, unlike the response during pseudoconditioning, it had an excitatory component (during the first 250 msec) followed by an inhibitory component during the final 500 msec of the CS-US interval (see Figure 5.3). At this point, however, the same conditioned response appeared to both CS+ and CS-, and it had much in common with the response of the previous session during pseudo-conditioning. Responses in all areas became successively better differentiated from day to day, and there was clear differentiation of responses between CS+ and CS- on day 3 of conditioning.

As to the function of the dentate gyrus, it seems that the

assumption presented in the previous experiments that dentate projects into the hippocampus information about the current tendencies to act can be slightly modified on the basis of the present study. There are some cases where the directions and trends are different between movement and dentate gyrus neuronal activity. Certainly the continuous behavioral inhibition which generally precedes negative reinforcement has a correlation with dentate units' inhibition. However, the behavioral momentary freeze, which is exhibited in the first quarter-second and is generated by both stimuli indiscriminately does not have a close correlation with dentate activity. On the third conditioning session, dentate response is differentiated already in the first quarter-second interval.

The course of the inhibitory dentate response across the CS-US interval is not comparable to the gross behavioral response. Whereas the dentate inhibition is larger to the aversive stimulus, behavioral inhibition is less pronounced. It now seems therefore that the two are separately correlated with a third variable and this may be the expectation of a positive or negative outcome.

The hippocampus proper had an excitatory response to both CS+ and CS-. These responses were well differentiated by the end of the experiment, being more pronounced to the CS+. The response to the aversive CS did not seem to be a result of stimulus generalization from the appetitive CS, because it was larger, in terms

of deviation from background firing rate, than that to a CS-having neutral instead of negative significance. However, it is possible that there is generalization from the positive signal and it is potentiated by arousal generated by the aversive US. The excitatory response of the hippocampus units to stimuli which cause opposite overt behaviors rules out the possibility of direct control over movement as a function attributed to the hippocampus by a few theories (Vanderwolf, 1971). These results fit well with interpretation of some studies recording θ rhythm in hippocampus (Konorski, Santibanez and Beck, 1968; Grastyan, Karmos, Vereckey and Kellenyi, 1966) who found a dissociation between the animal's behavior and the activity of the hippocampus.

Experiment 5: An Attempt to Produce a Sensory-Sensory Conditioning in the Hippocampal System

The previous two experiments demonstrated the possibility of initiation of a conditioned response in the hippocampus in a highly emotional state (aversive shock), in the presence (Experiment 4) or absence (Experiment 3, group II), of a positive reinforcing stimulus as part of the procedure. It is pertinent to learn whether a conditioned response can be evoked in the hippocampus in the absence of emotional, motivational, and operant factors. Morell (1960) has shown a temporary change in hippocampus in the process of sensory-sensory conditioning. In a preliminary fashion Hirano (personal communication) could not detect conditioned hippocampal unit responses in a sensory-sensory paradigm. The present experiment tries to solve this issue. In addition, in none of our previous experiments was a simple habituation procedure used. In all of them, an experiment started with a pseudoconditioning session. It might well be that some of the responses observed during these sessions were generalized from or sensitized by the responses to the unconditioned stimulus. The difference in the procedure may account for the differences between our results and those of Vinogradova (1970) who found, predominantly, rapidly habituating, inhibitory responses in the hippocampus of rabbits.

(i) Methods

Subjects

Twenty-two naive rats were used in the present experiment.

Procedure

The experiment was conducted in a single day and included two sessions. First, pseudoconditioning: two tones and a light flash were presented in random order, with a variable inter-trial (mean = 1 min). The tones were 1 and 10 khz, 400 msec in duration. The light flash, produced by a Grass PS-2 stimulator was 0.01 msec in duration. A single flash was used application of which involved production of a weak click. The second session consisted of a conditioning procedure: one of the tones, CS+, was correlated with the flash of light which was applied 300 msec after the onset of the CS+. The three stimuli were presented 150 times each, in each of the two sessions. Food and water were supplied ad libitum.

Data Collection and Analysis

The collection of the data and the analysis were similar to Experiment 2 (p. 61). Briefly, unit activity was sampled during 150 msec prior to onset of stimuli and 300 msec afterwards in 1.5 msec bins. Thus there were successive 100 bins for sampling background activity and 200 bins (300 msec) for post stimulus sample. Two types of analysis were performed: 1. Latency determination. The

rules of Experiment 2 were applied here for determining the response latency to the flash of light and the tone, for both sessions.

2. Changes in responses across trials. As aforementioned, data was grouped in 10 trial blocks each consisting of a string of 300 1.5 msec bins. There were 15 blocks for pseudoconditioning and 15 blocks for conditioning. All units in a given area were grouped and a two-way analysis of variance performed to detect changes within trials and across blocks of trials. In addition, the total activity during 300 msec post stimulation was averaged, subtracted from the corresponding background firing rate, and plotted in 10 trial blocks in a fashion similar to Experiment 1 (Figure 6.3).

(ii) Results

Activity of 120 units was recorded from 22 rats. Out of these, 16 units had to be rejected on quality control basis. The rest of the units were distributed in the following areas; medial septum, 9 units; lateral septum, 8 units; area CA3 of hippocampus, 16; area CA1, 21; dentate, 6; entorhinal cortex, 17; cingulate cortex, 11; subicular cortex, 16. A total of 104 units participated in the analysis of this experiment. The electrode locations are presented in Figure 6.1.

Distribution of Responses and Response Latencies during Pseudo-conditioning

For the most part, the flash of light turned out to be a better stimulus (Figure 6.2) than a tone. Most of the units, when responding, had a bigger responses to the flash of light than to the tone. Analysis of the response latencies in the various areas reveals (see Table 6.1) that among a medial septum units, 2 responded within 30 msec, one more within 42 msec, and two more had a late response (61-300) msec. Altogether, 7 out of 9 medial septum units responded to the tone. This whole group of units responded to the flash. The latencies of responses to the flash were somewhat longer: three units responded within 42 msec, two more within 60 msec, and the rest had a late response. It seems therefore that in the medial septum there were more short latency responses to tone than to light.

At the other end of the limbic forebrain system stands the cingulate cortex. Out of 11 units recorded here, three units responded to the light flash within 24 msec and three more had a latency of 25-30 msec. Three more units responded within 48 msec. The cingulate response to the tone is rather late, two units responded within 30 msec, and seven units had a late response. A comparison between medial septum and cingulate responses reveals that the septum seems to have a briefer latency to tone than the cingulate, whereas

Figure 6.1

Location of units recorded in the sensory experiment and the type of stimuli they responded to. The sections are redrawn from König and Klippel (1963).

