INVESTIGATIONS OF "SPATIAL" FIRING IN DORSAL HIPPOCAMPUS OF THE RAT

Thesis by

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ABSTRACT

"Spatial" units have been identified in micro-electrode recordings from dorsal hippocampus of rats. Such units fire at their maximum rates only when rats are in specific regions of the recording space, called the units' "fields", and seem to be independent of details of the animals' behavior. Spatial firing is consistent with a recent suggestion that the hippocampus functions as a "map" of the environment.

Recordings were made from dorsal hippocampus of rats as the animals performed a spatial alternation task in an enclosed T-maze. Spatial firing was identified in rats which had been selectively deprived of either visual, vibrissal, auditory or olfactory sensory inputs. Results are consistent with other evidence that hippocampal spatial firing is based upon multi-modal sensory cues.

Recordings were made from dorsal hippocampus of rats as the animals learned to shuttle and alternate in the T-maze when they had not been there before. In 11 out of 15 cases, spatial firing occurred during the first passage of the rat through the unit's field. In the other 4 cases, firing occurred for the first time within 15 minutes of the rat's first exposure to the field. Results are discussed in relation to theories of hippocampal function.

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INTRODUCTION

The hippocampus has probably been the subject of more extensive research than any other region of the mammalian brain. Since such investigations began over a century ago, hippocampal anatomy and electrophysiology have been explored in some detail. Nevertheless, comparatively little is known about the basic function of the hippocampus in the central nervous system.

In recent years, extracellular unit recording techniques have been used in conscious and freely-moving animals to provide new insight into the activity of the hippocampus. Among the phenomena revealed by these methods is the fact that in rats hippocampal neurons often fire in a characteristic way correlated principally with the position and orientation of the animals within the recording environment. Such "spatial" units have been identified with pyramidal cells, the principal, if not the sole, output neurons in hippocampus. From this fact alone, it seems clear that hippocampal function might be clarified by an investigation of spatial firing and its relationship to behavior. This report concerns two aspects of spatial firing recorded from hippocampus: the sensory inputs upon which it may be based, and the latency with which it initially appears in a new environment.

BACKGROUND

I. Theories of Hippocampal Function

Three general approaches have been taken in the investigation of hippocampal function. The most venerable approach has involved anatomical inference from details of the structure and connections of the hippocampus.[1] Thus, as part of the "Papez circuit", the hippocampus has been implicated in the control of affective behavior. Alternatively, because of the extensive olfactory input to surrounding areas and the close ties of the hippocampus to this so-called "rhinencephalon", it has been suggested that the hippocampus plays a role in olfactory behavior. Neither proposal now enjoys much support.

A more recent and certainly more productive technique has employed observation of the effects on behavior of hippocampal lesions.[1,2] Such studies have identified a number of interesting characteristics displayed by hippocampally-lesioned animals but not by normal animals. Among these are:

1) Deficits in memory for recent events (found almost exclusively in humans).[3,4,5]

2) Deficits in reversal training.[6,7]

3) Deficits in adjusting responses to changed reward contingencies or schedules.[8,9,10,11]

4) Greater resistance to extinction.[12,13]

5) Abnormal indestractibility to novel stimuli during behavior.[14]

6) Deficits in some passive avoidance tasks, but no deficits or even enhanced performance in active avoidance tasks.[15,16,17,18]

7) Deficits in spatial behavior and maze tasks.[19,20,21,25]

Several theories have been proposed to explain these results. The most important of these are: that the hippocampus is a mechanism for some form of inhibition;[1,22,23] that it is essential in directing spatial behavior;[24] that it is involved in learning and memory in general.[26]

Based ultimately upon Pavlov's ideas of a mechanism for extinction and habituation,[98] inhibitory theories suggest that the hippocampus mediates suppression of responses, of attention, or, more vaguely, of certain internal associations involved in learning. Such theories can be used to explain a large portion of the animal lesion data. For example, due to lack of internal inhibition, deficits might be expected in passive avoidance (withholding an inappropriate response) but not in active avoidance. On the other hand, Black et al.[27] have pointed out that the lesion literature is far from consistent in this area. Deficits are not always found in all types of passive avoidance. Furthermore, obvious spatial aspects are involved in many of the behavioral tasks traditionally used to assess the effects of hippocampal lesions. Thus, such tasks cannot clearly distinguish between the inhibition and spatial theories in particular.

The spatial theory pictures the hippocampus as the site of a "spatial map" containing a representation of places and objects in an animal's environment and their relationships to one another. By means of this map, animals navigate within a familiar region. Based upon sensory cues from the environment as well as information regarding the animal's movements, the map directs those movements and signals discrepancies between expected and actual features of the surroundings. The spatial theory is capable of explaining a great deal of the animal lesion data. It is not yet clear whether the theory can explain all the effects of such lesions. There are difficulties in testing such a theory. Subtle distinctions must be made

between place learning (which involves identification of a particular place), response learning (which involves recognition of a particular behavior within a sequence of behaviors) and cue learning (which involves responding to a specific, but non-spatial cue, such as a certain ambient light level). It is possible, nevertheless, that the spatial theory can reconcile the well-known differences between effects of lesions in animals and in humans. Generalizing the theory along the lines of Tolman's "cognitive map", one can extend hippocampal function to a mapping of concepts and events as well as locations in space.[24]

In humans, lesions of the temporal lobes produce a variety of symptoms. Prominent among these symptoms are deficits in memory for recent events. Nevertheless, in animals similar lesions have generally not produced comparable behavioral changes. Recently several other techniques have been applied to the study of hippocampal function. These include stimulation with low-level electrical current[28,30] or with chemicals,[29] histological or chemical analysis following learning,[32,33] and finally electrical recording of EEG[34] or unit[35] activity. While some of these studies have been used to support other theories of hippocampal function, such as its role in motivation,[36] most have been predicated upon the hypothesis that the hippocampus may be involved in learning and memory. Indeed, these data have provided most of the support for the learning theory as applied to animals. For example, low-level electrical stimulation of hippocampus enhances the acquisition of an operant task by mice if stimulation is applied soon enough after training.[37] Several studies have found specific changes in various chemical parameters of hippocampus after learning.[31,32] EEG[38] and unit firing[39] rates show changes correlated with learning. Data from unit recordings will be discussed further below.

II. Hippocampal Electrical Activity

Slow wave activity. In hippocampus, electrical slow wave activity is dominated by two general patterns.[40] In the rat, these are small-amplitude, fast (or desynchronized) activity at 40-60 Hz and large-amplitude, slow (or theta) activity at 6-10 Hz. The theta rhythm has attracted more attention due to its correlation with certain behaviors or (presumed) mental states. Green and Arduini[41] found that certain stimuli seemed to produce arousal in cats and that these stimuli also evoked hippocampal theta and neocortical desynchronization. This correlation has been studied extensively by Bennett.[42]

Hippocampal theta rhythm is apparently generated by synaptic endings on pyramidal and dentate granule cells. In the rat, moveable micro-electrodes have been used to make profiles of theta activity. These profiles show two loci for generation of theta, one in str. oriens of CA-1 and the other in the molecular layer of the internal blade of the fascia dentata.[43] The two loci appear to be 180 degrees out of phase, with a pronounced null zone between them. Theta can be recorded from other parts of hippocampus, as well as from surrounding areas of midbrain and neoortex. This activity is apparently the result of volume conduction from the two primary loci.

Hippocampal theta is under the control of cholinergic relay neurons located in medial septum.[44] Lesions in medial septum (or in fimbria-fornix) disrupt hippocampal theta,[45] and at the same time produce loss of ACh-E staining in hippocampus.[46] Micro-electrode recordings have revealed units in medial septum which fire in bursts phase-locked with hippocampal theta when theta is present.[47] Such firing is not accompanied by local slow wave activity in the theta range and is

found to recover from electrically induced seizure before the reappearance of hippocampal theta rhythm.

As mentioned above, there seems to be in the cat a good correlation between hippocampal theta and processes associated with arousal or attention. This correlation is not necessarily the same for other species.[48,49] In fact, there is apparently only one general behavioral correlate of hippocampal theta: its occurrence during paradoxical sleep. For other animals, behavioral correlates are voluntary movement in rat and guinea pig, and arousal or movement in the rabbit.

Unit electrophysiology. When recordings are made from micro-electrodes in dorsal hippocampus, units can be separated into two easily distinguished categories: "complex-spike" units and "theta" units.[50] 1) Complex-spike (CS) units can fire either single action potentials or complex spikes. The latter consist of several individual action potentials separated by 2-6 milliseconds with progressively smaller amplitudes (Figure 1). Complex spikes have been studied in detail using intracellular recording from presumed pyramidal cells in hippocampus by Kandel et al.[51,52] and by Fujita.[53] A typical complex spike is illustrated in Figure 2. Two inflections can be distinguished on the rising edge of the initial spike. The "A-B break" is similar to that observed in several other regions of the CNS. It is believed that this inflection separates the sequential generation of a spike in the initial axonal segment and its invasion of the cell soma. The "fast prepotential"[54] (FPP) has been tentatively identified as a dendritic spike, consisting of the passive summation at the soma of local active spikes occurring at major dendritic branch points. Such dendritic spikes have been found in other parts of the CNS[55] as well as in hippocampus of cat[56] and rat.[57] It may be that such prepotentials are confined

Figure 1

Examples of theta and complex-spike units recorded during surgery

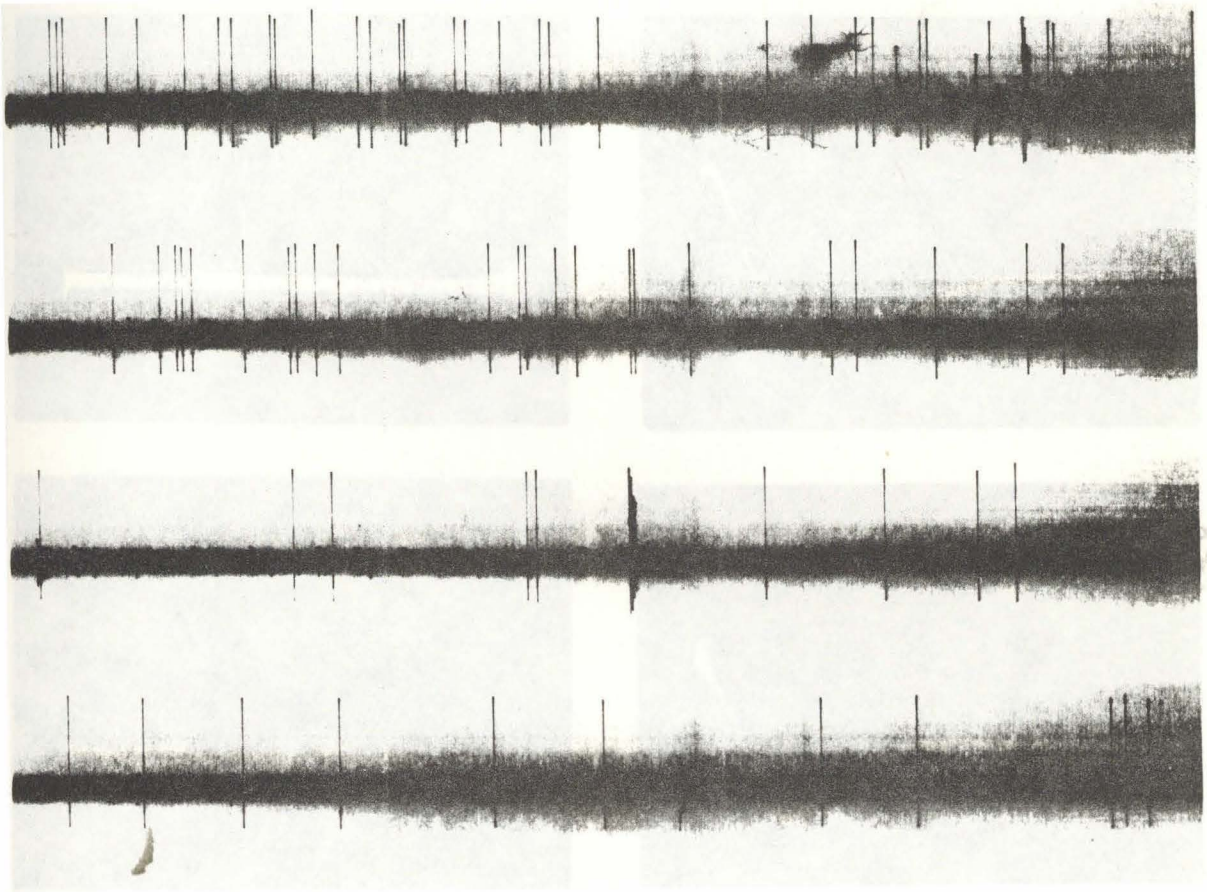
(A) Two large and one small CS spikes can be seen in this trace. Recordings obtained from single micro-electrode in dorsal hippocampus Regio Superior pyramidal layer in anesthetized (sodium pentobarbital) rat. Time scale: 1 cm = 0.2 sec.

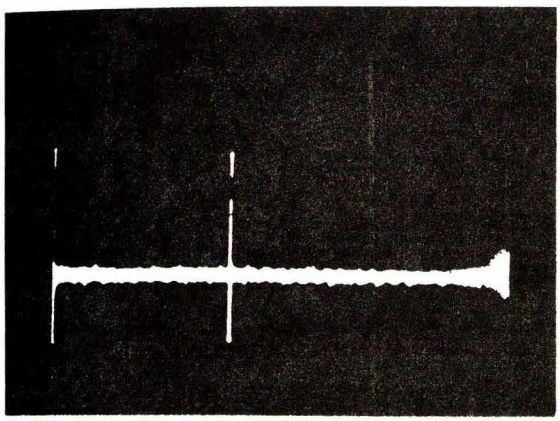
(B) A single complex-spike from the unit shown in Figure 6A. Time scale: 1 cm = 5 msec.

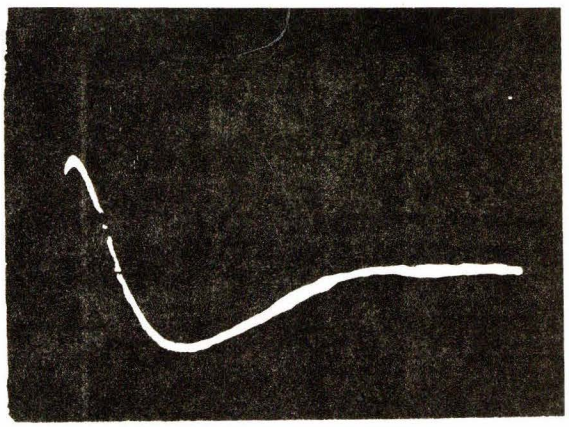
(C) Two individual theta spikes. Time scale: 1 cm = 5 msec.

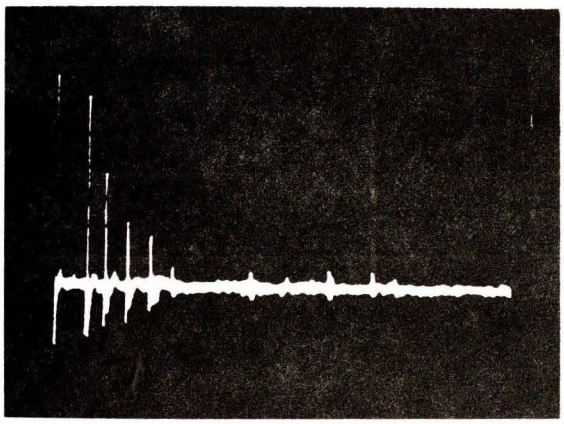
(D) Fast sweep of theta spikes, 6 sweeps superimposed. Time scale: 1 cm = 0.1 msec.

(E) Comparison of wave-shapes of theta and CS spikes. Free run during theta firing until first CS spike occurred. Time scale: 1 cm = 0.1 msec.









