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HIPPOCAMPAL UNIT ACTIVITY DURING WAKING AND SLEEPING BEHAVIORS

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HIPPOCAMPAL UNIT ACTIVITY DURING WAKING AND SLEEPING BEHAVIORS

by

Constantine Pavlides

A dissertation submitted to the Graduate Faculty in Psychology in partial fulfillment of the requirements for the degree of Doctor of Philosophy, The City University of New York

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Abstract

HIPPOCAMPAL UNIT ACTIVITY DURING WAKING
AND SLEEPING BEHAVIORS

by

Constantine Pavlides

Advisor: Professor Richard J. Bodnar

Rat hippocampal (CA1) complex spike (place) cells of freely behaving rats (8-arm maze) were recorded in pairs continuously during a number of waking (exploration (XPL) and still-alert) and sleeping behaviors (quiet-awake (QA), slow-wave (SWS), pre- rapid-eye movement (PREM) and rapid-eye-movement (REM) sleep). Pairs of units were selected that did not have overlapping place fields (PF). The rats were restricted from entering the place fields of both cells overnight and on the day of recording they were exposed to their diverse PF's independently and in a counterbalanced design. Following exposure of each cell to its PF, recording was made in the subsequent sleeping episodes and the firing characteristics of both cells were analyzed.

Following exposure, significant increases in the spiking activity of the exposed cell were observed in the subsequent sleeping states, which were not evident in the unexposed cell. The increased activity was observed in the rate of firing (spikes/sec.), the number of spikes within a burst, as well as the number of bursts displaying 2-4 msec. interspike intervals (ISI).

In a second, descriptive experiment, the firing characteristics of hippocampal (CA1 and CA3) place cells were analyzed during similar sleeping and waking behaviors as in the first study. Higher overall firing rates were observed in the CA3 than in the CA1 field. The majority of CA1 cells also fired at higher rates in SWS than in REM sleep, in contrast to CA3 cells which fired at higher rates in REM than SWS. Both higher bursting and greater numbers of spikes within a burst were detected in CA3 than in CA1. Significantly longer bursts were also seen in QA and pre-REM. Lower ISI's (for spikes within a burst) were found in the CA1 than the CA3 hippocampal field, and also during REM and XPL.

The findings suggest that neuronal activity of hippocampal place cells in the awake states may influence the firing characteristics of these cells in subsequent sleep episodes. The increased firing rates along with the greater number of spikes and the shorter ISI's within the burst, following exposure to a cell's place field, may speak for possible information processing occurring during sleep.

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in the defense committee. Dr. James Ranck and his associates provided valuable help in the initial equipment set-up, and electrode construction, and I like to express my appreciation. Also thanks to Dr. Ranck for his expert comments on the manuscript.

Finally, none of this would have been possible without the absolute dedication of Dr. Jonathan Winson to whom I am eternally indebted. He has not only been my mentor, throughout my graduate studies, but was also a second father to me. This dissertation is dedicated to him and to my dearest parents, Paul and Athena Pavlides.

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ABBREVIATIONS

Alv ..... Alveus

CA ..... Cornus Ammon (Ammon's horn)

CC ..... Corpus Callosum

CS ..... Complex Spike (cells)

Cx ..... Cortex

DG (GD) ... Dentate Gyrus

EC ..... Entorhinal Cortex

Fim ..... Fimbria

IPF ..... In Place Field

MF ..... Mossy Fibers

MTL ..... Medial Temporal Lobe

PP ..... Perforant Pathway

PREM ..... Pre Rapid Eye Movement sleep

Pyr ..... Hippocampal pyramidal cell layer

QA ..... Quiet Awake

REM ..... Rapid Eye Movement sleep

Sch ..... Schaffer collaterals

SAL ..... Still Alert

SWS ..... Slow Wave Sleep

CHAPTER 1

Introduction

It has been known for the past several decades that damage to the medial temporal lobe (MTL) produces deficits in memory. Recent research has allowed us to better comprehend memory function and to more accurately localize the brain structures involved. Currently, it is believed that structures within the limbic system, and more precisely the hippocampal formation, play a crucial role in such processes. Although there is considerable knowledge about memory processes at the neuropsychological level, the neural substrates involved are only vaguely understood. The objective of the present experiments was to provide a better understanding of mnemonic processes at the neuronal level.

O'Keefe and Dostrovsky (1971) discovered that the majority of units in the hippocampal formation of freely behaving rats increase their firing in correlation with the animal's position in space ('place cells'). A number of subsequent studies have further investigated the sensory correlates of these units. Several studies have also analyzed the firing of these units in a number of tonic behavioral states in the waking and sleeping animal. The findings indicate that besides the increased firing in correlation with spatial fields, rather high firing rates are also observed in the sleeping states, especially during slow wave sleep (SWS). One could postulate

that information is processed by increased neuronal firing. Since increased firing takes place during sleep, some information may be processed during these states. Furthermore, it could be hypothesized that with increased neuronal firing during the awake state, an increase may occur in the sleep states that follow.

Place cells offer a unique opportunity for testing the above mentioned hypothesis in that the extent of their natural, awake firing rates can be monitored and controlled (i.e. by allowing the animal to enter its place field or by restricting its entrance into such fields). For the dissertation, a pair of place cells with distinct and diverse place fields were recorded during the awake behaviors in order to establish their place fields. Following overnight isolation of both place cells from their place fields, each cell was exposed to its place field independently followed by extended recording in the sleep episodes that followed. An extensive analysis of the firing characteristics of these cells was then made during a number of behavioral states.