DISTRIBUTION OF SENSORY RESPONSES

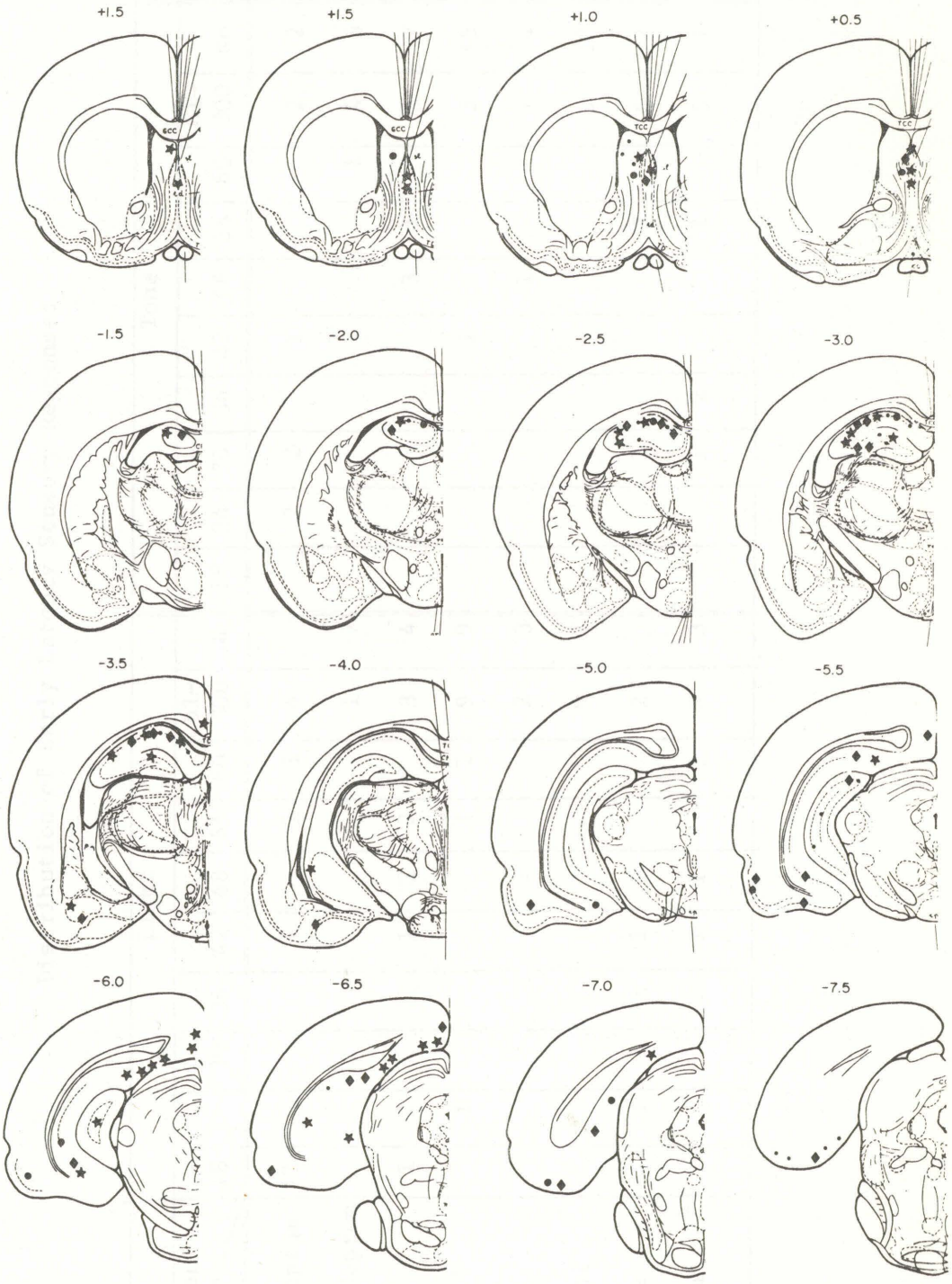


TABLE 6.1

Distribution of Early Latency Sensory Responses

	Light										Tone										Total
msec / Area	18	24	30	36	42	48	54	60	61-300	No	18	24	30	36	42	48	54	60	61-300	No	
Medial Septum	1				2		1	1	4			2	2		1				2	2	9
Lateral Septum									1	7								1	4	3	8
CA3	1	2		1	1	2	2		3	4						3	1		5	7	16
CA1		1					1	1	9	9					1				5	15	21
Dentate						1			2	3						1			1	4	6
Entorhinal						1			9	7									5	12	17
Cingulate		3	3	1	1	1			2				2				1		6	2	11
Subiculum				1	1	1		1	9	3			1	2			1		5	7	16

the reverse is true for light.

Within the hippocampus most of the units did not respond to the tone, and the general picture is similar to the one obtained in Experiment 2. However, most of the units in CA1 areas (Figure 6.3) had an inhibitory response, as opposed to an excitatory one, dominating the pseudoconditioned responses in Experiment 2.

The responses to the light flash were larger, and surprisingly enduring, although the flash itself had almost no duration. Area CA3 was the most responsive area to the flash of light. Three of its units responded within 24 msec, and six more within 54 msec.

Convergence of Sensory Information in the Hippocampal System

All areas had some units exhibiting convergence of responses; that is, responded to both stimuli. The two areas to show the highest percentage of convergence units were the medial septum and the cingulate (7 out of 9 and 10 out of 11, respectively).

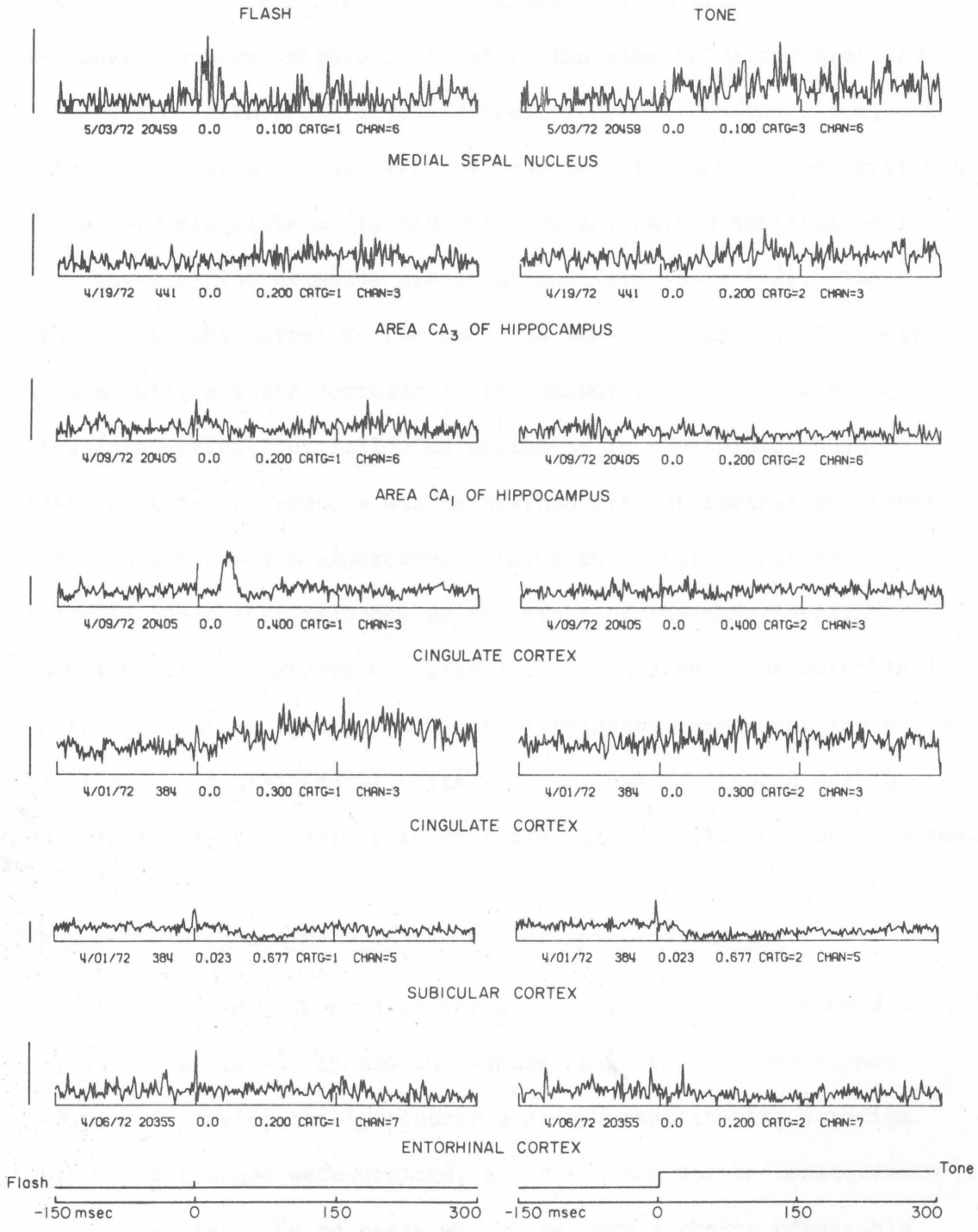
Changes in Responses across Trials

The purpose of this analysis was twofold: (1) to detect changes in response rate due to habituation, and (2) to detect possible changes in response rate following a conditioning procedure. (Due to some problems in data storage and retrieval, the number of units in this analysis does not correspond to the number of units analyzed above. Hence only a few of the studied areas ran through the present analysis.) The habituation process to the flash, if it

Figure 6.2

Averaged responses of individual units to a flash and a continuous tone. Each histogram is an average of 150 stimulus presentations in the first session of the experiment. A pair of histograms represents the response of the same unit to both stimulations. The bar to the left of each histogram represents a firing rate of 10/sec.

AVERAGED RESPONSES OF INDIVIDUAL CASES



occurred, occurred rather fast. In area CA1 habituation of unit responses occurred within 10 trials. The same holds for area CA3 units which, although attenuated their responses to the flash, also maintained a rather high response rate throughout the experiment. Septal and cingulate units did not show any marked habituation in the course of 150 stimulus presentations (see Table 6.2). The process of habituation to the tone was less pronounced. The only area showing a sharp decrease in the amount of response was the cingulate cortex, but following decrement in the initial high response rate, a response was maintained without further decrement throughout the whole experiment. Units in CA1 areas had an averaged inhibitory response at the start of the experiment. This inhibitory response was gradually attenuated. The entorhinal cortex maintained a slight inhibitory response throughout the whole experiment. Medial septal units seem to have habituated within 30 trials only to regain this response later, following conditioning.

Changes in Response Rate in the Process of Conditioning

None of the responses in any of the areas examined showed a marked or persistent change in response rate to the conditioned stimulus following the introduction of the conditioning paradigm. Only minor changes were noticed, and these may not be consequences of conditioning. In no cases was there seen a change comparable to the ones observed when food was the unconditioned stimulus. The

averaged curve (Figure 6.3) revealed a brief regain of a response in medial septal units that was lost 40 trials after the start of the experiment. The only prominent change that seems to have occurred was in CA1 group. Here, too, an inhibitory response present in the start of the experiment and habituated later was reinstated, only to attenuate gradually towards the end of the experiment.

(iii) Discussion

The present experiment demonstrated the existence of unconditioned, innate responses in two of the main afferent systems of the hippocampus--the medial septal nucleus, and the posterior cingulate cortex. It also demonstrated the relative absence or habituation of these responses in the main body of the hippocampus. In these afferent fields a convergence of sensory inputs could be seen. Analysis of response latencies in the septum and cingulate indicated that the sensory fibers probably get to these two areas from different sources and that they are independent of one another. Sensory input to the cingulate cortex was described by Mclean and his collaborators (Cuenod et al., 1965). They assumed that these fibers arrive from the geniculate (Casey et al., 1965) but no solid anatomical evidence exists to support this view. It is true, though, that the cingulate cortex gets sensory input from the neocortex.