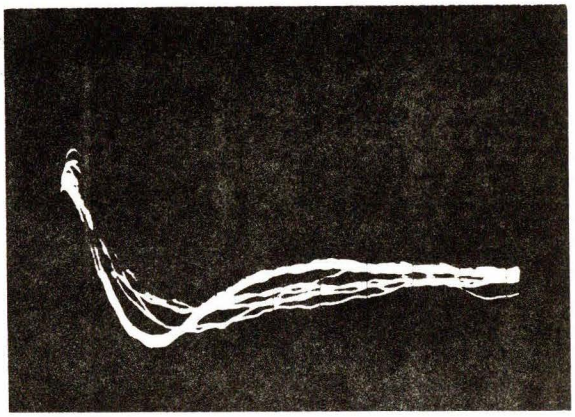
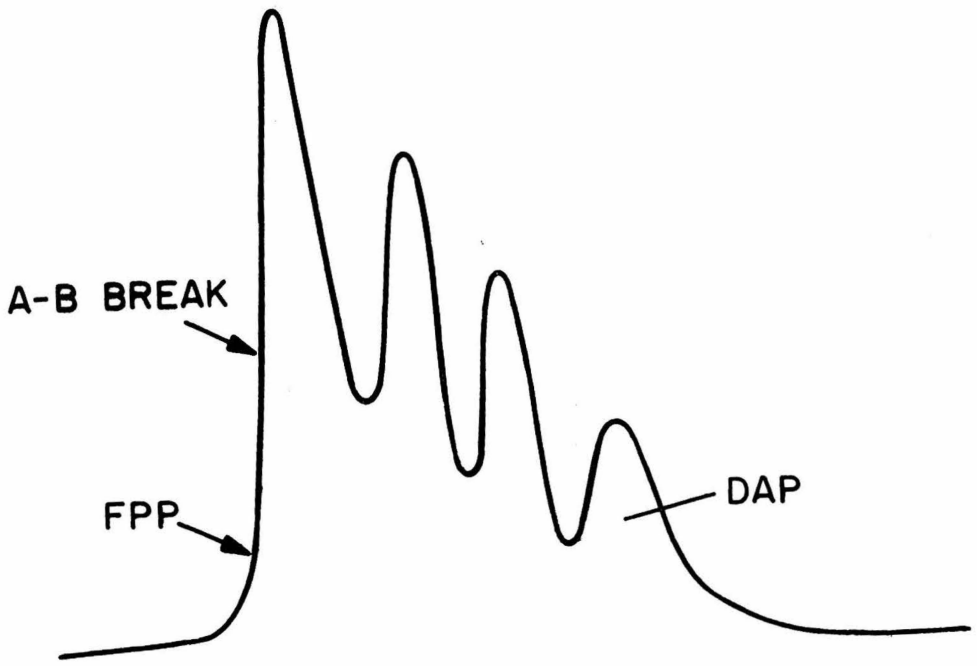


Figure 2

General form of a complex-spike from a hippocampal complex-spike cell. After a figure in Kandel and Spencer.[52]


A-B BREAK
FPP
DAP

to the more extensive apical dendritic tree in hippocampus. When spikes are elicited from pyramidal cells of hippocampal slices in vitro,[58] they show FPPs if produced by stimulation in str. radiatum, but not if produced by stimulation in str. oriens. Repetitive firing and the decline in amplitude and eventual termination of the complex-spike probably involve the pronounced depolarizing afterpotentials (DAPs) which are observable in intracellular recordings.[59] After single spikes, the DAP is generally about 10 mV high and lasts about 30 msec, with a passive, exponential decay.[52] During complex spikes, however, the successive DAPs sum and can reach magnitudes of 30-40 mV. It seems likely that this prolonged DAP results in a progressive Na-ion inactivation which terminates the complex-spike. A similar inactivation response has been observed in cerebellar Purkinje cells.[60] Recent work[59] indicates that the hippocampal DAP consists of two components, one a local event depending upon soma spike activity and membrane potential, the other derived from remote events, presumably occurring in the dendrites.

CS units generally fire at a low rate, ranging from zero to two spikes per second, in behaving animals. Although individual CS units may fire both single and complex spikes,[50] in observations to be reported here, most CS units produced either single spikes or complex spikes consistently. Occasionally, as will be described below under "correlates", CS units fire much more rapidly, reaching rates of 20 spikes per second or higher for short intervals. These high rates occur under circumstances which seem to be characteristic of CS units in hippocampus.

2) Theta units fire only single spikes. These can be distinguished from single spikes of CS units and from individual components of complex spikes by their shorter duration, quicker repolarization and extremely regular waveform

(Figure 1). Recently, extracellular recordings have been made from cells in hippocampal slices which may correspond to the theta units recorded from extra-cellular electrodes in intact animals.[61] Nevertheless, the electrophysiology of theta units has not been studied in detail. Theta units rarely fire at rates less than 10 per second and usually fire much more rapidly, often exceeding 50 spikes per second over intervals of seconds.

Localization and identification of theta and CS units. CS units can be recorded only from micro-electrodes whose tips are in or within about a hundred microns of the pyramidal layer of hippocampus or from those whose tips are within the hilus of the fascia dentata.[50] During a vertical penetration through CA-1 and CA-3 of dorsal hippocampus, their distribution corresponds well to the distribution of pyramidal cells in these regions: densely populated in a narrow zone in CA-1 and more diffuse in a broader zone in CA-3.

Theta units are more widely distributed than are CS units. When vertical penetrations are made with micro-electrodes in awake, unrestrained animals,[62] theta units are encountered almost exclusively above and within pyramidal layer in both CA-1 and CA-3. They occur most frequently in the top quarter of CA-1 (str. oriens) and in the top quarter of CA-3 (str. lacunosum-moleculare).[50] During implantation of chronic electrodes in anesthetized rats in this experiment, observations conformed substantially to the above description, save that theta units were found with maximum density just above pyramidal layer in each subfield.

Since CS units are confined to the vicinity of the pyramidal layer, while theta units are much more widely distributed, Ranck[50] has suggested that CS units are indeed pyramidal cells, while theta units correspond to hippocampal interneurons. This identification has been confirmed by Fox and Ranck during

electrophysiological studies in behaving animals.[63] In these studies, firing of CS units coincided with the occurrence of population spikes elicited by stimulation of septum, entorhinal cortex or commissural inputs to hippocampus. This was not true for theta units in general. Furthermore, CS units often showed antidromic activation, while theta units did not.

Correlates of unit firing. CS units and theta units are most clearly distinguished by the electrophysiological and behavioral events which routinely accompany periods of rapid firing. Both unit types show definite phase relationships to hippocampal theta rhythm when this is present,[50,59] but only theta units exhibit their highest rates consistently at such times. In the rat, the occurrence of hippocampal theta is correlated with "voluntary movements".[64] These movements include locomotion and changes of position, but do not include consummatory and grooming movements. In awake and unrestrained rats, a correlation can also be observed between voluntary movements and theta unit firing.[50,65]

Only one electrophysiological correlate has been reported for CS units. This is a general acceleration during deep slow-wave sleep.[50] On the other hand, several different behavioral correlates have been described. Ranck[50] classified CS units on the basis of their firing during a variety of operant and open-field behaviors. In his studies, most units could be assigned to one of four categories:

a) "Approach-consummate" units fired rapidly during successful appetitive and consummatory behaviors, such as retrieving and eating a food pellet.

b) "Approach-consummate-mismatch" units fired during unsuccessful, as well as successful appetitive behaviors and during consummatory behaviors.

c) "Appetitive" units fired most rapidly during approach and orienting behaviors, but not during consummatory behaviors.

d) "Motion-punctuate" units fired briefly at the end of presumed orienting movements or changes in direction of locomotion.

O'Keefe and Dostrovsky first suggested another behavioral correlate for CS units.[66] Using moveable micro-electrodes, these investigators observed the firing of hippocampal units in rats as the animals moved about or were manipulated on a small, raised platform. In recordings from dorsal hippocampus, 8 out of 76 units fired maximally only when the rat was in a particular part of the platform and (perhaps) facing in a particular direction. Five units did not fire at all in other parts of the platform while the other three had much lower but non-zero background rates.

Of the 8 units reported by O'Keefe and Dostrovsky, not all were from "freely-moving" animals in a strict sense. For the unit most extensively described in the paper, maximum firing occurred only when the animal was actually restrained by hand in a particular manner. Nevertheless, in all 8 cells a common factor seemed to be specificity for place, leading the authors to suggest that these cells, and the hippocampus in general might be involved in "mapping" the animal's environment. This hypothesis has subsequently been elaborated by Nadel and O'Keefe[24] and has been applied with some success to lesion experiments.[27]

In a recent report,[65] O'Keefe has described more extensive observations of "spatial units" in the rat, made while animals moved about an elevated three-armed maze in search of food pellets. O'Keefe confirmed previous descriptions[50] of the behavioral correlate of theta-unit activity in the rat and classified non-theta units into three categories. "Place units" (20/34) fired maximally only in certain regions of the maze. O'Keefe has applied the term "spatial field" to the region for which spatial unit firing is specific. "Misplace units" (6/34) fired maximally

only when the animal was within the unit's field and was actively sniffing there. Some units did not fit into either cateogry. These were classified as "other". O'Keefe has identified his place units with Ranck's approach-consummate units, and his misplace units with Ranck's approach-consummate-mismatch units.

In addition to qualitative description of spatial unit firing, O'Keefe reported the effects upon spatial firing of several types of manipulation. These included changes in room lighting, rotation of the maze and substitution of arms, introduction of novel objects and smells into the fields of units and holding animals in the same space in the testing room but out of contact with the maze. O'Keefe's results were virtually the same as those found in the preliminary work to be described below. Such observations permit the following general description of the phenomenon to be made.

Spatial units. A spatial unit fires bursts of single or complex spikes (10-20/sec) whenever the rat enters the unit's field within the recording environment. Firing continues, although often at a somewhat reduced rate, as long as the rat remains within the field. In other parts of the recording environment, the spatial unit's firing rate is usually less than two spikes per second. Many spatial units seem to be entirely silent in non-field areas, except possibly when the animal is asleep. Spatial firing is remarkably independent of the animal's behavior within the unit's field. Occasionally, firing is specific to a particular orientation of the animal, so that certain movements seem to elicit higher firing rates. Yet the same movements do not produce higher firing rates in other parts of the environment or when they take the animal to some other orientation within the field. In some cases, maximum spatial firing seems to occur only when the rat sniffs vigorously at a certain part of the field. In most cases, however, spatial firing

occurs reliably whenever the rat is within the field, whether the rat is passing through, exploring, sitting quietly, eating or grooming.

Spatial firing is rarely affected by minor changes made within or around its field. More substantial alterations can influence spatial unit firing. The most commonly effective change involves insertion of a physical barrier which forces the rat to move through the region differently. In this laboratory, such alterations usually eliminate spatial firing for that unit in a reversible fashion. O'Keefe[65] has also reported shifts in the extent or location of spatial fields as a result of alterations in the environment. Similar examples have been found in this laboratory, but observations of them have been inconclusive. The size and extent of spatial fields vary; those of most spatial units are small relative to the entire recording space. This is not invariably the case. O'Keefe has reported spatial units with fields covering substantial portions of the recording space, as well as units with apparently more than one separate field. Instances have been found in this study resembling each of these types of spatial firing. On the basis of his observations, O'Keefe has characterized spatial unit firing as a multi-modal (but not strictly sensory) response to location in space which is largely independent of behavior and motivation.

In early studies of spatial firing, a significant lack was the omission of quantitative measures of firing rates. This omission was remedied in the study of Olton et al.[67] Rats were trained to explore an eight-arm radial maze in search of food pellets. During this behavior, while the rat's activity was filmed on video tape, recordings were made from moveable micro-electrodes in dorsal hippocampus. During subsequent analysis, the video-tape record was used to determine where a rat was in the maze at each moment. On the basis of this determination, a

computer calculated average firing rates for each arm of the maze. Thirty-one CS units were observed in this manner. Twenty-eight showed average firing rates on at least one arm that were at least three standard deviations above the overall rates in the maze. These units could be classified into three groups: those specific to a single arm, those specific to more than one arm (not necessarily adjacent) and those showing a significant decrease in firing rate on one arm. Other observations were consistent with those previously reported.

Discussion. Quantitative methods have been employed in the work to be reported here, as well as in the work of Olton et al.[67] These methods lend credibility to descriptions of spatial firing, especially for those who have not observed the phenomenon directly in the laboratory. Nevertheless, in their present form, such methods share a serious defect. When the testing environment is divided into sections for the purpose of counting spikes before the measurements are made, then the boundaries of each such section will rarely coincide with those of the fields of spatial units. When numerical values are calculated for firing rates within each section, they may not reflect the actual specificity of spatial unit firing. Another limitation is that, for any single passage of an animal through the section containing a unit's field, calculated firing rate will depend upon details of the animal's behavior. That is, it will depend upon the relative amounts of time spent within that section but either inside or outside the unit's field. This circumstance will introduce spurious variance into average firing rates. Furthermore, it will make analysis of changes in firing rate difficult to interpret.

A point should be emphasized here concerning the phenomenon of spatial firing and the viewpoint of this paper in particular. In the spatial-mapping theory, Nadel and O'Keefe have specifically postulated that the hippocampus recognizes

places per se. While all observations of "spatial" units are so far quite consistent with this interpretation, no one has yet demonstrated clearly that such units exhibit a place-response in the sophisticated sense of the mapping theory. It is still possible, for example, that spatial units respond in a specific fashion merely to certain behaviors or movements which, in any given case, are incidentally localized to one region in the recording environment. When observations are made of even a single spatial unit in a freely-moving animal over long periods of time and under a variety of circumstances, such a trivial explanation seems most unlikely. Nevertheless, such observation cannot ultimately exclude all alternative explanations. In the absence of a definitive test of this point, the term "spatial" must be applied to hippocampal firing only in a limited sense.

This necessity is emphasized by a brief consideration of the literature concerning unit activity. For example, Segal and Olds[39] studied changes in hippocampal unit responses to an auditory signal during a classical differential conditioning paradigm. In a large number of CA-3 units and in some CA-1 units, they found a significantly increased long-latency response over several hundred conditioning trials. The results certainly seem to implicate the hippocampus in learning. In the experiment, however, rats had a good deal of freedom of movement and virtually no behavioral observations were made during the training. Thus, it is quite difficult to rule out systematic changes in behavior as a basis for the changes in unit responses.

After making careful behavioral observations, Ranck[50] discovered a number of behavioral correlates for hippocampal firing. These correlates involved primarily approach and consummatory activities and correlates could be described for a majority (about 60%) of the units observed in the study. In contrast, other experimenters[65,67,68] have reported clear spatial correlates for nearly all units

observed in hippocampus. In a subsequent review,[103] Ranck has modified his initial analysis to include some spatial correlation; O'Keefe and Conway[83] and Olton et al.[67] have mentioned that some spatial units may have additional correlates involving location of actual or expected reinforcement. The significance of this history, however, is that after making a large number of observations of the same general phenomenon, very careful experimenters could initially formulate quite different descriptions of what they saw. In this case, the discrepancy is probably due to differences in experimental design. Ranck's observations were conducted in a relatively small, rectangular box in which spatial features were not prominent. The other experimenters employed environments with salient spatial character and observed behavior of clearly spatial nature. Therefore, the different conclusions could be merely the result of different methods of observation. On the other hand, they could indicate that hippocampal function itself differs in environments which elicit different types of behavior. In either event, it is likely that further refinements will occur in observational techniques and that these or other circumstances will necessitate reassessment of the behavioral correlates of hippocampal unit activity.

GENERAL METHODS

I. Apparatus

Rationale. The experiment involved two different training and testing environments: a shuttle box, in which rats were pretrained and a T-maze, in which rats were tested during the sensory deprivation and time-course studies. In order to show clearly the initial appearance of spatial firing, it seemed important to obtain, from the beginning, a fairly rapid and repetitive sampling of unit firing in all regions of the T-maze. During preliminary work, it was found that when they had not been pretrained in some type of shuttling behavior, rats explored the maze erratically and slowly, even when very hungry. Such rats often spent long periods without moving. Pretrained rats usually moved rapidly through the maze from the outset and learned to shuttle and even to alternate at better than chance levels within minutes.

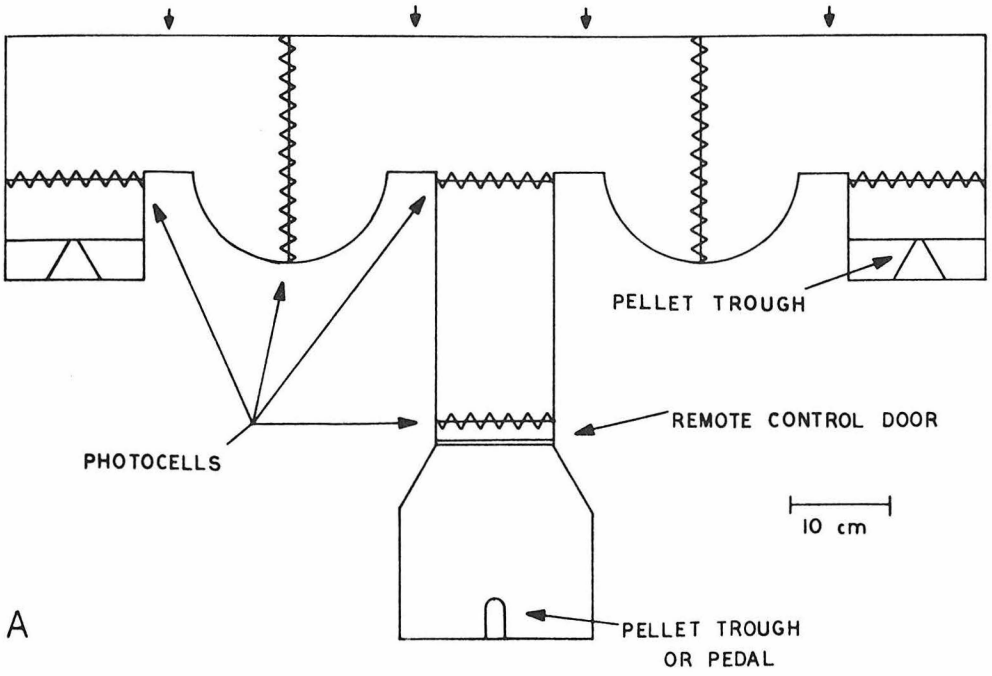
The shuttle box (Figure 3B) was rectangular, 20 cm high, constructed of clear plastic. The top was covered with clear plastic, with a hinged section at one end for access to the interior. The box rested upon a floor of thin metal rods with a spacing of about 1 cm between them. At one end of the box, a small metal pedal protruded from the end wall 3 cm above the floor. At the other end, a plastic trough received 45 mg food pellets from a dispenser mounted above it outside the box. A photocell beam was directed across the chamber at its midpoint 3 cm above the floor. The entire apparatus was housed in a light- and sound-attenuating chamber with dimensions 1.0 m x 1.2 m x 2.0 m. Illumination was provided by a 2.5 W incandescent lamp mounted 25 cm above the center of the box.