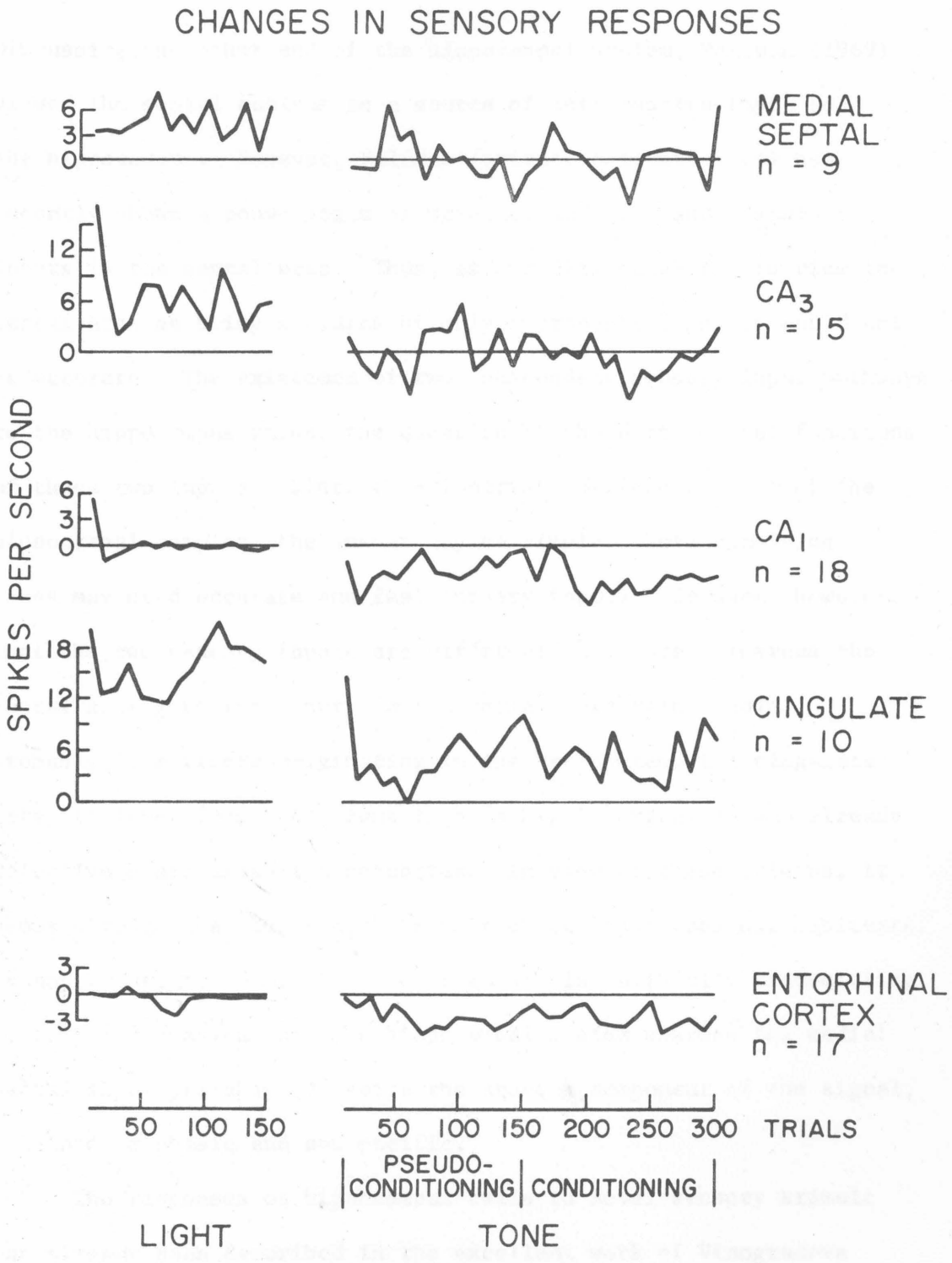
TABLE 6.2

Analysis of Changes in Sensory Responses

		Light (15 groups)		Tone (30 groups)	
		F	p<	F	p<
Medial Septum	across trials	1.35	N.S.	3.00	.01
	within trials	4.03	.01	1.49	N.S.
CA3	across trials	3.40	.01	1.80	.01
	within trials	5.31	.01	1.67	.05
CA1	across trials	2.43	.01	2.56	.01
	within trials	2.90	.01	2.23	.01
Cingulate	across trials	0.56	N.S.	2.58	.01
	within trials	4.42	.01	2.73	.01
Entorhinal	across trials	2.78	.01	1.73	.05
	within trials	1.23	N.S.	1.61	.05

Figure 6.3

Changes in response to both flash (left column) and tone (right column) in the course of the experiment. Every point in a histogram represents the mean firing rate during 300 msec post stimulation subtracted from the mean firing rate during 150 msec pre-stimulation period. Every point in a histogram is the mean of 10 successive trials.


CHANGES IN SENSORY RESPONSES
SPIKES PER SECOND
6
3
0
MEDIAL SEPTAL
n = 9
12
6
0
CA3
n = 15
6
3
0
CA1
n = 18
18
12
6
0
CINGULATE
n = 10
3
0
-3
ENTORHINAL CORTEX
n = 17
50 100 150
50 100 150 200 250 300
TRIALS
PSEUDO-CONDITIONING
CONDITIONING
LIGHT
TONE

Discussing the other end of the hippocampal system, Maclean (1969) viewed the septal nucleus as a source of introceptive input to the hippocampus. However, Feldman (private communication) has recently shown a convergence of sciatic, auditory and visual inputs in the septal area. Thus, as our data suggests, to view the septal area as being a source of only introceptive inputs would not be accurate. The existence of two independent sensory input pathways to the hippocampus raises the question of the differential functions of these two inputs. Since they innervate different parts of the hippocampal complex, the answer may be simple: both receiving areas may need accurate and fast sensory inputs. It seems however, that the two sensory inputs are different in nature. Whereas the septal area gets its input via the medial forebrain bundle, probably from fibers originating in the brain stem, the cingulate gets its input from what seems to be a highly organized and already selective brain area--the neocortex. In view of these origins, it seems strange that the cingulate is the area that does not habituate. In any event, it seems that the cingulate is faithfully transmitting sensory information into the hippocampal system whereas the medial septal input probably transmits the arousal component of the signal, and this is phasic and nonspecific.

The responses of hippocampal cells to novel sensory stimuli has already been described in the excellent work of Vinogradova (1970). She found a large proportion of hippocampal cells to respond by inhibition to sensory stimuli. This response had a long

latency and habituated rather fast. These results, not obtained in our previous experiments, were replicated here, in the present one. The difference, especially in CA1 responses, between a pseudoconditioning experiment in which food was presented intermittently as part of the experimental paradigm and the present experiment in which a pure habituation paradigm was used, is striking. Whereas previously the majority of CA1 units showed a slight excitatory response to the tone, here most of the units that did respond exhibited a dominant inhibitory response. This difference demonstrates the importance of an appropriate control for an appetitive conditioning that is, a proper pseudoconditioning. In addition, it is hard to imply from an appetitive pseudoconditioning paradigm what are the unconditioned responses to sensory stimuli. Except for this lesson, the difference between the responses in the two conditions demonstrates the importance of an arousal factor in modulating the hippocampal responses to a presumably neutral stimulus.

The lack of sbustantial changes in hippocampal responses to the conditioned stimulus after the onset of the conditioning paradigm is another striking result of the present experiment. Superficially, there is no apparent reason for the experiment to fail. The presence of a substantial, unconditioned unhabituated response to the conditional stimulus (flash) was demonstrated in at least the medial septum, CA3, and cingulate cortex. The conditioned stimuli are above threshold and at some time or another, units responded to these stimuli as well. To all intents and purposes

these are the necessary and sufficient conditions for the establishment of a conditioned response. Indeed, in sensory structures such as the colliculus, geniculate, and sensory cortex, a conditioned response has been demonstrated using essentially the same procedure (Hirano, private communication). However some factors, missing in the sensory paradigm, prevented the hippocampus from gaining the conditioned response. One is the general arousal factor. It seems quite clear that in the sensory paradigm rats are far less aroused than in any of the other experiments. This by itself could not be the major influencing factor, since in the aversive experiment, where rats were highly aroused, a conditioned response could not be established either. A common denominator to these two types of experiments is an absence of an operant behavior. It is conceivable that the hippocampus is involved in initiation of a purposive behavior and that in the absence of this only a minor response would be detected. However, some facts mitigate against operant behavior as a sole reason for the failure of the present experiment. In an experiment in which positive brain stimulation served as an unconditioned stimulus in a classical conditioning paradigm (Segal and Hiatt, unpublished), conditioned unit responses in hippocampus could be demonstrated in the absence of overt purposive behavior. One factor, common to the experiments in which a satisfactory conditioned response could be demonstrated, and absent in the experiments in which a failure of conditioning occurred, is the anticipation of a

positive outcome. This can account for the failure of conditioning in the sensory paradigm. A function of this sort was ascribed to many hippocampal neurons by Ranck (personal communication) and it is possible that one of the main functions of the hippocampus is to assess the rewardability of environmental stimuli. The importance of the other factors, missing in the sensory paradigm, cannot be ruled out unless the appropriate experiments are done--active avoidance (for the operant factor) and an intermittent supply of food incorporated in the sensory paradigm (for the arousal factor).

E. THE ROLE OF THE ENTORHINAL CORTEX IN THE DEVELOPMENT OF A CONDITIONED RESPONSE IN THE HIPPOCAMPUS

The previous experiments demonstrated that septal and cingulate inputs are insufficient for evoking conditioned responses in the hippocampus. This leaves the entorhinal input as the main possible input source for initiating a conditioned response in this system.

One of the most unexpected results in Experiment 2 was the long latencies of entorhinal units and the poor conditioned responses observed in this area within 300 msec CS-US interval. There were many reasons to believe that the entorhinal would be involved in conditioning. It was not a matter of chance that Lorente de Nŏ (1933) chose the entorhinal cortex for the demonstration of a reverberating circuit, much before this model was formulated by Hebb (1949). It is known to receive inputs from a variety of brain areas--cingulate cortex, pyriform cortex, neocortex, etc. Its main and very elaborated output pathway is considered to be the main input to the hippocampus. Therefore, the question was not whether the entorhinal cortex is involved in initiation of a conditioned response in the hippocampus or not, but rather, <u>how</u> is it affecting the hippocampus. An occasional observation of an entorhinal unit during conditioning initiated a series of experiments, one of which will be included here.

Experiment 6: A Mechanism for Short Term Memory in the Hippocampal System

The relative absence of conditioned responses in entorhinal units within 300 msec CS-US interval and the very long (150-300 msec) latencies of other units in this area suggested the possibility that even the units that did not respond would have done so if the CS-US interval will be long enough to allow a long latency response to develop. A support to this view was seen in the long (700-800 msec) latency response observed in some dentate units in experiment 1. The dentate is the main projection field of entorhinal efferents. In case there are long latency conditioned responses in the entorhinal area, what may the functions of these responses be, as the hippocampal units are already firing at that period and most of the overt preparatory acts have been executed? The following experiment suggests that it is not the exact timing of onset of this neuronal activity that is of functional significance, but rather the duration and the offset of this activity which is of value. In the present paper some observations will be described, on the basis of which an experiment was designed and will be reported as well.

(i) Prolonged Conditioned Entorhinal Unit Responses

Methods

Subjects

Rats with electrodes implanted in the entorhinal cortex, previously trained in the sensory conditioning paradigm, were used as subjects.

Procedure

Training consisted of a conditioning session followed by an extinction session. First a tone (CS+) was presented, alternating randomly with another one (CS-). Food pellets were delivered two seconds after the onset of the CS+. A tone (a trial) was delivered, on the average, once a minute. There were 150 trials in this session. Following was an extinction session in which the two tones and food pellets were presented in random order.

Data Collection and Reduction

Unit activity was sampled during 1 second pre-CS and two second CS-US interval in successive 10 msec intervals. Thus there were 100 intervals for the pre-CS period and 200 intervals from the onset of the CS to the application of the food pellets. Averages were made for the conditioning and extinction sessions, separately

for CS+ and CS- trials. In addition, the unit activity during application of the food pellets, in the extinction sessions, was sampled and averaged.

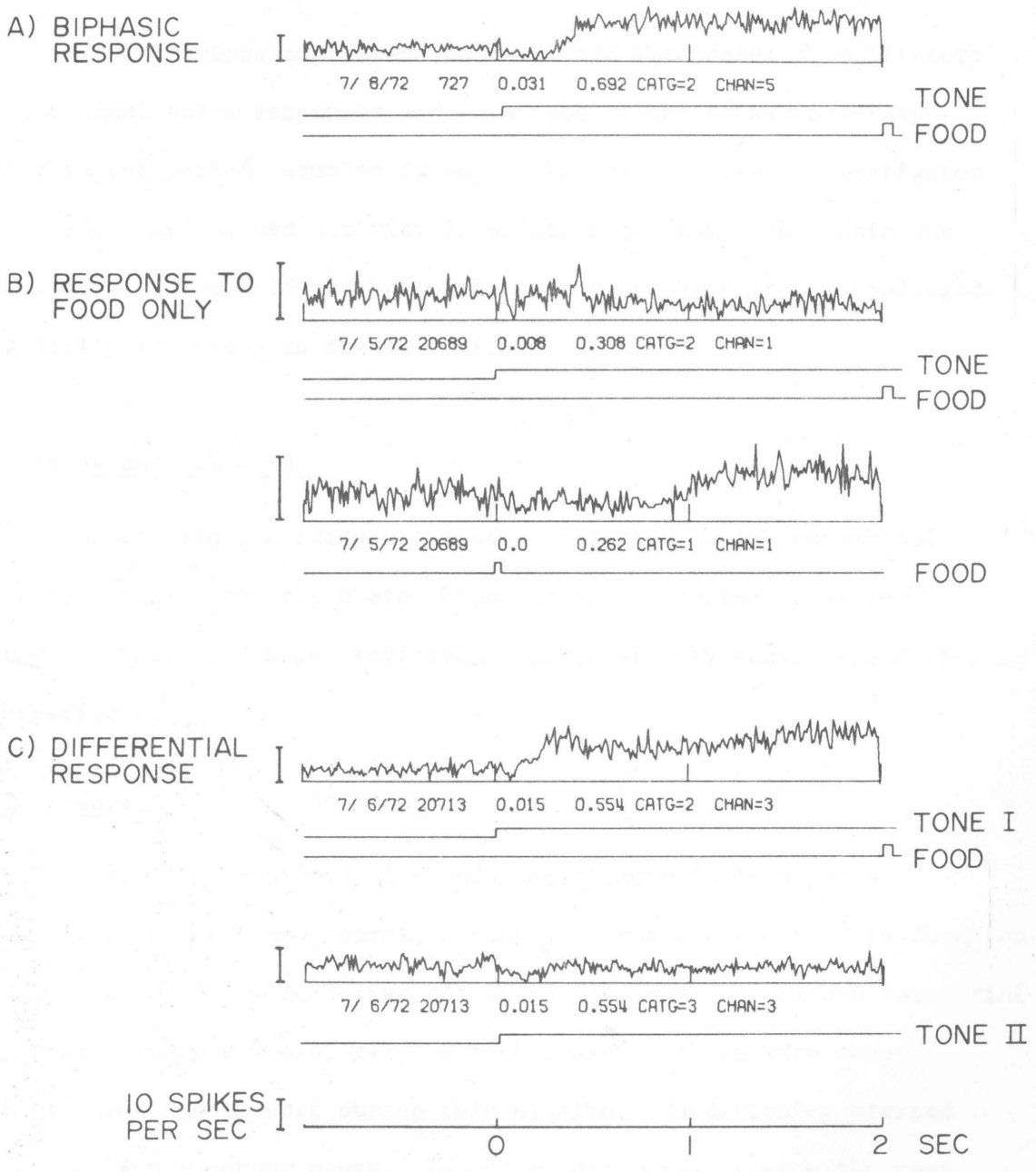
Results

Examples of averaged responses are presented in Figure 7.1. Case A shows a typical unit response to the conditioned stimulus. It had a small inhibitory response, which was followed, approximately 300-400 from the stimulus onset, by a large excitatory response. The firing rate of this response was at least three times as large as the background firing rate. This unit did not show any sign of decrement in firing rate throughout the CS-US interval. Case B is somewhat peculiar in that it did not have any excitatory response to the CS+ but did show this response only to the presentation of food pellets. Case C is similar to the first one and is presented here to demonstrate a differential response--excitatory to the CS+ and inhibitory to the CS- (similar cases were found in the dentate area, see Experiment 1).

Figure 7.1

Examples of averaged responses of three entorhinal units (A, B, and C). Underlying every histogram are the events that this histogram is related to. The bar to the left of each histogram represents 10 spikes/sec. Unit A histogram is an averaged response during 150 trials of the conditioning session. Unit B has two histograms; first one is taken during the conditioning session and the second during the extinction session. Both histograms of Unit C are sampled during the conditioning session. See text for further details.

CONDITIONED RESPONSES IN ENTORHINAL CORTEX



(ii) Time Constant of Prolonged Conditioned Entorhinal Responses

The previous experiment demonstrated late onset of excitatory entorhinal units responses and no offset of the evoked activity within the period sampled (2 sec). The present study investigates how long this evoked activity is maintained. Comparable data was accumulated for relevant brain structures to check if the prolonged activity is unique to the entorhinal cortex.

Methods and Subjects

Rats with recording electrodes implanted in the entorhinal cortex, subiculum, cingulate, hippocampus and septal areas were used. These rats were previously used in sensory-sensory conditioning experiment.

Procedure

Training consisted of pseudoconditioning followed by a conditioning session. First, a tone of 1 khz and 600 msec in duration and food pellets were delivered, in random order and random intertrial interval (mean = 1 min, range 9 sec--2 min). There were about 60 trials of each sort during this session. Conditioning started following a two-hour pause. In the conditioning session the tone was delivered, on the average, every minute. A food pellet was applied 600 msec after the onset of the tone. There were 120 trials

during the conditioning session.