The T-maze (Figure 3A) was constructed of clear plastic, 25 cm high.

Figure 3

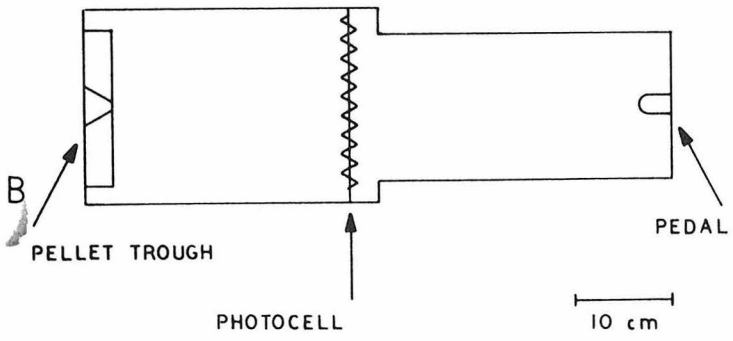
The shuttle box and T-maze

T-MAZE



A

SHUTTLE BOX



The top was covered with clear plastic, hinged at various points. The floor was a grid of metal rods. The left and right goal boxes contained plastic pellet troughs similar to that in the shuttle box. At the end of the central arm, the start box was furnished either with a pedal identical to the one in the shuttle box (for the sensory experiment) or with a plastic pellet trough somewhat different from that in the shuttle box (for the time-course experiment). Photocell beams were directed across the runways at the places indicated in the figure at a height of 3 cm above the floor. The maze was housed in a separate isolation chamber like that containing the shuttle box. Illumination was provided by a 2.5 W lamp over each of the arms and one over the choice point. The transverse runway of the maze was adjacent to a double glass window running along its length in the side of the isolation chamber. A pneumatically-operated guillotine door was located across the entrance to the start-box. This door could be operated quickly and quietly from outside the isolation chamber. The left and right arms could be blocked by manually-operated guillotine doors (at the points indicated by short arrows in Figure 6A). The running path could be changed by small hurdles and baffles inserted into the transverse runway.*

The shuttle box and T-maze were operated automatically by a system of 12-volt logic. The shuttle box system delivered a single food pellet when the pedal had been pressed for a preset interval. The pedal was reactivated for another trial when the rat broke the photocell beam in the middle of the box on his way

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*In the initial part of preliminary work, the T-maze had a simpler configuration than that shown in Figure 3. The older version can be found by consulting Figure 5.

to retrieve the pellet. In the T-maze, a rat could be required to press the pedal in the central arm (or to wait there for a food pellet if the pellet trough was in place) for a preset interval. The rat was then required to run to one of the goal boxes for further food reinforcement. The maze could be set to reinforce strict alternation (pellet delivered to one side only if the opposite side had been chosen previously) or to reward only one side. After each such trial, the rat could initiate the next only by returning to the start box.

II. Procedure

Recording: A 6-channel, single-sided multiplex telemetry system was used.[69] The transmitter is small (3 cm x 3 cm x 1 cm) and light weight (12 gm). While wearing the device, 300 gm rats could move virtually without restriction through the training apparatus. The six demultiplexed outputs from the telemetry receiver were fed in pairs into matched differential amplifiers. It was found that, with proper placement of common electrode in the surgical preparation, this procedure produced spike recordings of undiminished fidelity but virtually eliminated muscle artifact due to chew. The three differential amplifier outputs were recorded on magnetic tape, along with the combined outputs of the photocells and a voice-track description of the rat's behavior.

Training: Male Holtzman rats were used in the experiments. These weighed 275-325 gm at the time of surgery. For approximately one week prior to training, all rats were fed approximately 13 gm of food each morning and were maintained on a 12/12 reversed L/D cycle. Before training actually began, rats were food-deprived for one day. On subsequent days, rats were placed

individually in the shuttle box. In 3 hr daily sessions, each rat shaped itself to press the pedal at one end of the box and to retrieve a pellet at the other end. Training continued in this manner to a criterion of 200 trials at 5 sec delay in one session. Rats generally reached criterion in the shuttle box within 3-4 days. At this point in the time course experiment, rats were given food ad lib for 2 days prior to surgery. In the sensory deprivation experiment (except in the olfactory part), rats were trained further, now in the T-maze. In 3 hr daily sessions, each rat was allowed to shape itself to perform a strict alternation task in the T-maze, returning to the start box and pushing the pedal there between each trial to obtain further reinforcement. Training was concluded when rats reached a criterion of 200 trials at 5 sec delay in one session, with at least 75% correct responses. In all these cases, rats had been sensory-deprived before being introduced to the T-maze and during all pretraining there. For olfactory-deprived animals, the sensory deprivation occurred the day after surgery for implantation of recording electrodes. These animals were run, like the time-course animals, without pretraining in the T-maze.

Analysis: Recordings were first reviewed directly from the magnetic tape using a storage oscilloscope. Representative trials were then photographed on 35 mm film with a Grass kymograph camera for numerical analysis. The sample trials were chosen to cover all parts of the maze, with roughly the same number of left and right, correct and incorrect trials and with minimum artifact.

A close-spaced raster of horizontal lines could be superimposed on the data by turning on the scale illumination of the kymograph oscilloscope during filming. This scale was used to discriminate spikes. In all cases, a lower cut-off amplitude was chosen at least three times the amplitude of the background noise.

All spikes were counted which reached above this cut-off amplitude; no attempt was made to count complex-spikes as individual events. When spikes appeared which had distinctly different amplitudes, an arbitrary level was chosen by eye above the baseline cut-off. This level was used to assign spikes to one case or the other. Each trial was divided into segments using photocell traces filmed along with the data; these segments correspond to the six sections of the maze delineated by the photocell beams in Figure 3. The start box section was generally not scored and spatial firing occurring there is not included in the data. Within each segment, spikes were counted by eye and firing rates were calculated by dividing the number of spikes in a segment by its duration. Cumulative averages were then determined for each section by dividing total number of spikes in that section by total time spent there over all trials filmed.*

Criteria: Spatial firing was required to satisfy three conditions: 1) Spikes must resemble those associated with complex-spike cells, with a signal-to-noise ratio of at least 3 to 1; 2) As observed directly during the experiment, while the rat ran in the maze, the firing must show clear spatial character; 3) In the section (or sections) of the maze containing the field, the cumulative average firing rate must be at least three times that within any other section.

Figure 4 shows an example of spatial firing. The traces at the top represent passages of the rat from the start-box to a goal-box, one to the left and one

---

*In figures illustrating spatial fields, these cumulative averages are indicated to the nearest tenth of a spike per second, within the appropriate section of the maze. In a few cases, a section was scored separately for passages in different directions. These cases are denoted by arrows indicating the appropriate directions.

to the right. The smaller unit in the traces is a theta unit. The larger unit is a spatial unit. Its field is the narrow region indicated by cross-hatching in the central arm of the maze. In this case, the field is small compared to the size of the maze and spatial firing is specific and sharply delineated. Not all spatial firing shows this much specificity. Many examples have non-zero background firing rates in other parts of the maze.

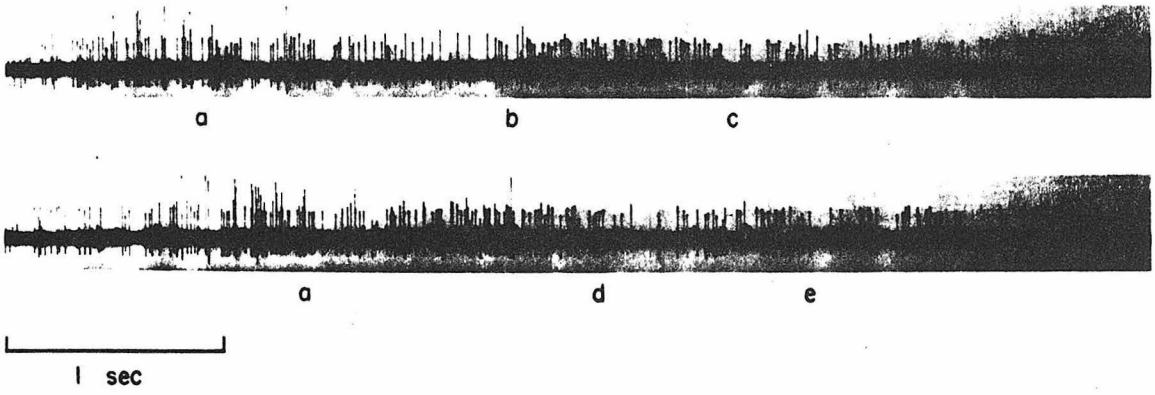
Surgery: Rats were anesthetized with Surital (thiamylal sodium, Parke-Davis, 45-60 mg/kg) and injected with atropine methyl-bromide (1 mg/kg, IP). Six wire micro-electrodes were implanted in either CA-1 or CA-3 of dorsal hippocampus (62.5 micron nichrome wire, enamel insulated with blunt, uninsulated tips prepared by cutting across with sharp surgical scissors). An uninsulated common electrode was implanted near the midline about 5 mm posterior to bregma to a depth of 4 mm below the surface of the skull. The wires were then led up through the pins of a plastic transistor socket and clamped into place by another transistor socket inserted into the first. The entire assembly was glued to small stainless-steel screws embedded in the rat's skull.

Anesthetic: Surital anesthetic was chosen for several reasons. Sodium pentobarbital was rejected because of the residual toxic effects it produces. These sometimes left animals incapacitated for 2 to 3 days following surgery. A shorter-acting anesthetic, ketamine (Vetalar, Parke-Davis) seemed to depress hippocampal unit activity too severely at anesthetic dosages. Surital produced satisfactory anesthesia in rats of 275-325 gm for 3 to 4 hours at dosages of 40-50 mg/kg. Residual effects were minimal. Surital's effect on hippocampal activity was intermediate to that of pentobarbital and that of ketamine. Background activity was markedly reduced. Spontaneous unit actity was depressed as well.

Figure 4

An example of a theta unit and a spatial unit recorded in a freely moving rat in the T-maze

Letters beneath each trace indicate the times when the rat's nose broke the photocell beams labeled similarly in the diagram of the T-maze. See p. 27 for explanation of numerical values.


a
b
c
a
d
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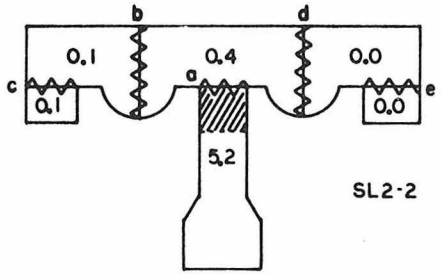
1 sec


b
d
0.1
0.4
0.0
c
a
e
0.1
0.0
5.2
SL2-2

However, many CS units seemed to be easily stimulated into firing brief bursts by the near approach of the electrode. In most cases, when electrode motion could be stopped immediately at this point, stable CS firing eventually appeared. Two problems were encountered in the use of Surital. There seemed to be only a small margin between anesthetic and lethal dosage. An overdose nearly always produced prompt and irreversible loss of all unit and background activity in hippocampus and overlying neocortex. This was followed eventually by death of the animal. Secondly, Surital did not keep well in solution, even when maintained at 5^{o}C. Within 5-7 days, solutions of Surital became cloudy. If used, these solutions produced symptoms of brain death similar to those of an overdose, even at below normal anesthetic dosages.

SENSORY STUDY

I. Background

Cues for spatial behavior. Several behavioral studies have been performed to find the most likely sensory basis for spatial behavior. These studies have provided neither consistent nor conclusive indications. In an investigation of the sensory basis for spontaneous alternation, Douglas[70] concluded that this is primarily vestibular, with perhaps a minor contribution from olfactory cues. Later investigations have been contradictory,[71] and one[72] has not ruled out the possibility of some kinaesthetic input. On the other hand, Steward et al.[73] have shown that rats could not learn to alternate turns in a (+) maze when approach arms were varied in random fashion. This result makes a kinaesthetic basis seem unlikely.

Hippocampal afferents. Anatomical evidence does not favor any particular sensory modality. There are two principal pathways into the hippocampus.[74,75] From midbrain and brain stem regions, fibers enter through the fimbria-fornix system. These include monoaminergic fibers (NA, DA and 5-HT) from brain-stem cell groups as well as cholinergic fibers from relay neurons located in medial septum.[76] Entorhinal cortex is the second major source of hippocampal afferents. From entorhinal cortex, fibers enter the white matter between cortex and subiculum. These fibers (the "perforant path") pass upward through subiculum to run transversely through subicular molecular layer into hippocampus. Some cross the hippocampal fissure to end in dentate molecular layer, while the rest continue into the molecular layer of hippocampus proper. In anatomical studies in the monkey,[77,78] it has been shown that fibers converge upon the entorhinal and perirhinal cortex from a wide range of cortical sensory association areas. These inputs

come from areas that subserve (at least) olfactory, visual, auditory and somaesthetic modalities. Thus, it seems likely that the hippocampus and fascia dentata can receive, through the perforant pathway, information from most, if not all, senses.

Unit recording studies. In a series of studies in the rabbit, Vinogradova[79] has reported significant responses to several intense sensory stimuli. Vinogradova does not provide precise information regarding the nature of the stimuli, nor does she report results of detailed behavioral observations. Thus, her data are somewhat difficult to interpret. This is particularly true in view of the results of Mays and Best.[80] These workers found a correlation between hippocampal responses to sensory stimuli and transitions from sleeping to waking in the rat. More compelling evidence comes from Green and Machne,[81] who found clear hippocampal unit responses to visual, auditory and vibrissal stimuli in curarized rabbits. Brown and Horn[82] found that in 60% of cat hippocampal units tested, a response could be elicited by external visual and auditory signals as well as by shocks to the optic nerve.

Finally, O'Keefe and Conway[83] have attempted to test directly the sensory basis of spatial firing in the rat. In their experiment, all but four very salient place cues were minimized in the environment around an elevated T-maze. Unit recordings from hippocampus showed that spatial firing was indeed dependent upon these cues, since it could be changed when some or all of them were removed. In some instances, firing could be changed by removing of only two cues; in others it could be changed only by removing all four. The cues were a light, a card, a fan, a buzzer as well as the presence or absence of reinforcement in the goal regions. Thus, it seems likely that spatial firing could be based, at least, on visual, auditory and perhaps tactile and reward-related cues.

II. Preliminary Study

Techniques. During the preliminary study, as well as during parts of the time course study, attempts were made to modify spatial firing in hippocampus as rats ran in the T-maze. This was done by changing various aspects of the maze around the unit's field. The manipulations included the following:

1) Thorough cleaning of all parts of a unit's field (soap and water, alcohol, steam).

2) Introduction of strong odors into the field region (ethyl alcohol, acetone, Aramis cologne, various foods).

3) Placing other rats into a unit's field for short periods in the absence of test animal.

4) Varying lighting levels and locations of light sources.

5) Placing a flexible, opaque mask over the rat's eyes.

6) Draping parts of the maze in black, white or striped cloth.

7) Placing a small speaker near the field region; the speaker emitted a series of sharp clicks.

8) Taping cardboard baffles along the roof of the maze on the inside, inserting doors or opening the top of the maze to change the acoustical properties.

9) Closing parts of the maze with doors or placing small hurdles or baffles in the rat's path near or within the field.

A final experiment tested the role of vestibular cues. A cylindrical start box, equipped with a rotating floor, was fitted to the T-maze. The floor could be spun by remote control at approximately 70 rpm. Four animals were habituated to being spun in the start box until they would re-enter it voluntarily. This was

done during shuttle (not alternation) training in the T-maze with one arm at a time blocked. The animals were then allowed to shape themselves to perform a strict spatial alternation in the entire maze, but were confined within the start box and spun for 30 seconds between each trial. The procedure was apparently able to disrupt vestibular inputs, since it occassionally caused rats to stagger and veer as they left the start box. Nevertheless, the rats learned to alternate as successfully and quickly as rats which were not spun. Furthermore, spinning seemed to have no effect on the spatial firing observed in these animals, although in two cases the fields were regions within the start box.

Numerical analysis was not used in the preliminary study. Instead, spatial firing was evaluated by eye, using a storage oscilloscope and the audio monitor. Firing was considered to be gone after a given manipulation if it could not be discerned in the oscilloscope traces and could not be detected in the audio for ten successive trials following the change in the environment.

Results. Table 1 contains the results obtained for all rats in the preliminary study. By far the most common finding was that a given manipulation did not affect spatial firing. When an effect could be found, it usually involved disappearance of spatial firing altogether. Such changes seemed to occur immediately; that is, firing was absent from the first time the rat re-entered the field. Changes in firing could usually be reversed by reversing the alterations in the environment. If this procedure was carried out repeatedly, however, firing often either disappeared entirely or became relatively insensitive to the alteration being made.

In several instances, spatial firing was affected by purely visual changes. One case involved changes in lighting as well as the placing of objects around

Table 1

Results of manipulations on spatial firing - preliminary study

Firing	Field	Visual	Auditory	Olfactory	Barrier	Spin
Lg-4	Choice point (return only)	+	-	-	+	
L9-2	Choice point	-	-	-	+	
L11-2	Right of CP	-	-	-	+	
A2-2[a]	Right arm	-	-	-	+	
A2-5	Right arm	-	-	-	+	
A5-4	Right arm and CP	+	-	-	-	
P2-3	Left of CP	-	-	-	+	
N1-1	Choice point (return only)	-	-	-	-	-
N2-4	Start box	-	-	-		-
N2-10	Right arm	-	-	-	-	-
N3-10	Start box	-	-	-		-
N4-2	Left arm	+	-	-	-	-

[a]Fields of A2-2 and A2-5 were not the same and their firing was influenced by hurdles in different positions.