Data Reduction and Analysis

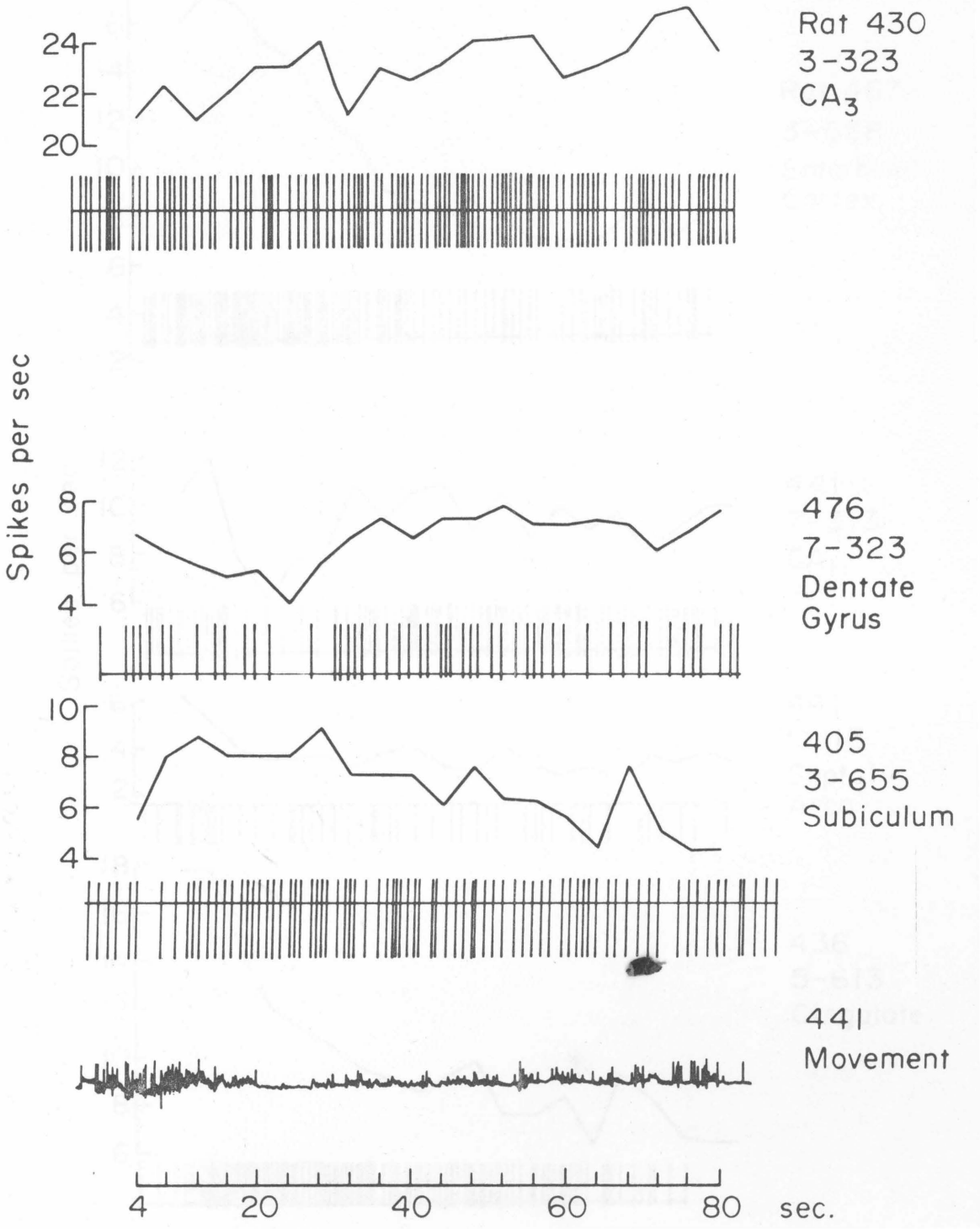
Unitary spikes were amplified and fed into a waveform discriminator which used spike height and fall time to select a predetermined waveform. Upon identification of a spike, the discriminator generated a binary pulse which was fed to a binary counter. The counter was set to produce an output for every given number of unitary spikes, depending on the unit firing rate (range = 2-20 spikes per output). This output was recorded with a Grass Model 7 polygraph. Unitary activity was monitored continuously throughout the experiment. Post stimulation time histograms for periods as long as 90 seconds were prepared manually.

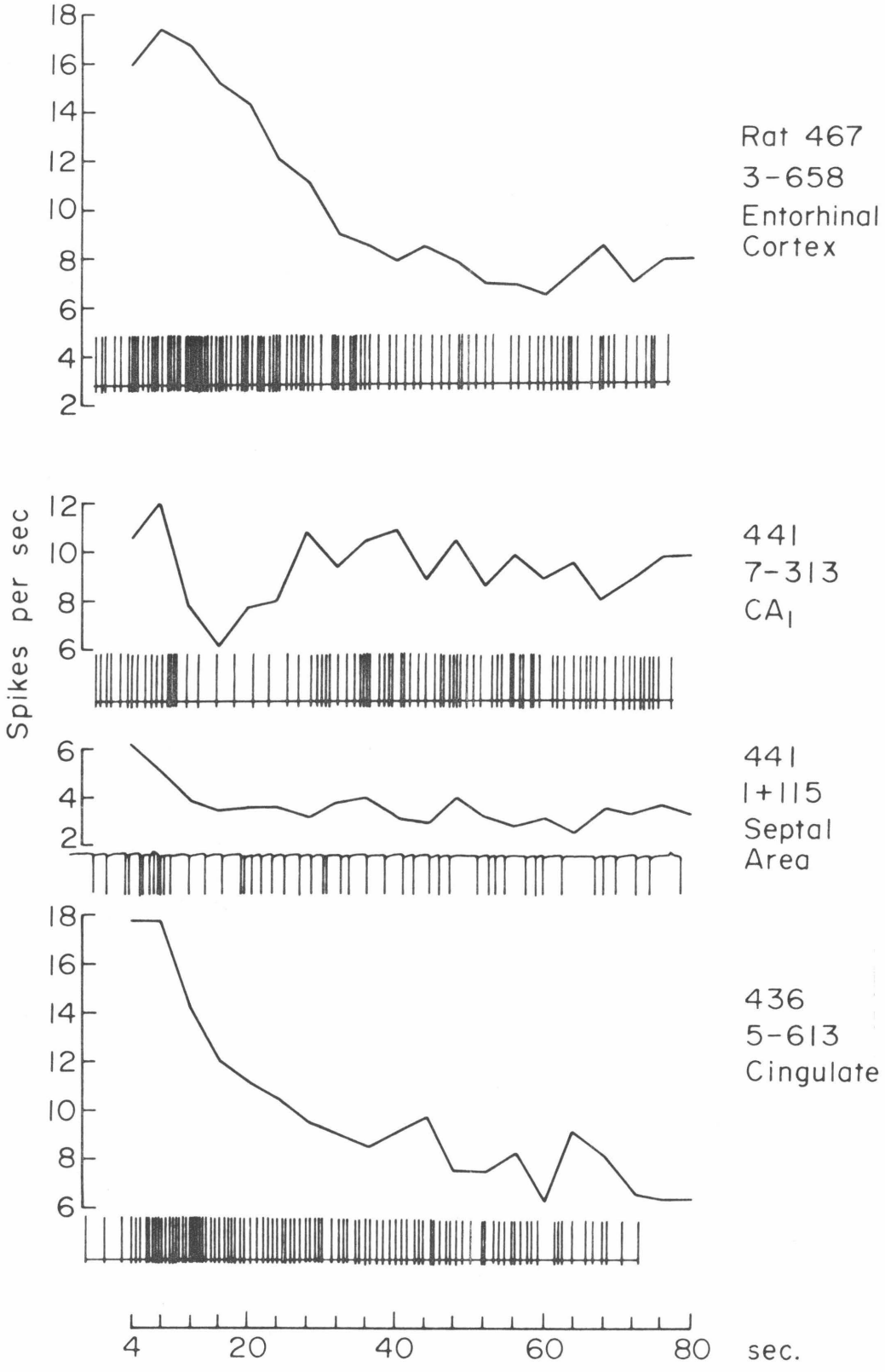
Results

Data was gathered from 24 units in the following areas: septum-3 units; CA3-2 units; CA1-8 units, entorhinal cortex-5; dentate-2; cingulate cortex-2, subiculum-2. Units in the main body of the hippocampus did not show a gross trend in the intertrial interval. Both dentate units reduced firing rate for 10-20 second after the trial and then returned to previous firing rate. The same was true for four out of ten units in CA3-CA1 areas of the hippocampus (see Figure 7.2). Septal units that were activated by the conditioned stimulus subsided within 20 seconds from the end of the trial. The most striking results were achieved by

Figure 7.2

Single traces and corresponding post stimulus time histograms of single units sampled during the intertrial interval. The histograms are made of successive 20-30 trials. The spikes in each trace were redrawn from a polygraph paper and represent a preset number of unitary spikes.


Rat 430
3-323
CA3
24
22
20
Spikes per sec
8
6
4
476
7-323
Dentate
Gyrus
10
8
6
4
405
3-655
Subiculum
441
Movement
4
20
40
60
80
sec.


Spikes per sec
Rat 467
3-658
Entorhinal
Cortex
441
7-313
CA1
441
1+115
Septal
Area
436
5-613
Cingulate
4
20
40
60
80
sec.

the entorhinal units. All five of them were turned on late in the CS-US interval and this activity subsided gradually, to return to background firing rate only after approximately one minute. It should be noted that no similar phenomenon was observed during pseudoconditioning.

(iii) A Correlation between Entorhinal Firing Rate and Hippocampal Response Rate

The two preceding experiments demonstrated the uniqueness of entorhinal units in that they were turned on very late within the CS-US interval but once an entorhinal unit was turned on, it would return to the pretrial firing rate long after the units in the rest of the system were back to stable firing rate. The question then is: is this of any functional value? Based on stimulation studies, one would expect that increased entorhinal activity would facilitate hippocampal response to septal stimulation. If this is true, then one would expect to find that hippocampal responses to a conditioned stimulus applied within one minute from the last trial, while entorhinal units are still more active, would be larger than responses to the conditioned stimulus applied later, when entorhinal units are less active. The following experiment was to test this hypothesis.

Methods

Subjects were 18 rats.

Procedure

The same procedures described in the previous experiment were used (page 155).

Data Reduction and Analysis

All trials in the conditioning session were divided by means of a timer, into two categories: (1) those applied within one minute from the previous trial (SITI), and (2) those applied more than one minute from previous trial (LITI). The distribution into the two categories was random and there were approximately 60 trials in each category. Unitary activity was sampled during 300 msec before application of the tone and 600 msec CS-US interval, in successive 3 msec bins. All trials for a given category were averaged to yield a post stimulus time histogram. This histogram was reduced in the following manner: the mean of 100 background sample points was calculated. The mean firing rate in successive periods (120 msec; 40 bins each) of the CS-US interval was converted into standard scores by subtracting it from background firing rate and dividing by the standard deviation about the mean of this background rate. This procedure yielded 5 successive standard scores for every unit. In addition, the mean of response rate for the whole CS-US interval was calculated and subtracted

from the background firing rate.

Results

Background firing rate, response firing rate and standardized response scores were obtained for 90 units distributed in the following areas: septum-16 units, CA3-10, CA1-25, dentate-7, entorhinal-11, subiculum-11, and cingulate cortex-10 units. The placements of these units are presented in Figure 7.3.

Background Firing Rate

A Wilcoxon matched pair test (Siegel, 1956) was applied to compare background firing rates, in the various areas, in SITI and LITI. The only group of units to show a larger mean firing rate in SITI than in LITI was the entorhinal group. In fact, all 11 units sampled in this area showed this difference. This result was already observed in the previous study. No other group of units passed this test. It should be noted that CA1 group had a somewhat larger background firing rate in LITI than in SITI, but this was not significant statistically ($T = 91.5$, $0.5 < p < .10$ two tail test).

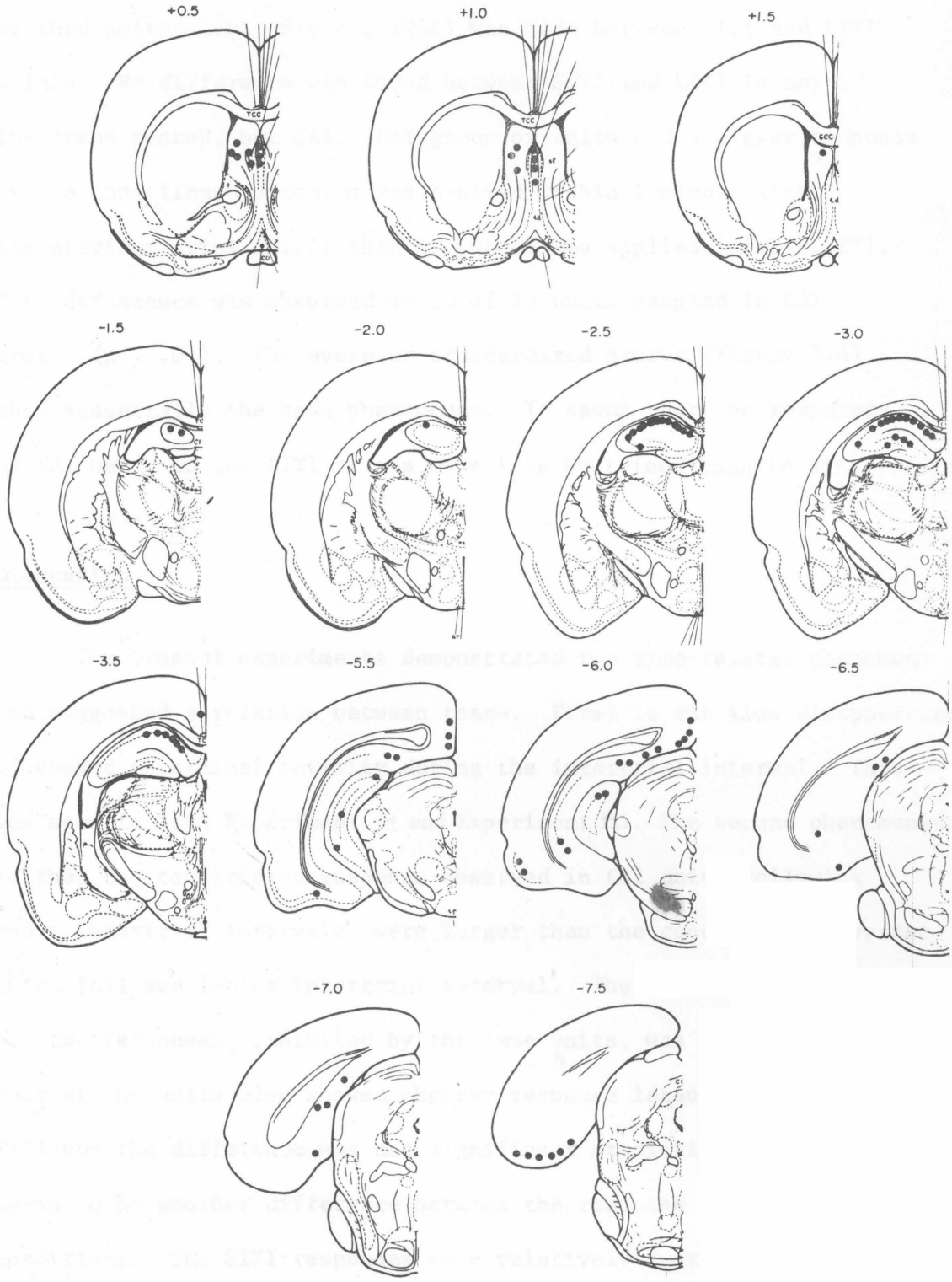
Response Firing Rate

For every unit, the mean firing rate during CS-US interval was subtracted from the background mean and a comparison (Wilcoxon

Figure 7.3

Distribution of the placements of the recorded unit in the main experiment. Sections are taken from König and Klippel.

DISTRIBUTION OF RECORDED UNITS



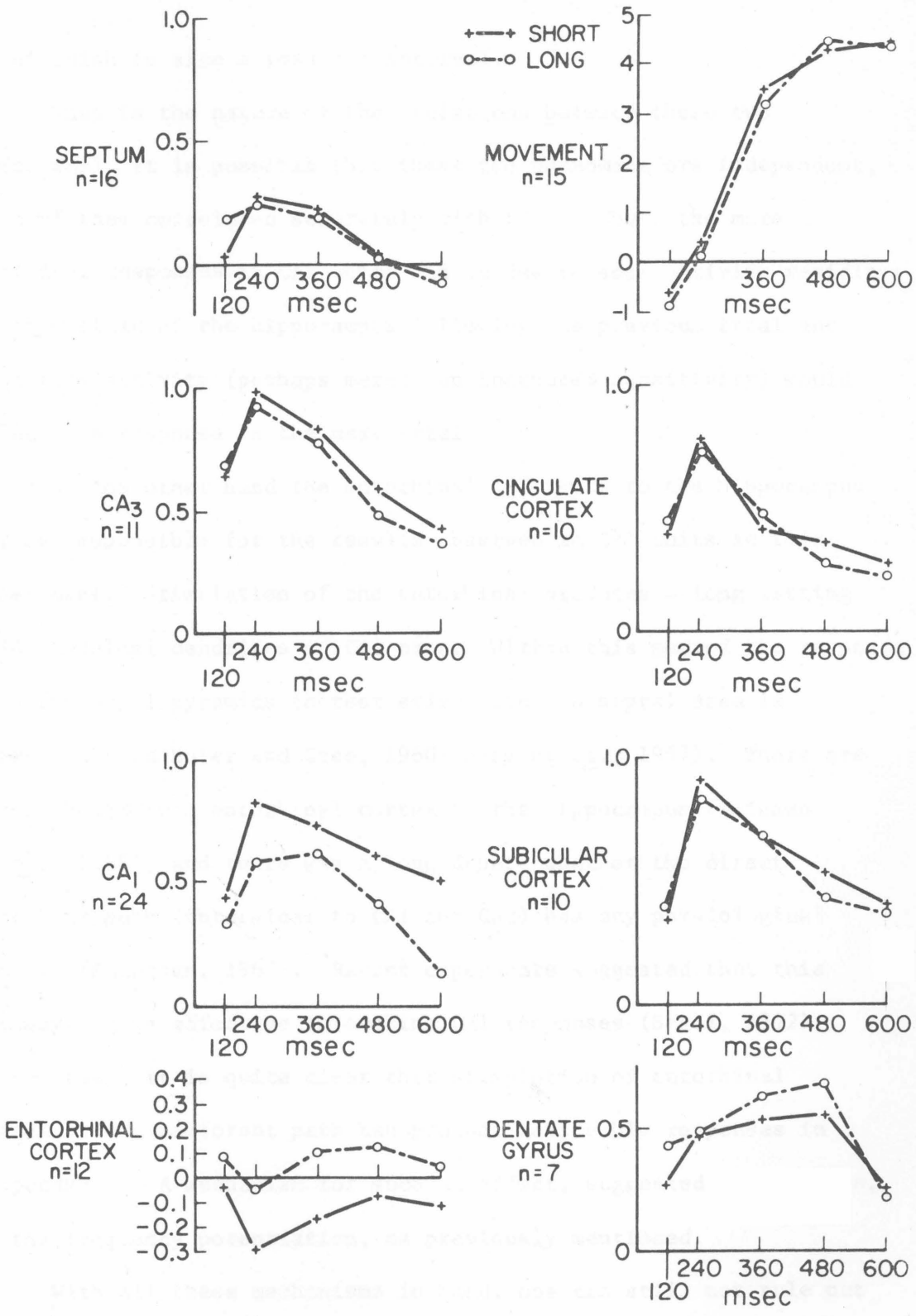
matched paired test, Siegel, 1956) was made between SITI and LITI trials. No difference was found between SITI and LITI in any of the areas tested, but CA1. CA1 group of units had a bigger response if the conditioned stimulus was applied within 1 minute after the previous trial (SITI) than if the CS was applied later (LITI). This difference was observed in 20 of 25 units sampled in CA1 area ($p < .01$). The averaged standardized scores (Figure 7.4) show essentially the same phenomenon. It seems that the responses of CA1 units in the LITI trials were less sustained than in SITI trials.