A plus (+) indicates that firing was eliminated by the change in the environment. A minus (-) indicates that no substantial alteration in spatial firing occurred.

the field region outside the maze. The second involved applying or removing the rubber mask. The third was affected by placing a striped cloth over the maze. No clear example was found of spatial firing eliminated by purely auditory stimuli, although one case was found during the experiment itself which may suggest an acoustical effect. The field of SL1-6 was well out in the right arm of the maze. In this case, when the left arm was blocked at the choice point, spatial firing ceased. The effect occurred in spite of the facts that the rat could not see and that firing was normally maximum when the rat was proceeding outward, away from the left arm.

Surprisingly, in no case was spatial firing clearly influenced by purely olfactory cues. Spatial firing was uniformly unaffected by thorough cleaning of the maze. Obtrusive odors occasionally prevented a rat from entering a region for some time, but did not seem to affect spatial firing there when an animal finally did re-enter the region. On the other hand, a possible olfactory effect has been found during subsequent work. In this case, the test animal was removed from the maze for a short time and another male rat was allowed to run about there. When the test animal was returned, spatial firing had changed. Firing no longer occurred in the right arm, as it had initially, but occurred in the left arm. Although obvious cues, such as fecal boli from the interpolated rat, had been removed before the test rat was returned, non-olfactory cues cannot be ruled out absolutely. It may be significant that, in this case, the interpolated rat had been at one time a cage-mate of the test animal. In most other cases tested in this manner, the interpolated rat was from another group and was often a female.

By far the most frequently effective manipulations were those involving the insertion of doors, hurdles or baffles into the rat's running path. One of the more reliable examples is given in Figure 5. The hurdle was a rectangular obstacle made of white plastic; this blocked the entire width of the passage and was 10 cm high.

Discussion. It is difficult to interpret clearly the observations described above. When they are specific to only one sensory modality, environmental alterations rarely affect spatial firing. When they are effective, alterations usually differ in detail from one case to another. As a result, the preliminary study soon involved an unmanageably large repetoire of manipulations and their variants to be tested for each rat. Under such circumstances, a negative result would not be very meaningful for any given manipulation. Some changes influence spatial firing fairly reliably; but these changes involve the insertion of physical obstacles and certainly present a broad spectrum of sensory cues to the animal. Taken altogether, the observations suggest that spatial units do not, in general, respond to a single sensory modality and that, in any given case, spatial firing may be a response to a large number of different cues characteristic of the field.

III. Experiment

Rationale. To test the sensory basis of spatial firing in hippocampus more economically, it was decided to approach the question in another way. If spatial firing can be observed in animals which have been selectively deprived of all input in a given sensory modality, then cues of that type must not be essential for the phenomenon in general. This conclusion might not be very surprising for a sense such as hearing. For another, such as olfaction, it could be significant.

Figure 5

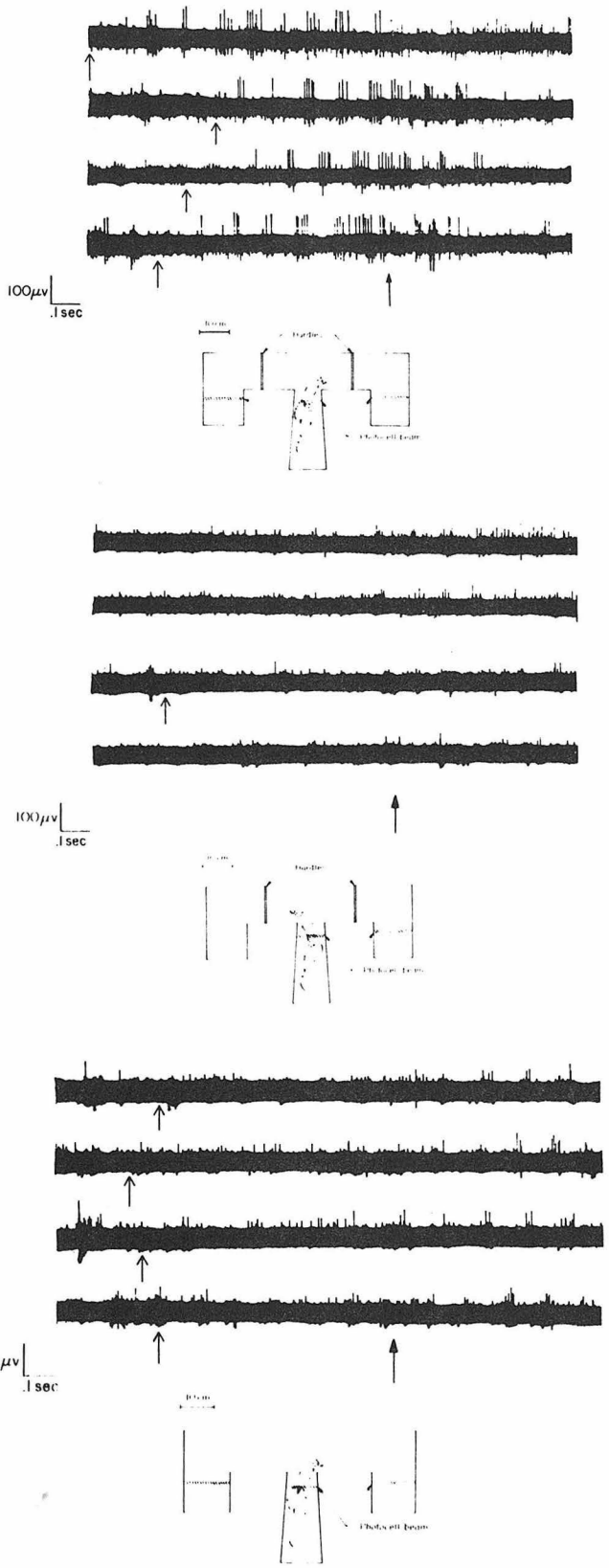
Illustration of spatial firing affected by change in testing environment

TOP: Rat turns to right and surmounts hurdle.

MIDDLE: Rat turns to right with hurdle removed.

BOTTOM: Rat turns to left and surmounts hurdle.

Large arrows indicate times at which the rat's nose broke the center arm photocell beam. Small arrow indicates the times when the rat cleared the center arm photocell (traces adjusted to put these times in register).


100μv
.1 sec
100μv
.1 sec
100μv
.1 sec

In this experiment, 4 modalities were selectively interrupted: sight, hearing, vibrissal input and olfaction. The experiment was initially intended to compare the frequency of occurrence of spatial firing between animals deprived of different senses. Such a study might indicate the relative importance of each modality. This purpose was abandoned for the following reasons: 1) In all animals used in these experiments, the yield of spatial firing has been roughly comparable, one or two cases from each animal implanted with 6-8 electrodes. This fact indicates that, at most, small differences exist between occurrence of spatial firing in differently sensory-deprived animals. To demonstrate such differences, if they exist at all, would require a very large number of animals. 2) Several sources of bias would have made results suspect in any event. Surgical technique improved somewhat over the course of the experiment, but it would have been difficult to quantify the extent or effect of this improvement. In at least some cases (i.e., blind and deaf rats), sensory deprivation had to be done well ahead of the time of implanting electrodes. This necessity introduced the possibility of selection effects during surgery, as well as that of experimenter bias. Finally, sensory deprivation produced variable behavioral effects. Deaf rats could not use the sound of the pellet dispensers as a secondary reinforcer or time cue. These animals required more extensive training than others and did not always perform as well in the maze when delays were required in the start box. Vibrissal-deprived animals sometimes behaved erratically, appearing confused and apprehensive. Two such animals could not be trained at all. Thus, the four sensory-deprived groups were not equivalent. In terms of the hypothesis being tested, it seemed possible that in some animals the disruption of behavior might be due to a failure of the very function being examined.

In view of these limitations, a more modest aim was adopted, to discover whether or not spatial firing was at all possible in each type of sensory-deprived animal. For this demonstration, the criterion was taken to be identification of at least two instances of spatial firing in at least two different animals for each modality.

Methods

1) Vision: Visual input was eliminated in either of two ways. In the first method, rats were fitted with a close-fitting silicon-rubber mask (Figure 6). The mask completely covered both eyes but did not extend over the rat's ears or vibrissae. Shallow depressions were molded into the mask's inner surface so that it did not bear directly on the rat's eyes. The edges of the mask were sanded thin to avoid pressure points there. The mask was screwed into place against a threaded teflon adapter glued with the telemetry connector onto the rat's head and could be applied or removed within about ten seconds. Rats habituated rather quickly to the mask, even when it was put in place for the first time. Overt signs of irritation, such as frequent scratching or grooming, usually disappeared within one or two minutes. When the mask was applied properly, the rat's eyes could not be seen from the outside under the closest scrutiny. Nevertheless, although the mask itself was quite opaque, the possibility could not be entirely discounted that traces of light leaked around its edges. For this reason, two rats were permanently blinded.

In this method, a rat was anesthetized with Surital (see Surgery Methods) and mounted in a stereotaxic apparatus. The rat's eyelids were retracted with hemostats and the eyes dissected out with forceps and surgical scissors. Bleeding was controlled by swabbing the eye socket with adrenaline chloride. The wounds

Figure 6

The rubber mask

were cleaned and packed with topical antibiotic (Neosporin, Borroughs Welcome Co.) but were otherwise left undressed. Rats were injected with penicillin and allowed to recover. In all cases, rats tolerated this procedure remarkably well and seemed fully recovered within 3-4 days after surgery.

2) Vibrissae: Vibrissal input was eliminated simply by shaving the rat's vibrissae off close to the skin with electric clippers. The procedure was repeated every third day in order to maintain the visible length of vibrissae well below 1 mm. No assay of the method was undertaken.

3) Audition: Neomycin, kanamycin and other aminoglycosidic antibiotics can cause permanent loss of hearing when administered chronically and in sufficient dosage.[84] The effect seems to involve suppression of membrane potential changes in the chochlea, where these antibiotics are selectively accumulated.[85] Recent work[86] indicates that neomycin interferes with the metabolism in cochlear membranes of phosphoinositide. This phospholipid component has been implicated in controlling membrane permeability there.[87]

In this study, six rats were given daily injections of neomycin sulfate (200 mg/kg subcutaneous) for a period of 60 days. A control rat was given equivalent daily injections of sterile saline. During the drug treatment, one animal began to lose weight consistently and was discontinued. Another rat died abruptly after the second day of treatment. The remaining rats gained weight at an apparently normal rate (neomycin rats: 1.2 gm/day; control rat: 1.4 gm/day) and showed no unusual physiological or behavioral changes.

At the end of the drug treatment period, all animals were tested for hearing loss. They were then placed on ad lib food and water for several days

until training sessions began. After the completion of the recording phase of the experiment, each animal was again tested for hearing loss. Hearing tests were conducted as follows: 1) Rats were tested for the presence of a Preyer reflex (pinna twitch)[89] in response to two types of auditory stimuli. A metal cricket was held 3 to 5 cm above and behind the rat's head and snapped at irregular intervals. A Galton's whistle, tuned to around 10 KHz, was held 10 cm above and behind the rat's head (pointed away from the rat) and sounded at irregular intervals. The Preyer reflex was assayed first by direct observation and then by holding the rat close to a small light source and observing its shadow projected onto a grid pattern about 1 meter away. Finally, the possibility was tested that neuromuscular pathology might have suppressed the Preyer reflex. This was done by inserting a stiff hair (actually a rat's whisker) into the rat's outer ear. 2) Rats were tested for an acoustic startle reaction[90] in response to the cricket and whistle. Each rat was placed within an opaque cylindrical enclosure, which was then covered with a clear plastic lid. The rat was allowed to remain quietly within this chamber for 20 minutes. At the end of this time, the rat was tested for a visible startle reaction to either the cricket or the Galton's whistle when these were sounded just outside the chamber. Finally, rats were tested for startle reactions to air-puffs from a plastic syringe aimed through a hole in the cylinder and to sharp raps on the table on which the cylinder rested. After each stimulus, rats were left undisturbed in the cylinder for 20 minutes.

A rat was considered to be deaf if, in both pre- and post-experimental testing sessions, it displayed neither Preyer reflex nor visible startle reaction to purely auditory stimuli, but <u>did</u> give an ear twitch response to mechanical stimulation and displayed one clear startle reaction to air-jet or vibration.

4) Olfaction: Intranasally applied $ZnSO_4$ produces acute peripheral anosmia in a number of animals, including the rat[91,92,93] (see Alberts,[88] for a review of early work). For example, 3 weeks after $ZnSO_4$ treatment in the mouse,[93] the olfactory eipthelium is completely disrupted and contains no identifiable olfactory receptors. In addition, degenerative changes have been found in the olfactory bulb after $ZnSO_4$ treatment. These include a complete absence of olfactory nerve fiber inputs and apparent atrophy of the glomeruli. Behavioral effects include inability to locate buried food pellets[94] (the most common assay), deficits in feeding behavior in rat pups,[95] as well as various symptoms of short-term systemic toxicity.[91]

The simplest and most common method was used here for perfusing rats with $ZnSO_4$. On the day after electrode implantation, each rat was anesthetized with ether and suspended head-downward from a ring stand. A small diameter plastic tube was inserted well up into one nostril until a rubber cement cuff pressed against the external nare. About 3 cc of 5% (0.32 M) $ZnSO_4$ was slowly infused through the tubing. Most of the Zn solution drained out through the other nostril. If more than a few drops drained through the animal's mouth, the speed of injection was decreased. The same procedure was followed through the other nostril. The rat was allowed to hang upside-down until it had recovered consciousness and was then returned to its home cage.

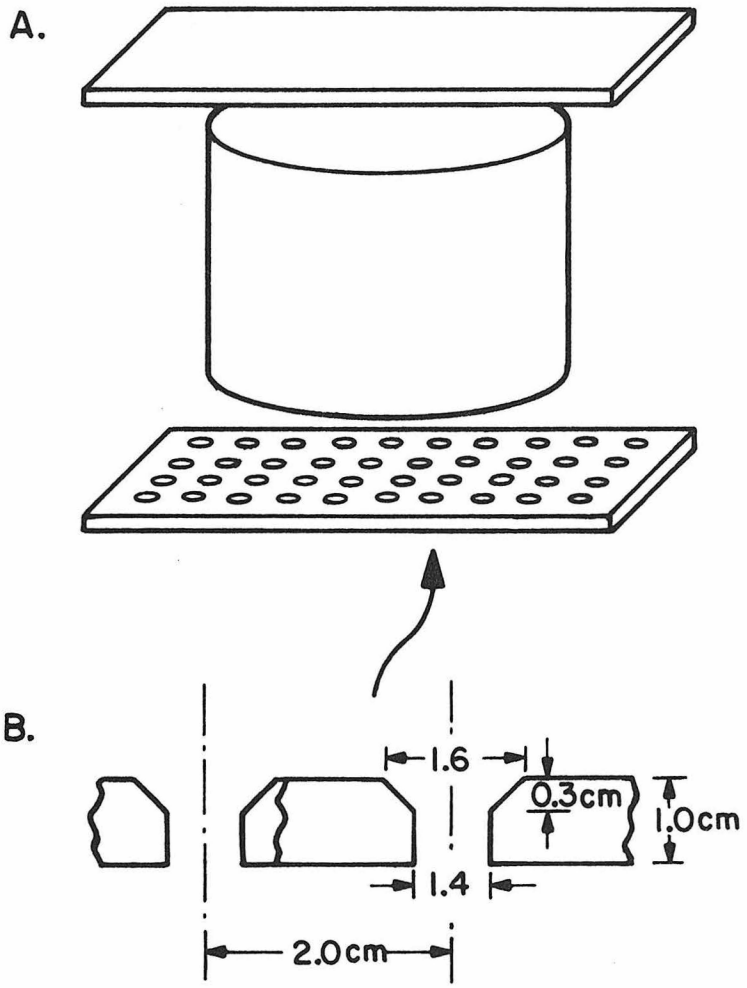
After one to three days of recovery, each rat was tested for olfaction. The testing apparatus (Figure 7A) consisted of a cylindrical chamber resting on a separate plate of clear plexiglass 1.5 cm thick. The floor plate was pierced by an array of regularly spaced holes (Figure 7B). The holes were shaped so that a rat could, with moderate effort, recover a 45 mg Noyes food pellet from the

Figure 7

Apparatus for olfactory test

(A) Chamber and floor plate.

(B) Detail: Cross-section of floor plate showing holes.


A.
B.
1.6
0.3cm
1.0cm
1.4
2.0cm

bottom of a hole once the pellet had been located. It seemed unlikely that pellets could be located by any means other than smell or vision. During the olfactory tests, observations supported this assumption. In fact, several tests indicated that rats could not (or, at least, did not) use vision to locate pellets at the bottom of holes, even when vision was not impaired. In addition to the deep holes, two shallow depressions were drilled into the plate near the center to a depth of about 2 mm. When placed in one of these depressions, a food pellet protruded slightly above the surface of the plate.

For the test, the grid floor plate was placed upon a clean, flat surface and the cylindrical wall was placed upon the plate. A single food pellet was placed at the bottom of each of 5 holes randomly spaced around the floor (although no pellet was placed in a hole adjacent to or partly covered by the wall). In addition, a pellet was placed in each of the two shallow depressions. Twenty minutes before testing began, each rat was blindfolded with the rubber mask described under Methods and was replaced in its home cage. At the end of this time, the rat was removed from its cage and placed into the test chamber. The chamber was then covered with a clear plastic lid. At the end of five minutes, if the rat had not found all five deep pellets, it was removed from the chamber. The grid floor was lifted, leaving the remaining pellets in place. The rat was returned to the chamber and allowed another five minutes to discover the remaining pellets. This test and (if necessary) the control were repeated at least three times in each session. Between each test, the rat was returned to its home cage and the grid floor and the surface on which it rested were cleaned with soap and water and swabbed with ethyl alcohol. At least two such sessions were administered to each rat, one given before the recording day and one after. A rat was considered to

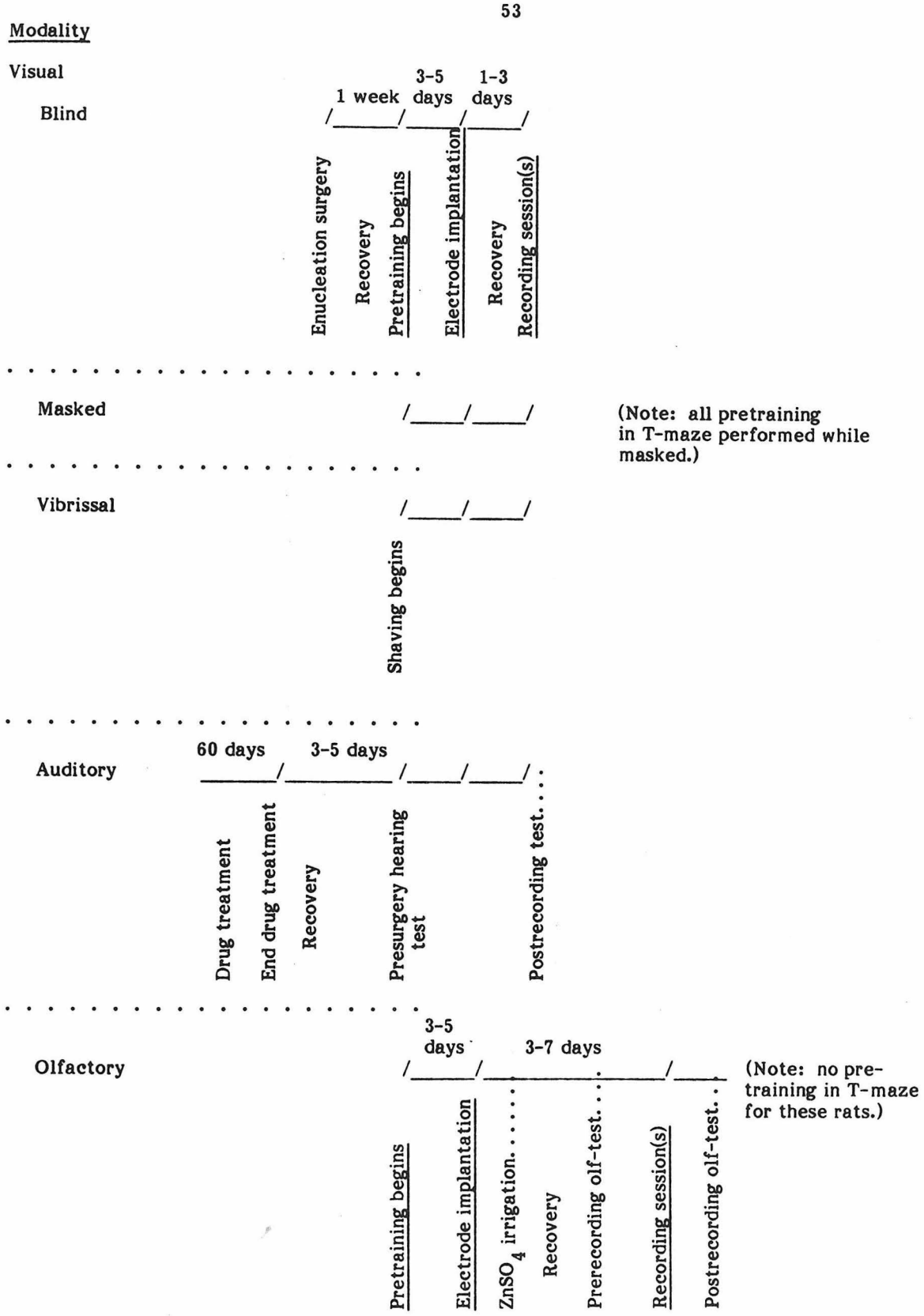
be anosmic only if, during each test, it recovered both shallow pellets but none of the five deep pellets and if it recovered all of the pellets during the subsequent control period.

5) Schedules and methods of observation: Schedules are given in Figure 8 for the preparation and testing of sensory-deprived animals. In all except the olfactory-deprived group, animals were pretrained thoroughly to alternate in the T-maze before the day of recording. For the olfactory-deprived animals, testing procedures were the same as those described below in the time course experiment. On the recording day, the telemetry was connected to the test animal and it was placed into the T-maze and allowed to perform the alternation task. Observations were conducted entirely with the lights in the experimental room turned off. The experimenter sat next to the isolation chamber and observed the rat's behavior. Hippocampal spike activity was monitored using an auditory monitor and storage oscilloscope. During recording sessions, a continuous recording was made on magnetic tape of a verbal description of the rat's behavior. Events were tallied on a check-off sheet with time markings. When spatial firing was observed, its field was mapped and it was characterized on the basis of spike height, waveform and pattern of firing. When possible, each rat was confined to the region of the maze where spatial firing occurred. Observations were made of the field's extent while the rat explored the smaller space. Finally, as in the preliminary study, tests were made for the effects on spatial firing of various changes in the environment.

Between tests of each animal, the T-maze and grid floor were cleaned with soap and water and swabbed with 95% ethyl alcohol. This step was taken as a precaution in spite of the observation that neither thorough cleaning nor introduction of strong odors has appreciable effect on spatial firing.

Figure 8

Schedules for rats in the sensory deprivation experiment


Modality
Visual
Blind
1 week
3-5 days
1-3 days
Enucleation surgery
Recovery
Pretraining begins
Electrode implantation
Recovery
Recording session(s)
Masked
(Note: all pretraining in T-maze performed while masked.)
Vibrissal
Shaving begins
Auditory
60 days
3-5 days
Drug treatment
End drug treatment
Recovery
Presurgery hearing test
Postrecording test. . . .
Olfactory
3-5 days
3-7 days
Pretraining begins
Electrode implantation
$ZnSO_4$ irrigation. . . . .
Recovery
Prerecording olf-test. . . .
Recording session(s)
Postrecording olf-test. . .
(Note: no pre-training in T-maze for these rats.)

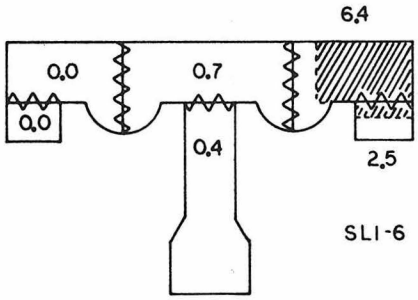
Results

1) Vision: No visual test was performed on masked or blinded animals. Masked rats can perceive at most very general information about ambient light levels. Enucleated rats can receive no visual information at all. Spatial firing was found in two non-visual animals, one (SL-1) masked and the other (SL-2) blinded. Results are summarized in the drawings in Figure 9. It was noticed that sightless rats generally moved confidentially and surely through the maze, rarely bumping into walls or hooking the telemetry on corners of the maze.

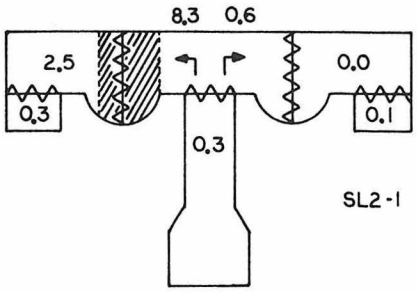
2) Vibrassae: No test was performed of the effectiveness of shaving the vibrissae. Animals probably receive sensations from their vibrissae even after these have been shaved off flush with the skin. Nevertheless, it is unlikely that such animals can gain any meaningful information about the environment in this fashion. Of the four vibrissal animals prepared, two were dropped from the study because of poor behavior. One rat would not move from the outset and was discarded after two days of inactivity in the shuttle box. The other rat performed so erratically in the T-maze that the data were useless. Four examples of spatial firing were found in the remaining two rats (DW-1 and DW-2). These are illustrated in the drawings in Figure 10. The two vibrissal-deprived animals which could be run successfully did not display the same symptoms of disorientation observed in the other two rats. DW-1 and DW-2 tended to nudge the walls of the maze as they moved through it and often hooked the telemetry on corners as they turned or pivoted; nevertheless, they moved confidentially through the apparatus and seemed to show normal levels of activity.

Figure 9

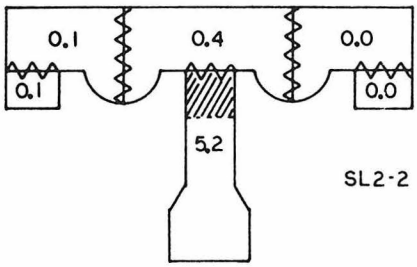
Fields of spatial firing found in non-visual animals



SL1-6



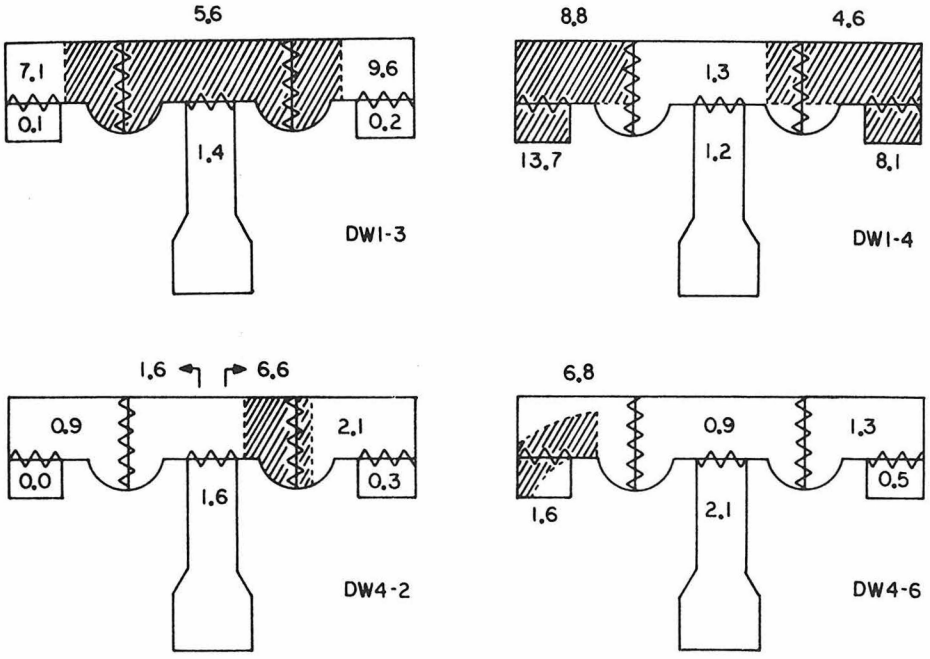
SL2-1



SL2-2

Figure 10

Fields of spatial firing found in vibrissal-deprived animals


5.6
7.1
9.6
0.1
0.2
1.4
DW1-3
8.8
4.6
1.3
13.7
1.2
8.1
DW1-4
1.6
6.6
0.9
2.1
0.0
0.3
1.6
DW4-2
6.8
0.9
1.3
0.5
1.6
2.1
DW4-6

3) Auditory: Four neomycin-treated animals survived the drug administration period. Of these rats, three satisfied the requirements for the presurgery hearing test; the fourth showed a small, but noticeable Preyer reflex and was discarded. One rat died during surgery. Spatial firing was found in each of the remaining rats (ND-1 and ND-5). These examples are diagrammed in Figure 11. Both rats also satisfied the requirements for deafness in the post-experimental hearing test. A single obvious difference was noticed between the neomycin-treated rats and normal rats. Apparently, neomycin-treated rats could not hear the sound of the pellet dispensers in the shuttle box and T-maze. For this reason they were slower to learn the behavioral tasks in each apparatus. Once proficient, however, they seemed to behave as well as normal rats when no delay was required in the start-box.

4) Olfaction: Three animals were subjected to the $ZnSO_4$ treatment. One did not pass the pre-recording olfactory test, since it located several pellets during each test. The other two animals (A-1 and A-2) satisfied the olfactory test for anosmia in both pre- and post-recording sessions. Table 2 summarizes test results for these rats and for three control animals (not treated with $ZnSO_4$ but implanted with hippocampal recording electrodes). Spatial firing was identified on three probes in the anosmic animals. These examples are illustrated in Figure 12. Rats showed definite signs of toxicity on the day following $ZnSO_4$ treatment and, to some extent, for several days after. Symptoms included lack of appetite, listlessness, and a persistent hunched posture. When these symptoms had largely disappeared, a characteristic behavior was noticed which was lacking in untreated animals. $ZnSO_4$ animals paused quite often and groomed their muzzles and noses extensively. Such grooming behavior frequently followed periods of vigorous

Figure 11

Fields of spatial firing found in auditory animals

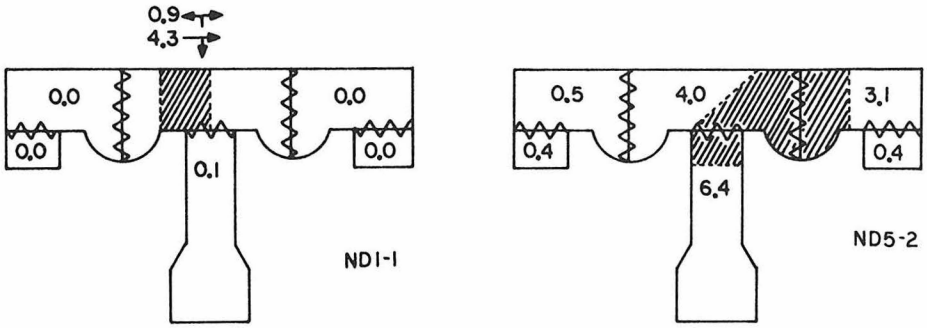

0.9
4.3
0.0
0.0
0.0
0.0
0.1
ND1-1
0.5
4.0
3.1
0.4
0.4
6.4
ND5-2

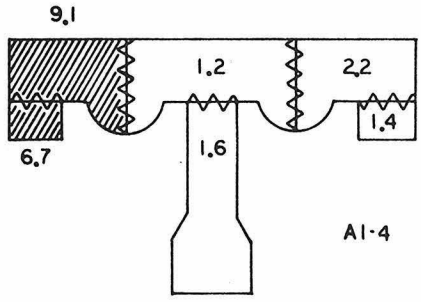
Table 2

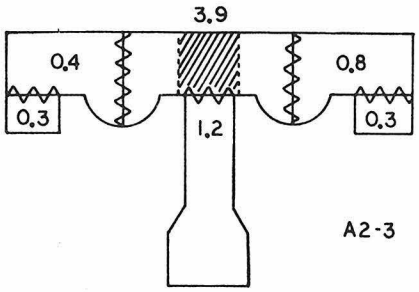
Results of olfactory tests

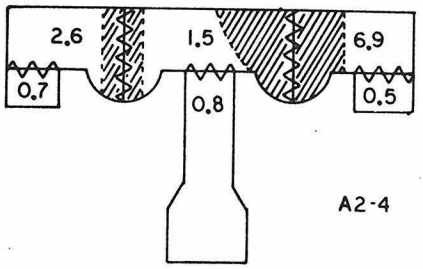
Rat	Time of session	# Tests	Average time to recover 5 pellets	
			Test period	Control period
Controls (N = 3)	———	12	1 min 43 sec	---
A1	Pre-recording	5	None recovered	0 min 38 sec
	Post-recording	3	None recovered	0 min 44 sec
A2	Pre-recording	3	None recovered	1 min 41 sec
	Post-recording	5	None recovered	0 min 16 sec

Figure 12

Fields of spatial firing found in $ZnSO_4$-treated animals


9.1
1.2
2.2
1.4
6.7
1.6
A1-4


3.9
0.4
0.8
0.3
0.3
1.2
A2-3


2.6
1.5
6.9
0.7
0.5
0.8
A2-4

sniffing, although the sniffing itself and the frequency with which it occurred were unremarkable.

IV. Discussion

Several studies have documented the physiological effects of ototoxic drugs upon the chochlea. During chronic administration, these drugs depress the microphonic response of the cochlea recorded from the round window and cause degenerative changes in the organ of Corti.[96] Several behavioral methods exist for testing hearing loss.[89,90,97] This experiment employs the two most commonly used and simplest tests. It seems likely that the Preyer reflex test is a minimal test. In a more complicated behavioral assay of hearing loss,[97] simultaneous observations were made of auditory discrimination performance and Preyer reflex. The Preyer reflex showed an elevation of its threshold that either paralleled or followed after reduction in discrimination performance. The acoustic startle response test was added because it was simple to perform and offered a behavioral assay perhaps more directly related to the experiment. It should be noted, however, that Harpur[90] did not find significant differences in acoustic startle response between neomycin-treated (35 days, 100 mg/kg/day) rats and normal rats. In addition, he found a pronounced and long-lasting habituation of the response. It seems clear that in testing hearing loss, the most reliable behavioral indication is elimination of the Preyer reflex.