Discussion

The present experiments demonstrated two time-related phenomena and suggested a relation between these. First is the slow disappearance of evoked entorhinal activity during the intertrial interval. This was seen in both Experiment ii and Experiment iii. The second phenomenon is that the conditioned respones observed in CA1 units following short intertrial intervals were larger than the conditioned response which followed longer intertrial interval. The difference between the two responses, exhibited by the same units, was mainly in size. Most of the units also showed shorter response latencies in the SITI but the difference was not significant statistically. There seems to be another difference between the response in the two conditions. The SITI responses were relatively sustained through the CS-US interval, while the LITI responses were less so and tended

Figure 7.4

Averaged standard scores of the responses of units in the various areas to conditioned stimuli applied after short or long intertrial intervals.


SEPTUM
n=16
1.0
0.5
0
120
240
360
480
msec
SHORT
LONG
MOVEMENT
n=15
5
4
3
2
1
0
-1
360
480
600
msec
CA3
n=11
1.0
0.5
0
120
240
360
480
600
msec
CINGULATE
CORTEX
n=10
1.0
0
120
240
360
480
600
msec
CA1
n=24
1.0
0.5
0
120
240
360
480
600
msec
SUBICULAR
CORTEX
n=10
1.0
0
120
240
360
480
600
msec
ENTORHINAL
CORTEX
n=12
0.4
0.3
0.2
0.1
0
-0.1
-0.2
-0.3
DENTATE
GYRUS
n=7
0.5
0
120
240
360
480
600
msec

to diminish in size across the interval.

What is the nature of the relations between these two phenomena? It is possible that these two phenomena are independent, each of them correlated separately with time. Thus, the more efficient responses in CA1 units can be due to some activity residing in this field of the hippocampus following the previous trial and that this activity (perhaps merely an increased sensitivity) would affect the response in the next trial.

On the other hand the entorhinal afferents to the hippocampus may be responsible for the results observed in CA1 units in this experiment. Stimulation of the entorhinal produces a long lasting EPSP in apical dendrites of CA1 area. Within this period the response of hippocampal pyramids to test stimulation in septal area is augmented (von Euler and Gree, 1960; Adey et al., 1957). There are two pathways from entorhinal cortex to the hippocampus (Raisman et al., 1965), and there was a long debate whether the direct perforant path (entorhinal to CA1 and CA3) has any physiological effects (Andersen, 1967). Recent experments suggested that this pathway can be effective in evoking CA1 responses (Segal, 1972). In any case, it is quite clear that stimulation of entorhinal cortex or the perforant path can produce noticeable responses in hippocampus. A mechanism for such an effect, suggested by Andersen, is the frequency potentiation, as previously mentioned.

With all these mechanisms in hand, one can still not rule out

the possibility that the effects observed in the present experiments in CA1 units are local. To solve this issue and to further demonstrate the dependence of hippocampal conditioned responses upon entorhinal activity the following experiments were done and will be summarized briefly. The first experiment tried to imitate the entorhinal effects on the hippocampus by stimulating the perforant path just prior to application of the conditioned stimulus. The conditioned responses were compared to a regular trial in which no pre-CS stimulation was applied. Both types of trials were applied with at least 1 minute intertrial interval. The results of this experiment showed a larger CR when pre-CS stimulation was applied. Application of the stimulation has no noticeable effect otherwise. In another experiment the pathway from entorhinal to hippocampus was partially damaged. As expected, two phenomena were observed: first, the conditioned responses in the hippocampus were smaller than those obtained in the normal intact animals. And second, there was no difference between SITI and LITI trials. These series of experiments strengthen the view that the entorhinal cortex plays an important role in the initiation of a conditioned response in the hippocampus.

F. GENERAL DISCUSSION

Before trying to integrate the experimental data to yield a cohesive picture of the hippocampal activity during conditioning, a brief summary of the main data is pertinent. The basic paradigm in this series of experiments was of a classical conditioning type. A continuous tone (CS+) was followed by a food pellet (Experiments 1,2,3,4 and 6), electric shock (Experiments 3,4), or a flash of light (Experiment 5). A second tone (CS-) alternated randomly with CS+, was uncorrelated with food (Experiment 1,2,3, and 5), or correlated with shock (4).

The animal's behavior, detected by recording head movements, did not change much upon presentation of an insignificant tone. In the first few trials there was inhibition of movements, corresponding probably to orienting behavior, but no increases in movements were detected. Some 11-30 trials after initiation of a conditioning paradigm the rats started to respond to the CS+ by showing a general excitatory behavior. It took approximately 20 more trials to initiate a specific retrieval behavior (crossing photocell, Experiment 1). The conditioned response was well discriminated and was not evoked by CS-. When extinction procedure started, the conditioned response gradually subsided and disappeared within 50-60 trials (Experiment 2). The latency of a conditioned excitatory response was 120-200 msec from presentation of the CS. When the unconditioned

stimulus was an aversive shock, inhibition of occasional movements was pronounced during the CS-US interval (Experiments 3 and 4). There was no difference, in amount of response, between short and long intertrial intervals.

Some striking differences were found between units of the fields of the hippocampal system. The dentate gyrus, not responding to tones in a pseudoconditioning appetitive paradigm, was first to acquire a conditioned response (Experiment 1), and first to extinguish (2). Its conditioned response was phasic (1), and its latency was long (2). Dentate exhibited an excitatory response to food-related CS and an inhibitory response to an aversive related CS (3 and 4). There was no difference between the conditioned responses to the CS after short and long intertrial intervals. In many of these aspects dentate resembled the detected behavior of the animal.

Area CA3 of the hippocampus exhibited the shortest conditioned response latencies in the system. It followed dentate in the establishment of a conditioned response (Experiment 1) and its extinction (2). It did not maintain responses to the tone during pseudoconditioning, although some responses to the first 10 tone presentations were noticed (2). Its response was sustained throughout a one second CS-US interval and was specific to the CS+ (Experiment 1). It did show a response to a strong sensory stimulation (flash, 4), but this response was not conditionable. It had an excitatory response to a negative CS if previously conditioned with a positive

CS (3), but this was significantly smaller than the response to a positive CS (4).

Area CA1 was similar in many respects to CA3. It had sustained (1), long latency (2), conditioned response to positive CS. This response was specific (1). An excitatory response was present to an aversive CS if pretrained with a positive CS (3), with a smaller intensity (4). The acquisition of the conditioned responses took more trials than the acquisition in area CA3. There was no complete extinction within the period tested. There were inhibitory responses of small magnitude to tones in a sensory paradigm and there seemed to be transient inhibitory responses to the tone when correlated with a flash of light. CA1 area was the only one to show a significantly better unit responses to a conditioned stimulus if this was applied shortly after a previous trial.

The Afferents to the Hippocampus

Medial septal nucleus had the shortest latencies observed in the system. The initial phasic component of these responses was not altered by a conditioning procedure. A later component (100-300 msec) was enhanced by conditioning. The response was not reduced much by an extinction procedure although some attenuation could be observed (2). Medial septal units had short latency responses to a flash of light as well. Lateral septum is in fact considered as an output stage of the hippocampus. It did not have a

response to the tone during pseudoconditioning and the conditioned responses by itself was small in magnitude (due probably to inhibitory effects exerted by medial septum (Defrance et al., 1972)).

Entorhinal cortex had long (240-300 msec) latency conditioned responses to an appetitive CS (Experiment 2) and these excitatory responses were maintained for a rather long period (up to approximately one minute). Some entorhinal units had a differential response, that is, were inhibited as a response to CA-. Ohter units responded only to the presentation of food and not to the CS (6).

The cingulate cortex maintained unconditioned responses to sensory stimuli. A conditioned response was added to the preexisting response and was not extinguished readily in the extinction procedure. Its main efferent field, the subiculum, followed the cingulate in the buildup of a conditioned response and in its resistance to extinction.

The question of how the hippocampus operates can be broken up into two questions: the first one is what are the optimal conditions for evoking learned responses in the hippocampus, and the second is how the hippocampus operates under these conditions. The series of experiments presented above seems to indicate that the best conditioned responses were evoked to a stimulus which preceded food. Both stimuli which preceded unavoidable shock or a flash of light failed to produce as large a response as the stimulus which preceded food. Moreover, there were conditions that improved responsiveness of hippocampal units in the appetitive

paradigm: a shorter pseudoconditioning session in combination with shorter CS-US interval (Experiment 2) reduced the number of trials needed to exhibit conditioned responses; an overtraining session (results not reported for Experiment 1 and reported in Experiment 2) increased the number of responsive units; short intertrial interval increased the amount of response in comparison to long intertrial interval. As already discussed in Experiment 5, it seems that the main difference between the appetitive and the other paradigms is the presence of anticipation of a positive outcome, or the reward factor. The arousal factor seemed to be present to some degree in all experiments. A mechanical requirement--the presence of an unconditioned response to the US was present as well. In addition, there was an indication that conditioned responses may be evoked in the hippocampus in the absence of operant behavior (i.e. when brain stimulation served as a reward).

Which of the pathways can be considered as the pathway carrying the reward factor into the hippocampus? The immediate candidate for this function is the medial septal nucleus, a site at which stimulation was first found to be rewarding (Olds and Milner, 1954). Not only that, it is closely linked to the medial forebrain bundle and the lateral hypothalamus, both associated with reward functions. This candidate however, did not seem to be the critical one in our experiments. Units in this area did not show as drastic a change between pseudoconditioning and conditioning and between conditioning and extinction, as did the hippocampus.