A further caution should be mentioned regarding the test of hearing. Hearing loss cannot be unequivocally established by tests which document merely those sounds to which an animal does not react in certain ways. It would be difficult to cover all possible ranges and types of sounds audible to rats, such as very

high frequency sounds. On the other hand, the metal cricket used here can be expected to have produced many very high frequencies. Furthermore, recordings of cochlear potentials indicate that ototoxic effects occur preferentially for higher frequencies.[96]

If rats passed both the acoustic startle and Preyer reflex tests, they behaved in all other circumstances as though they could not hear. These animals did not appear to react to loud noises of any kind, so long as care was taken that some other stimulus did not reach the animal, such as vibration or air current produced along with the sound. In the experimental apparatus, these animals could learn to pedal press for delivery of a pellet and could, with some difficulty, learn to do this for relatively long intervals (5-10 seconds). However, on the latter task, their performance was always erratic. They often continued to press the pedal long after the pellet dispenser had fired. This error was rare in normal rats. It could be greatly reduced in the neomycin-treated rats by the addition of a solenoid which struck the grid floor at the same time the pellet dispenser fired.

Olfactory tests have generally employed food location as the tested behavior.[88,94] This assay was modified somewhat for use in this experiment. The new method should be an improvement over previous versions in that its controls for general activity and motivation are more convincing. Such improvement seemed necessary in view of the toxic effects produced by the treatment and their effect on activity levels. The test still cannot be regarded as conclusive. It is likely that other odors can be more easily detected by rats than can food, among them perhaps pheremones laid down by the test animals themselves.[104] This possibility is emphasized by the incident mentioned in the Results of a change in spatial firing after intrusion of another rat.

The reliability of the food location test was supported by observations made during the experiment. In order to retrieve a food pellet from one of the feeding troughs in the maze, a rat had to rear, arch its neck and push its nose down into the trough. Normal rats quickly learned to ascertain the presence of a pellet merely by sniffing briefly at the trough and did not rear at all when no pellet was there. $ZnSO_4$-treated rats tended to rear as though recovering a pellet on every visit to a feeder, whether a pellet was actually there or not. It is likely that these animals were using tactile, rather than olfactory cues to find the food.

No assay was performed of the effectiveness of the visual or vibrissal deprivation techniques. It seemed safe to assume that the blinded animals, in particular, could not be identifying locations in the apparatus visually and that the shaved animals could not be doing so by means of their vibrissae.

The sensory deprivation experiment provides additional indirect evidence that spatial firing in hippocampus of rats is a multi-modal response and that, in any given instance, no single type of cue is absolutely essential. For at least two modalities, vision and vibrissae, it has been shown that spatial firing can occur in animals totally deprived of meaningful cues of each type. For the other two modalities tested, hearing and olfaction, the evidence supports, but does not firmly establish the same conclusion.

TIME COURSE STUDY

I. Rationale

Lesion studies have demonstrated that in the rat dorsal hippocampus is involved in spatial behavior.[2] There is, however, no direct evidence implicating spatial firing itself in this function. An attractive possibility is that such firing is the substrate within hippocampus for a "map" that is involved in guiding the rat's spatial behavior.[24] To test this possibility, it may be useful to examine the time course with which spatial firing develops in a place to which the rat has not previously been exposed. If it does form a functional basis for spatial behavior, then spatial firing should appear at least as quickly as spatial behavior itself. Preliminary observations have indicated that spatial firing may often appear within a few minutes after rats first encounter a new environment or a new aspect of a familiar environment. The following study was undertaken to investigate this characteristic more rigorously.

II. Experiment

Method. Apparatus and methods were essentially the same as those described previously for the sensory-deprivation experiment. The following procedural differences distinguished the time course study. After rats had reached criterion in the shuttle-box, they were given food ad lib for two days. Recording micro-electrodes were then implanted. On the second day following surgery, rats were again allowed to run in the shuttle-box for one or two sessions until criterion had once more been reached. On these days, a plastic dummy telemetry was attached to the connector on the rat's head. On the day after a rat had again

reached criterion, the functional telemetry was connected to the rat and the rat was placed in the shuttle-box. Observations were conducted as described for the sensory-deprivation experiment. The rat was then transferred without delay from the shuttle-box to the start-box of the T-maze. With the door to the start-box closed, several pellets were delivered to the pellet trough there. The rat was allowed two minutes to consume the food and to recover from any excitement caused by handling. At the end of this time, the tape recorder was started and the door to the start-box was opened. The rat was allowed to explore the T-maze and to shape itself to shuttle and alternate as required. Continuous recordings were made of hippocampal spike activity and of the rat's behavior for 100 trials or for one hour. At the end of this time, the rat was screened for spatial firing and the effects of various manipulations were tested.

Analysis. Data were analyzed using essentially the same methods as in the sensory-deprivation experiment. For each case of spatial firing found in the T-maze, data were transferred to 35 mm film for the trials containing the first two or three consecutive passages of the rat through the field. In cases for which spatial firing did not clearly occur during these initial exposures, successive trials were filmed until spatial firing did occur. Trials were then filmed at intervals throughout the experimental run to establish approximate steady-state values.

Results. Criteria were the same as those for the sensory-deprivation experiment. Thirteen rats were run successfully in the time course experiment. (One of these was an anosmic rat from the sensory-deprivation group.) From these rats, recordings were obtained of 18 instances of hippocampal firing which satisfied the first two criteria. In 15 of these cases, condition 3 was also satisfied. Results

are presented in Table 3. In 11 out of 15 cases, apparently fully developed spatial firing occurred on the very first passage of the rat through the relevant part of the maze. Figure 13 summarizes the calculated firing rates for these 11 cases for the first and second passages through their fields. For this figure, rates in each case were normalized, so that the cumulative average firing rate within the section(s) containing the field was 1.0. Normalized rates were then averaged over all 11 cases for the first and second passages. This was done separately for the sections containing the fields (open circles) and for all other sections combined (filled circles) to give an indication of background activity.

Figure 14 shows diagrams of the fields of all spatial firing in the time course study, together with graphs of the calculated firing rates during the course of each recording session. The field diagrams have been described above. In the graphs, the vertical axis (NFR) is the normalized rate in either the section containing the field or all other sections.

Figure 15 shows a particularly clear example of first-time spatial firing. In this case, the field was a region near the right corner of the choice point. During the initial trial, the rat (J11) moved out of the start-box (a), reached the choice point (b), and turned promptly to the right. Passing through the right arm photocell beam (c) to the goal-box (d), the rat retrieved and consumed a food pellet, turned (e) and retraced its path to the start-box. Spatial firing occurred as the rat rounded the corner of the choice point on the way out and on the way back to the start-box. Rat J11 traversed the maze with unusual celerity during the initial trials of the recording session. In addition, there was little other spike activity on probe J11-5, so that in this case it is easy to see the initial appearance of spatial firing.

Table 3

Spatial firing reported in time course study

Example[a]	Cumulative average field rate	Cumulative average background rate	Spatial firing 1st passage	Field rate 1st Passage	Field rate 2nd Passage
J-1-4[b]					
prompt	4.2	0.7	+	3.6	4.4
delayed	8.5		-	0.3	0.0
J-1-4[c]	10.0	2.3	+	10.5	8.2
J-2-3	7.8	1.5	+	6.3	5.3
J-3-4	6.6	1.5	-	0.3	1.2
J-4-1	4.5	0.9	+	4.3	5.3
J-4-5	4.8	1.1	+	6.5	3.8
J-5-1	4.8	0.2	-	0.0	0.1
J-5-6	9.6	0.5	+	4.9	14.6
J-6-1	8.0	0.4	+	8.8	6.0
J-8-2	5.6	1.4	+	3.5	5.5
J-10-6	2.9	0.2	+	2.1	2.9
J-11-5	13.5	0.9	+	13.3	11.4
J-12-3	6.7	0.4	-	0.0	4.5
A-2-3	3.9	0.6	+	2.9	3.1
A-2-4	6.9	1.0	-	1.8	5.0

Table 3 - continued

All firing rates are given as unnormalized values in spikes/second. Cumulative average field rate was found in each case by dividing total number of spikes occurring in sections containing the unit's field by total time spent there. Cumulative average background rate was found by dividing total spikes occurring in all other sections by total time spent there.

[a]In the designation of spatial firing, J represents the experiment, the first number the rat, the second number the probe from which firing was recorded.

[b]In these two regions, firing could not be unequivocally distinguished electrophysiologically.

[c]Distinctly different, smaller spikes than those occurring in the transverse arms. J-1-4 fired quite specifically in the center arm near the entrance to the start-box.

Figure 13

Average firing rates for promptly-appearing spatial firing during first and second passages

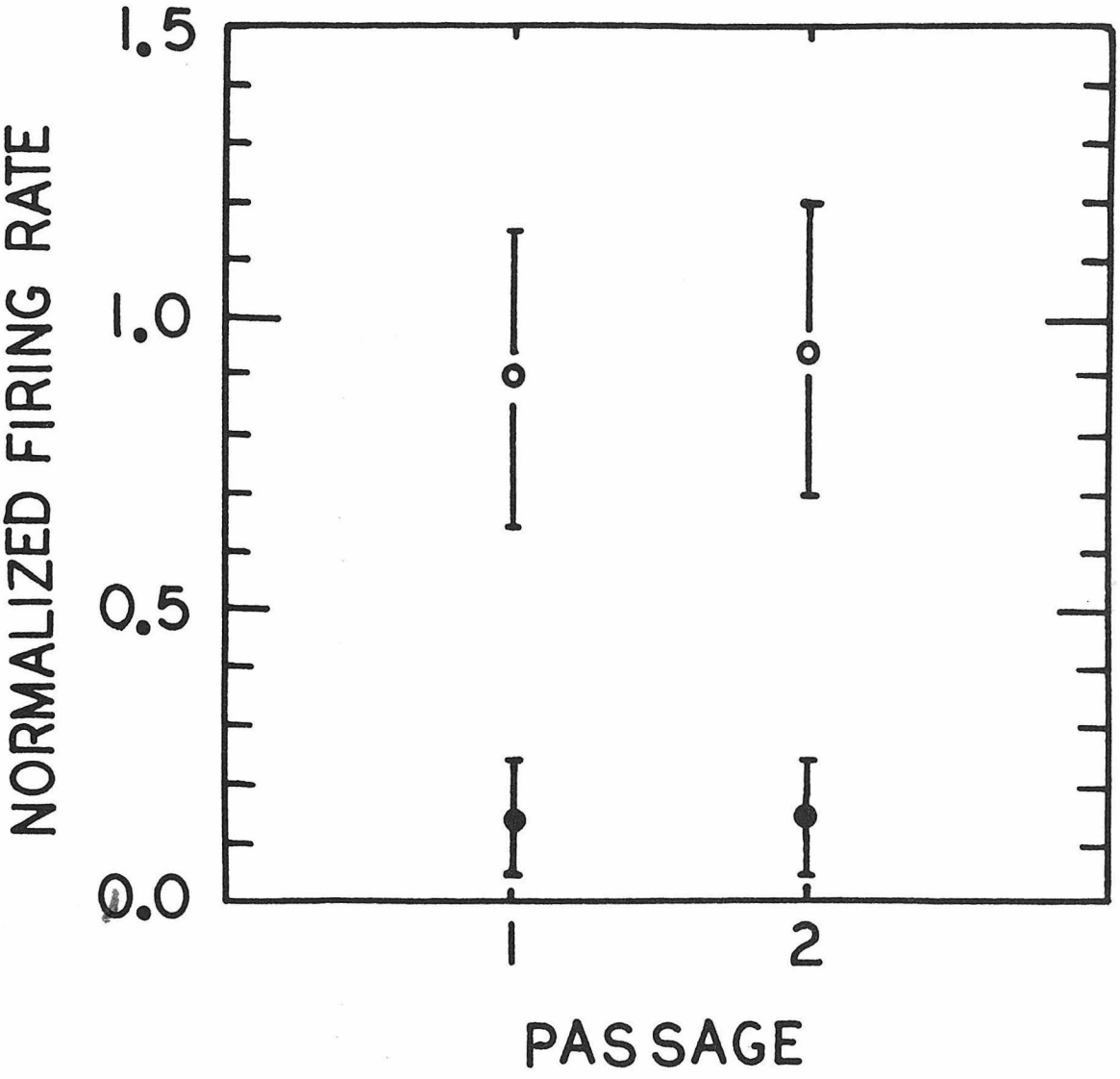
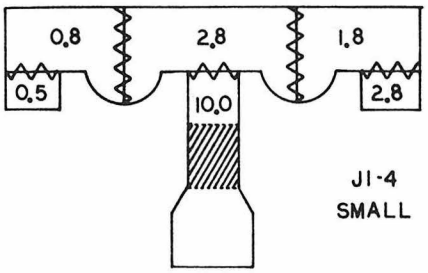
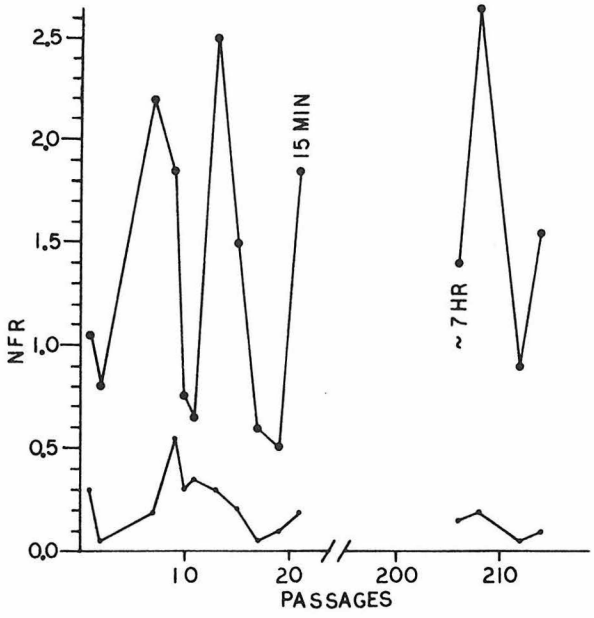

NORMALIZED FIRING RATE
1.5
1.0
0.5
0.0
1
2
PASSAGE

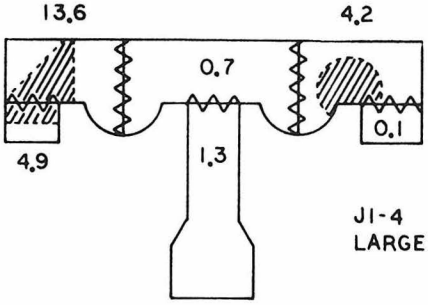
Figure 14

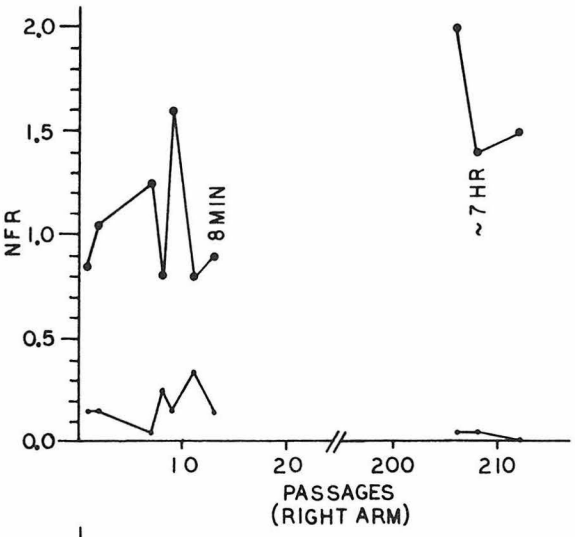
Spatial fields and general time course of calculated firing rates for all examples in time course study

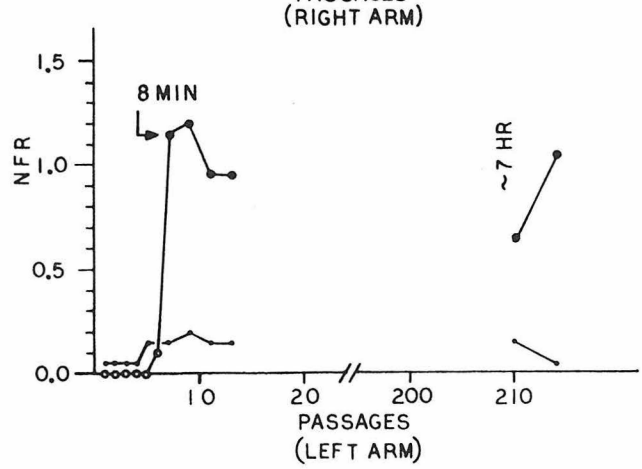
One passage is counted as a complete traversal of the field by the rat in any direction (if the spatial firing is not specific to orientation), or in the proper direction (if the spatial firing is specific to some orientation). For examples in which firing does not occur on the first trial, the time is indicated when spatial firing does first occur. Open circles represent trials for which no spatial firing was observed. Other times are indicated to give an idea of the intervals over which data are shown. Vertical scale is normalized-firing-rate, as described in the text.

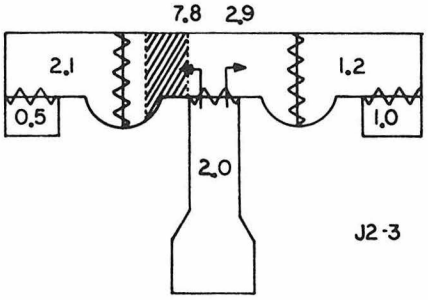

0.8
2.8
1.8
0.5
10.0
2.8
J1-4
SMALL

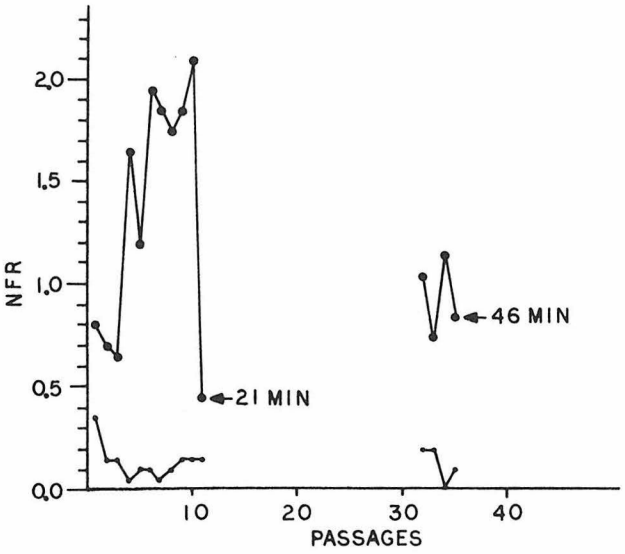

NFR
15 MIN
~7 HR
PASSAGES
10
20
200
210

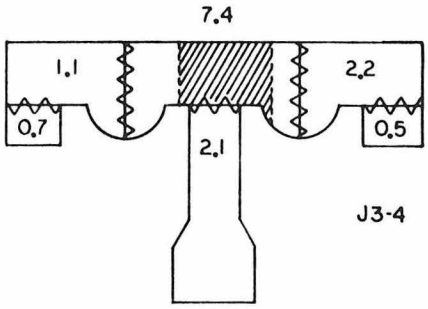

13.6
4.2
0.7
0.1
4.9
1.3
J1-4
LARGE

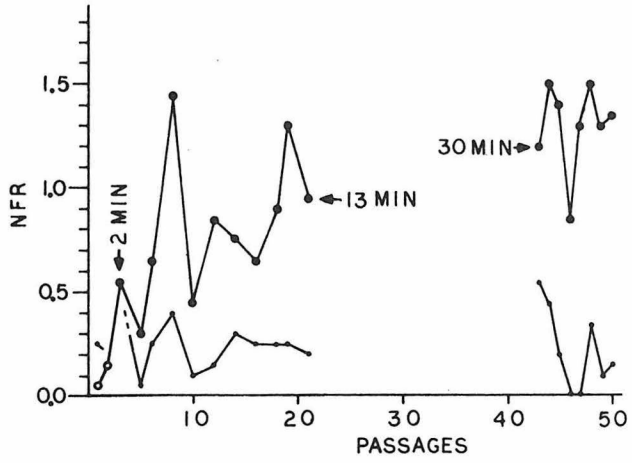

NFR
8 MIN
~7 HR
PASSAGES
(RIGHT ARM)

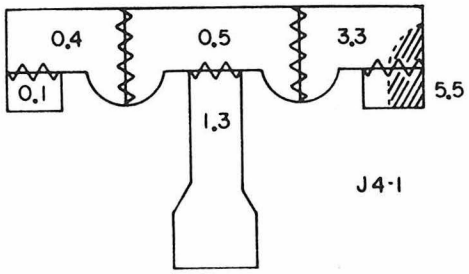

NFR
8 MIN
~7 HR
PASSAGES
(LEFT ARM)

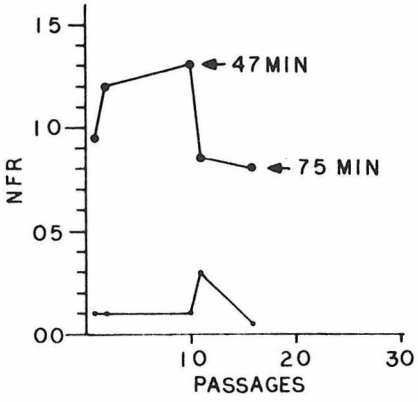

7.8
2.9
2.1
1.2
0.5
1.0
2.0
J2-3

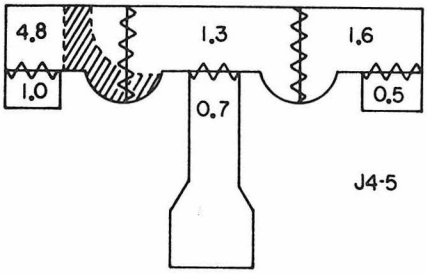

NFR
2.0
1.5
1.0
0.5
0.0
21 MIN
46 MIN
10
20
30
40
PASSAGES

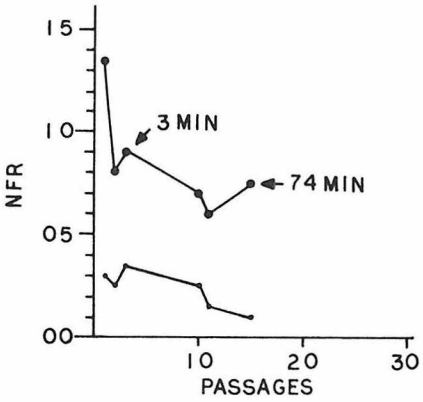

7.4
1.1
2.2
0.7
0.5
2.1
J3-4

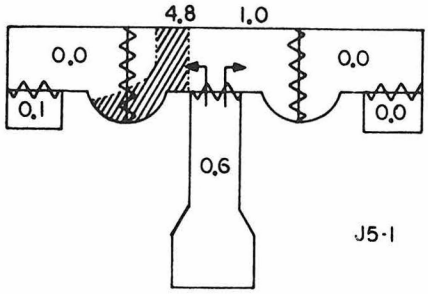

NFR
1.5
1.0
0.5
0.0
2 MIN
13 MIN
30 MIN
10
20
30
40
50
PASSAGES

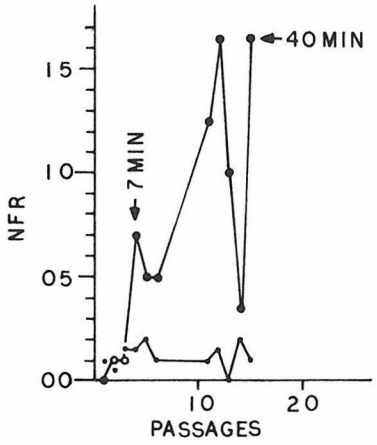

0.4
0.5
3.3
0.1
5.5
1.3
J4-1

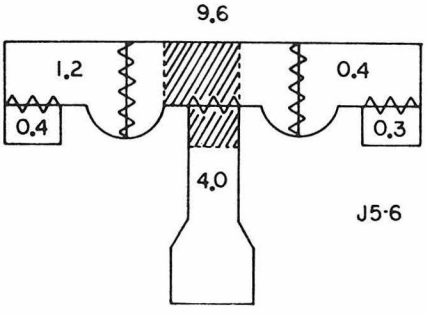

NFR
15
10
05
00
47 MIN
75 MIN
10
20
30
PASSAGES


4.8
1.3
1.6
1.0
0.7
0.5
J4-5


1.5
1.0
0.5
0.0
NFR
3 MIN
74 MIN
10
20
30
PASSAGES


4.8
1.0
0.0
0.0
0.1
0.0
0.6
J5-1


1.5
1.0
0.5
0.0
NFR
40 MIN
7 MIN
10
20
PASSAGES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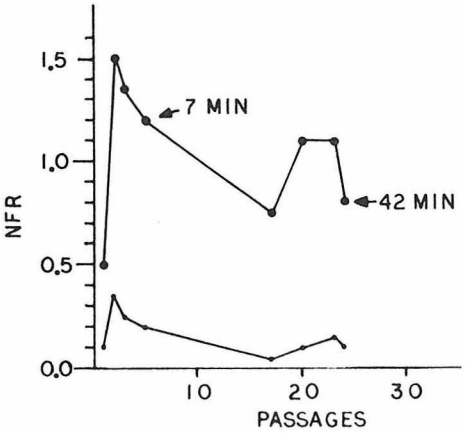
4.0
J5-6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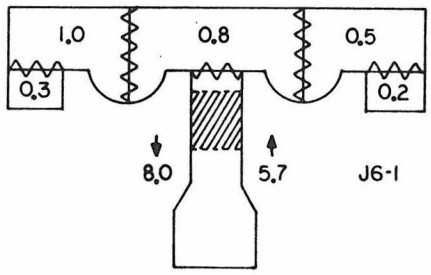
10
20
30
PASSAGES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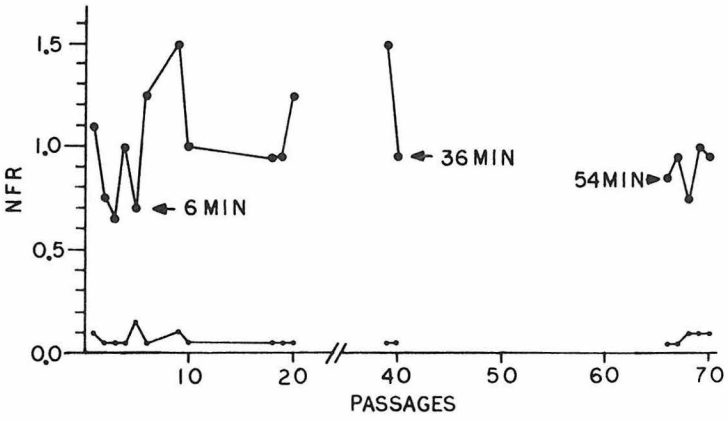
J6-1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0.5
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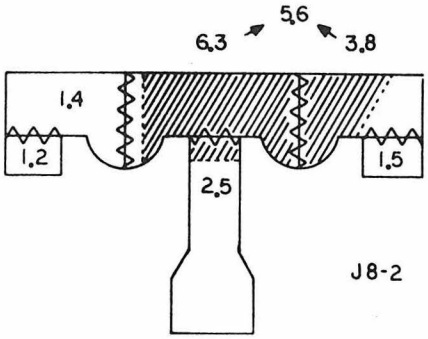
6 MIN
36 MIN
54MIN
10
20
40
50
60
70
PASSAGES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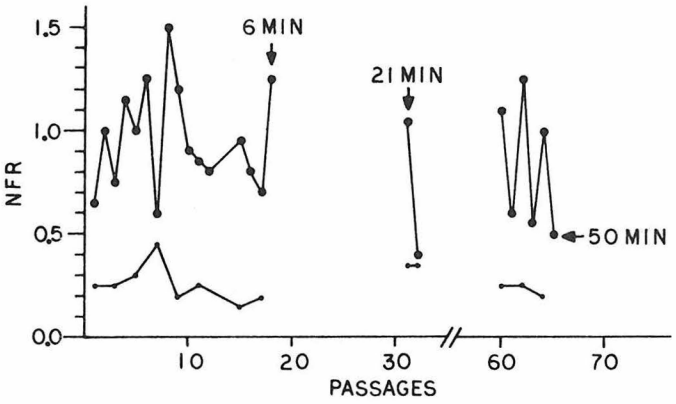
6.3
3.8
1.4
1.2
1.5
2.5
J8-2


NFR
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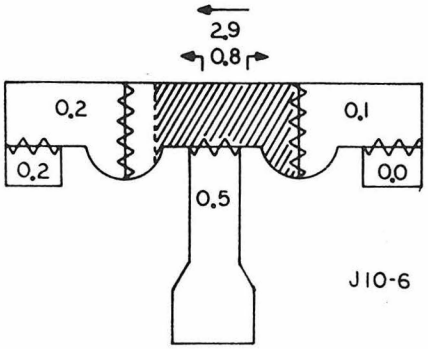
0.5
0.0
6 MIN
21 MIN
50 MIN
10
20
30
60
70
PASSAGES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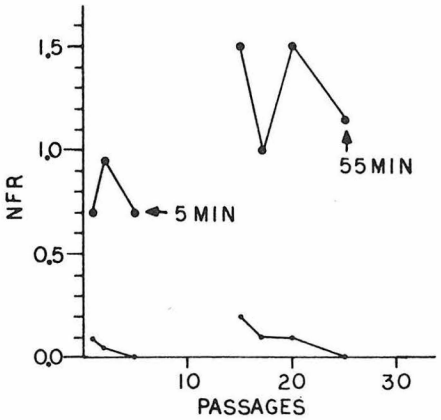
0.2
0.1
0.2
0.0
0.5
J10-6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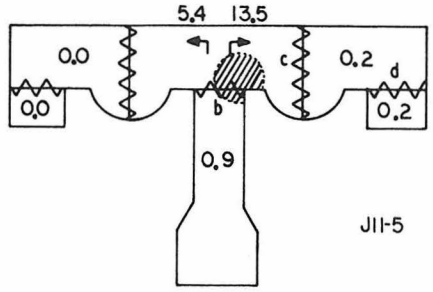
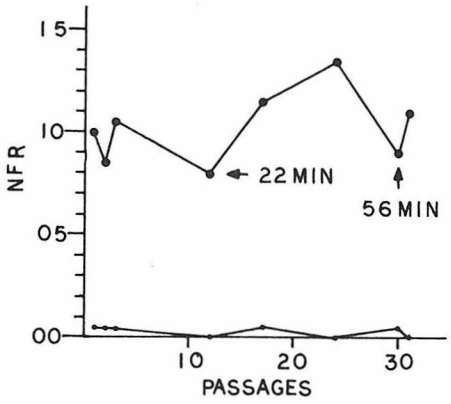
1.0
0.5
0.0
55MIN
5 MIN
10
20
30
PASSAGES


5.4
13.5
0.0
0.0
b
c
0.2
d
0.2
0.9
J11-5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1.0
0.5
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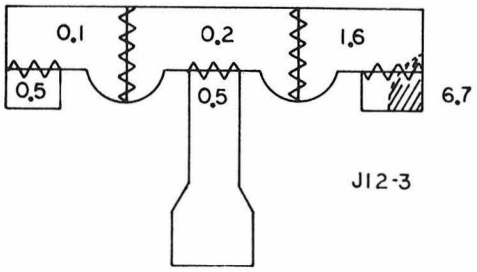
22 MIN
56 MIN
10
20
30
PASSAGES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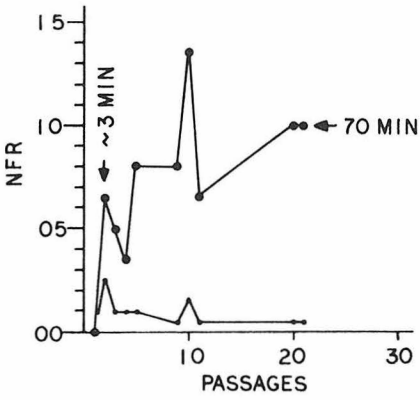
0.2
1.6
0.5
0.5
6.7
J12-3


NFR
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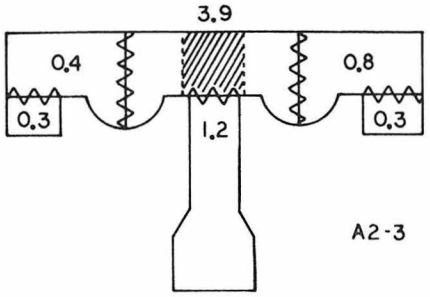
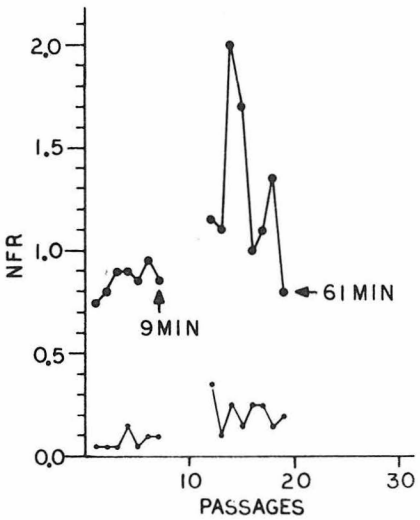
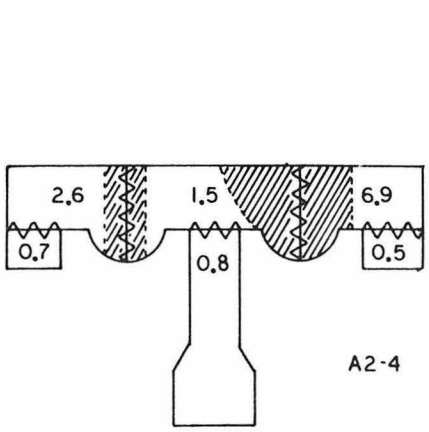
0.5
0.0
~3 MIN
70 MIN
10
20
30
PASSAGES


3.9
0.4
0.8
0.3
1.2
0.3
A2-3


NFR
2.0
1.5
1.0
0.5
0.0
9MIN
61 MIN
10
20
30
PASSAGES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6.9
0.7
0.8
0.5
A2-4