In fact, in many cases, as for example the sensory paradigm, there seemed to be a failure of transmission between septum and hippocampus, that is, CA 3 units did not respond to septal input. It seems that the septum maintains a sensory--arousal function, that is, responds to any strong or meaningful stimulus. The cingulate cortex did not seem to carry the reward factor either for the same reasons mentioned above. In fact, based on the sensory experiment (5), anatomical (Cragg, 1965) and physiological (Maclean, 1969) data, it seems to transmit into the hippocampal system elaborated sensory information. This leaves us with the third and combined pathway into the hippocampus--the direct entorhinal path passing through CA1 apical dendrites and ending on CA3 pyramidal cells, and the entorhinal-dentate-CA3-CA1 pathway. The dentate seems to be the best candidate for transmitting reafferent rewarding messages into the main component of the hippocampus-CA3. It preceded the CA3 area in both acquisition and extinction of a conditioned response. Reduced dentate activity (as was the case for the food-shock situation, Experiment 4), was correlated with slowing down of acquisition of a conditioned response in the hippocampus. Absence of dentate activity, or, in fact, inhibition of dentate unit activity, was correlated with lack of initiation of a conditioned response in the hippocampus (Experiment 3). Lack of dentate and entorhinal responses as was the case in the sensory paradigm, prevented the establishment of hippocampal conditioned responses, although other inputs were present.

There are reasons to believe that the entorhinal cortex exerts its effect on the hippocampus differently via the two connections--the direct and indirect pathways. First is the difference in origin of the two pathways--lateral and medial entorhinal, respectively. Second is the different terminal areas on the hippocampus (see Figure 1.3). Third is the difference in physiological properties--stimulation of the direct pathway produces a long EPSP in apical dendrites of CA1 units that lasts for several hundreds of milliseconds but does not overlast the stimulation period, whereas the indirect pathway has a facilitatory effect on dentate cells that lasts for hours, in the acute preparation (Lomo, 1971). Finally, our results: Experiments 4 and 6 indicated that there are conditions in which CA1 units may increase the intensity of response and concomitantly decrease latency of response. The entorhinal cortex was held responsible for these modifications. In these experiments there were no concomitant changes in dentate or septal units. Moreover, Experiment 3 demonstrated that CA3 is not entirely dependent upon dentate input for the maintenance of a conditioned response. It seems then that entorhinal afferents may have two different effects on the CA3-CA1 system, or perhaps effects in two different time domains. All in all, entorhinal cortex did not seem to exercise its effects within the CS-US interval. Its response latency was too long and the hippocampal responses were sustained

already when entorhinal input arrived. The striking phenomenon in entorhinal unit responses were their long duration. This long duration, high frequency firing may produce in dentate cells increased reactivity similar to the one observed following tetanic stimulation of the perforant path. The dentate cells will maintain their "charge" for a long period, even after entorhinal activity has subsided; they will "discharge" whenever tested again, perhaps by CA3 input or septal input. This "discharge" will form a phasic response (Experiments 1, 4) that will be generated to both CS+ and CS-. The direct entorhinal input to CA1-CA3 has a shorter time constant and seems to be more precise. This effect is exerted only while entorhinal units are still under the influence of the previous trial (Experiment 6). It should be noted that the entorhinal input is highly restricted to certain lamellae of the hippocampal sheet (Blackstad and Andersen) and it seems that a selective facilitation is the mode of operation of the entorhinal influence. This may explain the high percentage of nonresponsive units in CA1 area observed in Experiment 1.

The mechanisms of conditioning in the hippocampus seem to be the following: short-latency non-specific and unconditioned input enters the hippocampus from the septum. Occasional driven hippocampal response to the septal input are depressed by means of the strong local negative feedback loops which further maintain the theta rhythm originally driven by the septum. Upon conditioning,

when entorhinal input selectively preloads the pyramidal cells by producing long EPSP's on their apical dendrites, their response to septal input is heterosynaptically facilitated. The additional dentate input further facilitates CA3 responses and may in addition affect the local inhibitory process; the CA3 is thus considered as a major junction point upon which information is converged both spatially and temporally. (If there weren't a place like this we would have to produce one, if we want to have learning centers in the brain.) Selective spatial and temporal effects are achieved by the CA3-CA1 connections. Here a CA3 cell can activate only a subset of CA1 elements to produce enormous possible combinations of reactive CA1 cells.

This model is simple. Although not explicitly proposed, it has ample support from previous work on the hippocampus. It was demonstrated (Adey, Sunderland and Dunlop, 1957; Von Euler and Green, 1960) that conditioning shock to the entorhinal facilitates hippocampal responses to test stimulus applied to the septum. With a similar approach Shaban (1970) demonstrated a reduction in theta intensity and in responsiveness to sciatic stimulation following a lesion in the perforant path. The physiological basis for entorhinal and septal inputs to the hippocampus have long been studied (see Introduction). As to the occurrence of such a mechanism during conditioning, an indirect support can be seen by the work of Holmes and Adey (1960) and with a slight modification in the work of Maclean and his collaborators. These seem to fall in line with

results of the series of experiments which form the basis of this thesis.

In regard to the two main questions posed in the Introduction: A. What is the hippocampus doing? The evidence for this is only indirect since it was suggested (Douglas, 1967) that hippocampal lesioned rats can acquire and perform a classically conditioned response with ease. This however does not mean that the hippocampus is not involved in learning in the normal animal.

The long latency of conditioned hippocampal responses and the relatively few unconditioned sensory responses there make it unlikely that the hippocampus is involved in sensory integration. (This conclusion would not hold for the rabbit; see Vinogradova, 1970.) The fact that the conditioned responses are initiated after or simultaneously with the initial unconditioned behavioral responses to a stimulus (freezing) make it unlikely that the hippocampus is closely linked to some innate behaviors. This is supported by results of Experiment 3 and 4, where dissociations between hippocampal activity and behavioral freeze were detected. The hippocampus is probably not closely linked to the performance of the conditioned response and this is demonstrated in the extinction data (Experiment 2) and in Experiment 6. It is conceivable that the hippocampus is involved in some sort of temporary acquisition and storage of information related to motivational factors. The proposed mechanism of hippocampal circuitry makes it ideal for such purposes. It is tempting to speculate that "information" related to the current

environment is stored in this structure and compared to what is demanded by actual signals coming from the environment. The "information" is probably a limited set of disjoint acts that are of relevance to the environment and are changed upon changes in the environment. An "act" is a temporally arranged series of instructions that are needed to produce an instrumental, voluntary behavior. According to this hypothesis the more variable the environmental demands the more variable the hippocampal responses will be. The more familiar the animal is with the environment, and the fewer the behaviors demanded the less variable the hippocampal responses will be. This would fit with the overtraining data (Experiments 1 and 2) and with results of Experiment 4. It is easy to test these hypothesis in further experimentation. This mechanism provides the animal with the flexibility needed in new environments (and missing in hippocampal animals) and also with smoothness in reacting to an old and familiar stimulus.

B. What can we learn about learning from the way the hippocampal acts? There are a few criticisms that almost every neurophysiological model of conditioning has failed to satisfy. One is how an unconditioned response (to US) can affect the rsponse to CS applied prior to US. The present model suggests that the effect is not exerted only backward but also forward. This is similar to Sperry's suggestion of the existence of a central facilitatory state (Sperry, 1955). The present model has refined this statement and proposed a source

and an effect of this facilitation. It should be noted that this model is tentative and needs more experimental evidence. There are some questions the answers to which are still inconclusive. One is the problem of the difference between pseudoconditioning and conditioning. In both of these paradigms the presence of food should facilitate the response to the tone, and why then did the hippocampus respond to tone during conditioning only? The fact of the matter is that the hippocampus (mainly CA3-1) did indeed respond to the tone during pseudoconditioning, in a way different from that exhibited during the sensory paradigm, when food was not available, but still the response was much smaller than the responses in the conditioning session. It is assumed that the dentate is involved in the concurrent matching of CS and US related activities, acting backward from the US. (It is interesting to note that the dentate was the only area to show progressive decrement and increment of latencies during conditioning and extinction, respectively;see Figures 3.6 and 3.7). Moreover, the relations between the dentate and CA3 were demonstrated in a number of experiments (1,2,3,4) but the exact physiological mechanism underlying dentate effects has still to be refined.

Another problem is how specificity is achieved, that is, how does the hippocampus "know" that the present tone is 10 Khz and not 1 Khz and what makes it respond only to the CS+? A few possibilities should be considered for this matter. One is that

the selection of the stimulus is done prior to the arrival of the message to the hippocampus. This is not likely to be the case since the short latency septal responses were neither specific nor preferring the CS+. It is still possible that the septum conveys a differential sensory message since many of its units responded primarily to one tone and less to the other both during pseudoconditioning and conditioning. In that case the "decision" of which tone to react to will be spared for the hippocampus proper. The possibility of preferred modality and frequency for septal units can easily be tested. The specificity of the hippocampal response would then be determined by the concurrent activity of dentate and entorhinal inputs on subset of CA3 cells. This activity may result in a permanent structural modification in CA3 cells, the nature of which has still to be worked out.

Finally, there is the question: Are there structural changes in the hippocampus as a consequence of conditioning or can a "dynamic trace" model account for the conditioned responses observed in this structure. The sophisticated neurophysiologist would immediately say "both." This however can be easily tested (in an extension of Experiment 6) by recording hippocampal unit responses to a previously established conditioned stimulus after a long resting period.

It is understood that further experiments are needed to test some of the assumptions presented above. It is nice to have these hypotheses. It is fun to test them.

In discussing recent cellular approaches to the study of learning in the mammalian brain, Bureš and Burešova state (p. 370), "A careful analysis of latencies of conditioned responses may indicate the sequence of activation within the reacting population but even this method does not make it possible to identify the critical change with precision." This statement is correct. However, the use of this and additional methods can make it possible to identify the critical change. The present work attempts to demonstrate this point.

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