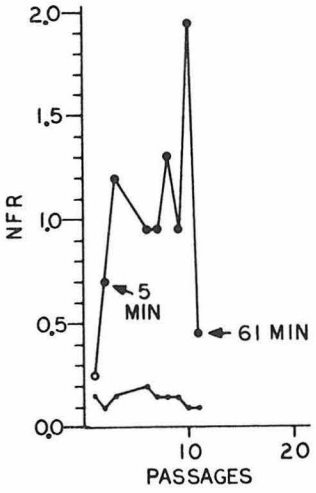
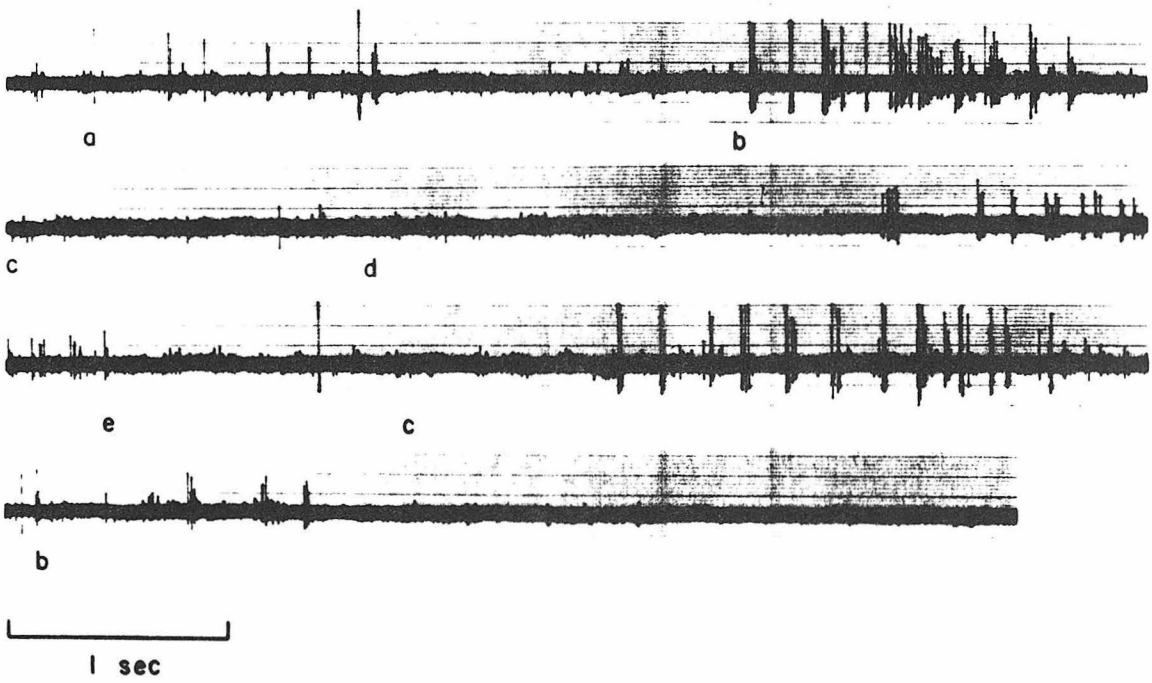

2.0
1.5
1.0
0.5
0.0
NFR
5
MIN
61 MIN
10
20
PASSAGES

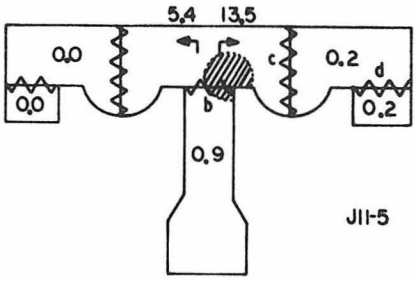
Figure 15

Spatial firing from J11-5 during first passage through field

TRACES: Recording during first passage out and back through field.

DIAGRAM: Field of J11-5


a
b
c
d
e
c
b
1 sec


5,4
13,5
0,0
0,0
b
c
0,2
d
0,2
0,9
J11-5

Calculated firing rates sometimes showed substantial variability from trial to trial. It was found, however, that such variation was due mainly to differences from trial to trial in the way rats passed through the maze. (As mentioned previously, this is an unavoidable concomitant of scoring a fixed partitioning of the maze.) In each case, when the taped data were reviewed with careful attention to the audio description of the rat's behavior, it was found that rather uniform spatial firing occurred whenever the rat actually entered the field. An extreme example is shown in Figure 16. The sample trace represents part of a single trial to the left for animal J5. The letters b, d, and e below the trace indicate the times at which the rat's nose broke the photocell beams labeled similarly in the diagram of the maze. Other letters correspond to actions described for this trial on the audio track. During the trial, the rat left the start-box (a) and moved slowly to the choice point (b), where it turned right and paused for some time sniffing and looking into the right arm. Eventually, it turned back (c) and moved into the left arm. J5-1 fired extensively just before the rat passed through the photocell beam in the middle of the left arm (d), after which the rat moved quickly to the left goal-box (e). In the choice point section during this trial (trial 22, the 14th passage through the field), the firing rate was only 35% of the cumulative average rate there. This low value is due primarily to the long pause which the rat made in the choice point before turning left into the field.

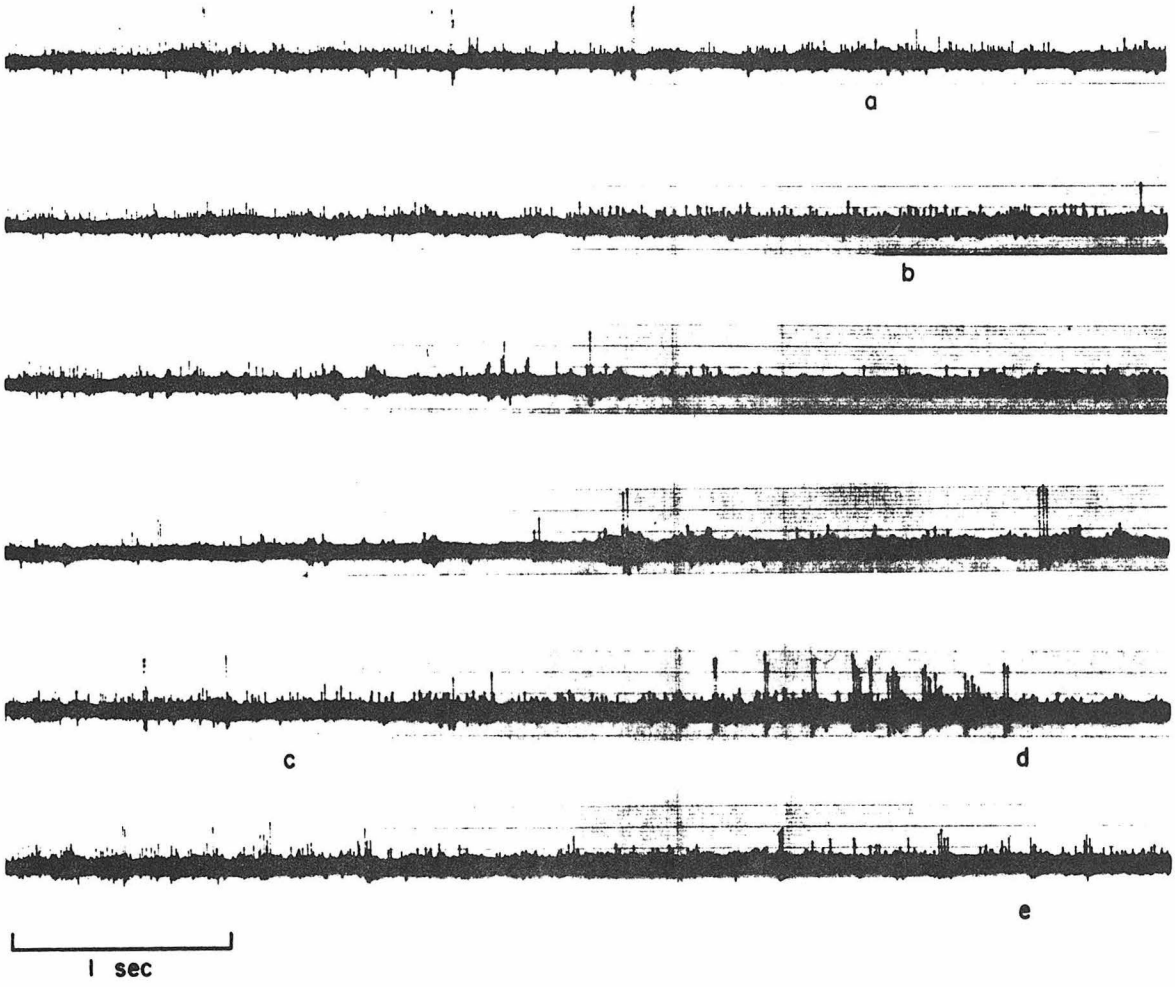
In four cases, spatial firing appeared some time after the rat's initial exposure to the field. In addition, on probe J1-4, where spatial firing occurred from the first trial in the right arm, similar firing began somewhat later in a distinctly separate field in the left arm. In each of these cases, firing appeared for the first time within the first 15 minutes of the runs. In no case did spatial

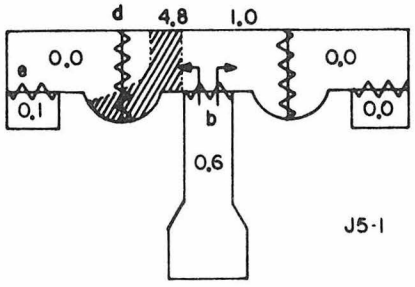
Figure 16

Spatial firing from J5-1 during a single passage (out) through field

TRACES: Recording during trial 22.

DIAGRAM: Field of J5-1


a
b
c
d
e
1 sec


d
4.8
1.0
0.0
0.0
a
0.1
b
0.0
0.6
J5-1

firing appear later during the runs or during the subsequent screening and manipulation periods. These latter periods usually extended the total time for observation in the maze to 2 hours or more.

It is interesting, however, that spatial firing was often observed to appear overnight on probes on which none had been the day before. Since a comparable number of examples disappeared overnight, it may be that both changes were due to shifts in electrode position.

III. Discussion

In 11 of the 15 admissible cases of spatial firing in this study, such firing occurred on the first passage of the rat through the relevant part of the maze. Of the two cases not satisfying criterion 3, one showed definite spatial firing in the outer corner of the left-hand goal-box on the first three visits there. Subsequently, the rat adopted a more economical method of leaving the goal-box, by pivoting past the inside corner. Since the rat no longer passed through the field, a meaningful average rate could not be obtained for this example by the methods used here. It is not included in Table 3. Nevertheless, during the testing period after the time course study, when the rat was confined to the left-hand goal-box, firing occurred as it had initially whenever the rat turned into the left outside corner.

If the delayed spatial firing of J1-4 is regarded as a separate case on the basis of its different time course, then the results indicate that in 12 out of 17 instances of spatial firing, the firing occurred during the first passage. It is possible that a second type of spatial firing occurs in hippocampus that requires a longer period of exposure to a new environment. The data are not adequate

to determine this issue. Spatial firing seems to be based upon a wide range of sensory inputs. The exact nature of these inputs is not known. In addition, spatial firing can be influenced by relatively small shifts in a rat's apparent attention. For example, the field of J2-3 was a small band across the left arm near the choice point. During the manipulation part of the experiment for this rat, a 3 cm high hurdle was inserted across the left arm through the middle of the field. Spatial firing no longer occurred on J2-3 in this region when the rat passed through it by quickly surmounting the hurdle. On several occasions, however, the rat hesitated before going over the hurdle and lowered its head slightly to explore the hurdle and the floor beneath it. In each such instance, vigorous firing did occur. It is important that this result be taken in proper perspective. The effect of the hurdle is neither general nor controllable enough to be very significant in itself. Furthermore, it is not clear whether the effects of the hurdle resulted from actual modification of the sensory cues associated with the field or merely from an enforced shift of the rat's attention away from a certain set of the cues. It is to illustrate this latter possibility that the above example has been described.

In all cases observed, once spatial firing had first appeared, its occurrence and its general character were stable. Appreciable changes were not observed in either extent of the field or pattern of firing there. This finding is consistent with other observations made during this work, where several cases have been observed of spatial firing with the same characteristics over many hours for up to four consecutive days. (This was true, for example, of SL2-2 in the sensory-deprivation experiment.)

It is true that, in a sense, the T-maze was not a "new" environment. In some of their characteristics (materials used in construction, general behavior

required there), the T-maze and the shuttle box were similar. Indeed, although in 9 of the cases reported here no spatial firing was observed in the shuttle box, in the other 6 cases it was observed there. In these cases, spike activity appeared to be the same as that eventually occurring in the T-maze. The observation is not necessarily surprising. Olton, Branch and Best[67] have reported that for complex-spike units recorded from hippocampus using moveable micro-electrodes 90% show spatial firing in a single experimental environment. Such observations suggest extensive redundancy in hippocampal spatial firing. Together with the multiplicity of places to which a rat may be exposed in its lifetime, this implies that a given spatial unit must be able to represent distinct regions in many different environments. It seems safest to conclude, then, that the time course study does not address the ultimate origin of spatial firing in any case.

It is not likely, in any event, that continuity of operant behavior can explain the prompt appearance of spatial firing in the T-maze. Extensive observations have been made of over 50 examples of spatial firing during work in this laboratory. No consistent behavioral correlate of spatial firing has been found (other than occasional acceleration as rats sniff at places within their fields). Furthermore, during the preliminary work for this experiment, one rat was run successfully in the T-maze without any kind of pretraining. Although the rat's behavior was too erratic to allow analysis by the methods used here, spatial firing was observed in this animal during the rat's first passage through its field.

CONCLUSIONS

The hippocampus has been implicated in learning and memory, in inhibition and in spatial mapping. The first theory is supported mainly by observations of the effects of hippocampal lesions on humans and by a variety of animal studies involving lesions, stimulation, electrical recording and histochemical analysis. This evidence has been reviewed in the Introduction. In general, human and animal lesion data have been incompatible, in spite of attempts to reconcile them. Recently, in discussing studies of unit recordings from hippocampal formation of humans, Halgren et al.[102] have suggested that in humans memory deficits result from damage to the hippocampal gyrus rather than to hippocampus itself. This possibility may explain the lack of effect upon learning and memory generally found in animal lesion studies. These have given support primarily to theories of hippocampal function in inhibition.

The spatial theory derives ultimately from observations of spatial units in hippocampus; it is supported also by reinterpretation of previous lesion data and by lesion experiments specifically designed to test this theory. Recently, additional support has come from observations of the effect of lesions on hippocampal EEG. Winson[99] has reported that when they abolish hippocampal theta in the rat, medial septal lesions also disrupt performance of a previously-learned spatial task. Unfortunately, a conclusive test has not been reported which would clearly distinguish inhibitory and spatial function in animals. It remains possible that the hippocampus performs several functions, perhaps at the same time, perhaps only under different circumstances.

The data reported here are consistent with the spatial-mapping theory. In reports from other laboratories,[65,67] spatial units have been described that

seemed to utilize the presence or absence of reinforcement as a spatial cue. O'Keefe has identified these with Ranck's approach-consummate-mismatch units. Such units ("misplace units") fired at increased rates during sniffing which accompanied non-reward when their fields encompassed a goal area. In many instances during the present experiments, spatial firing occurred at its maximum only during active sniffing within the field. However, this acceleration did not appear to be specific to goal areas. It often occurred when rats sniffed vigorously within spatial fields well removed from a goal-box and during activity which did not lead at all directly to a goal-box. Only one instance was found of spatial firing which depended clearly on reward. This was observed during preliminary work for the time course study. A spatial unit fired in the left-hand goal-box, but only when a food pellet was present. No other non-spatial correlate could be seen. In interpreting this case, consideration must be given to the differences normally observed in goal-box behavior between rewarded and non-rewarded trials* and to the possible influence on spatial firing of small shifts in attention.**

The sensory-deprivation experiment provides additional evidence that "spatial" firing in hippocampus can be based upon a number of different sensory modalities. Minimally, the data show that spatial firing can occur in the complete absence of visual and vibrissal information about the environment and probably also in the absence of auditory and olfactory information. Little insight is provided into the problem of hippocampal function or into the fundamental nature of "spatial" firing itself.

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*See discussion of olfactory-deprived animals.

**See discussion of time course experiment.

In the time course experiment, results indicate that many hippocampal spatial units are "assigned" to their fields during the rat's first exposure to a new environment. Therefore, it is clear that spatial firing can appear early enough to play a functional role in guiding spatial behavior in a new place. Several behaviors seem necessarily to involve recognition of spatial aspects of the environment. Some take place after only one exposure to a new place. Notable among these are spontaneous alternation and one-trial step-through avoidance. Both complex maze performance and spontaneous alternation are disrupted by hippocampal lesions. However, spontaneous alternation may recover within two weeks.[100] Furthermore, in most cases, hippocampal lesions do not disrupt acquisition of one-trial step-through avoidance tasks.[27] Thus, it is not clear that there is a necessity for early appearance of spatial firing.

In general, observations of "spatial" firing have not ruled out other interpretations of hippocampal function. For example, it is possible that the hippocampus does subserve internal inhibition. Spatial firing could be the neuronal correlate of inhibition specifically directed toward movement through a certain place. This possibility is made less likely by the observation that such firing seems to be independent of whether or not the movement actually occurs. In the T-maze, spatial firing has often been observed when a rat paused in the choice point and merely looked down one arm in the direction of a spatial field before turning into the other arm. The inhibition argument is further weakened by numerous examples of fields which seem to be specific to no particular orientation or direction of movement through the field. On the other hand, the hippocampus may subserve merely the inhibitory input to behavior so that the final behavior need not correspond in all cases to events in the hippocampus.

Observations do not support a general involvement of the hippocampus in learning. In fact, in the time course experiment, spatial firing appeared fairly abruptly in all cases. This step-like time course does not resemble the more gradual learning curves associated with most learning or those reported for hippocampal units themselves in a semi-classical conditioning paradigm[39] or for hippocampal EEG in a classical conditioning experiment.[101]

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