Hippocampal Involvement in Mnemonic Processes.

A- Hippocampal Lesions

Most of our knowledge of memory and its pathology is derived from a clinical case (patient H.M.), first described by Scoville and Milner (1957). Following bilateral hippocampectomy, this patient developed a severe compound memory deficit. His amnesia consisted of both an

anterograde deficit (an inability to form any new memories) as well as a retrograde component that included a period of approximately three years prior to surgical intervention. Work on other clinical cases with bilateral MTL abnormalities revealed similar deficits (patient P.B. and F.C.- left medial temporal resection with right temporal dysfunction; Penfield and Milner, 1958). In most of these cases, there is a scarcity of information concerning the precise location and extent of the lesion. Recently, however, there has been a report of a case (patient R.B.) in which an episode of hypoxic-ischemia was shown histologically to have produced a discrete, and total, bilateral lesion of the CA1 region of the hippocampus (Zola-Morgan, et al., 1985). This patient showed no signs of dementia and yet displayed a severe anterograde amnesia as well as a mild retrograde amnesia. Although a number of other such cases have also been reported (Volpe and Hirsh, 1983; Volpe, et al., 1985), to date this is the only clear, histologically verified case of a circumscribed hippocampal lesion producing a marked memory deficit.

In an attempt to more precisely localize the structures in the medial temporal lobe that are responsible for amnesia, animal models have been developed in both monkey (Mishkin, et al., 1982; Squire and Zola-Morgan, 1983) and rat (Olton, 1984). Early experiments in monkeys suggested that a combined amygdalo-hippocampal lesion was required for an amnesic syndrome to develop (Mishkin, 1978). A trial-unique, delayed-non-matching-to- sample task was used in which an animal is initially presented with a single stimulus and, following a specified time delay, it is then presented with two stimuli, and is

expected to detect the novel stimulus. Monkeys, in which the lesion was restricted to either the hippocampus or the amygdala, performed at 91% and 94% correct respectively. In contrast to these mild deficits, animals with combined hippocampal and amygdaloid lesions were severely impaired, performing at 60% correct (chance levels are 50% in this test). Later studies using a somewhat different testing procedure and longer test delays showed a much greater memory deficit with hippocampal lesions alone (Mahut, et al., 1982). There are a number of possible explanations given for these discrepancies (for review see Zola-Morgan, 1984). The role of the amygdala in memory processes at this point remains somewhat unclear. In all, however, both human (R.B.) and monkey data emphasize the important role played by the hippocampus in mnemonic function.

Extensive research on amnesia has also been carried out in rats. A basic paradigm used is one in which food deprived rats are tested in a T-maze or an eight arm radial maze in either a working memory or a reference memory task. In working memory or trial-dependent tasks, the animal must remember which arms of the maze were previously visited. In reference memory or trial independent task, the animal must remember to make similar responses, such as always turning left in the T-maze, to obtain a reward. The findings indicate that while normal rats acquire these tasks rapidly and perform with a high degree of accuracy (Olton, et al., 1977; Olton and Samuelson, 1978), rats with lesions placed in the hippocampal system (fimbria-fornix, entorhinal cortex) performed reference memory tasks but not working memory tasks (Jarrard, 1978; Walker and Olton, 1979). Indeed, lesions restricted

to the CA1 field of rats by experimentally-induced cerebral ischemia are sufficient to produce the full working memory deficits (Davis, et al., 1985; !986; Volpe, et al., 1984; 1985).

The clinical evidence, along with the experimental work in animals thus provide a strong link between the hippocampus and specific forms of memory. Furthermore, two very important distinctions concerning memory processes have been made. One distinction is the subdivision of memory into short term (in the range of a few minutes) and long term (in the range of hours to many years) components. Human subjects with hippocampal lesions display intact short term memory (STM) in that information is retained as long as the material does not exceed STM capacity or as long as it is actively rehearsed. As described, hippocampal lesions have a devastating effect on the formation and/or retrieval of long term memory. A second distinction has been made for human memory dealing with specific facts (declarative- extrapersonal or episodic-autobiographical) as compared to information about procedures (procedural memory; Squire, 1986). Similar dissociations in animal studies occur between trial-dependent (working) and trial-independent (reference) memory (Olton, 1983). Hippocampal lesions destroy declarative-episodic (Squire, 1986) or working memory (Olton, 1983) while having little effects on procedural or reference memory. From these findings it appears that the hippocampus is required for declarative-episodic or working memory. Furthermore, consolidation of such memory is a dynamic process that appears to take as long as

several weeks in the mouse (Squire and Spanis, 1984) to several years in humans (Squire, et al., 1975; Squire, 1986).

Although strong evidence now links the hippocampal formation to memory, and the various memory components have been elucidated, still very little is known about the detailed neurophysiological mechanisms that underlie memory consolidation, storage and its retrieval.

B- Neuroanatomy of the Hippocampal Formation

Evidence for the role of the hippocampus in memory is also supported by neuroanatomical findings which indicate close interconnections with other brain regions implicated in mnemonic processes. This section will present some neuroanatomical findings involving the intrinsic and extrinsic interconnections of the hippocampus primarily in the rat. The hippocampus (the dentate gyrus (DG) and Ammon's horn (CA)) is an allocortical structure intimately related to the limbic system. The subicular complex and the entorhinal cortex (EC), that lie caudally, make intricate connections with the hippocampus proper and along with the hippocampus have been collectively referred to as the hippocampal formation (Blackstad, 1956).

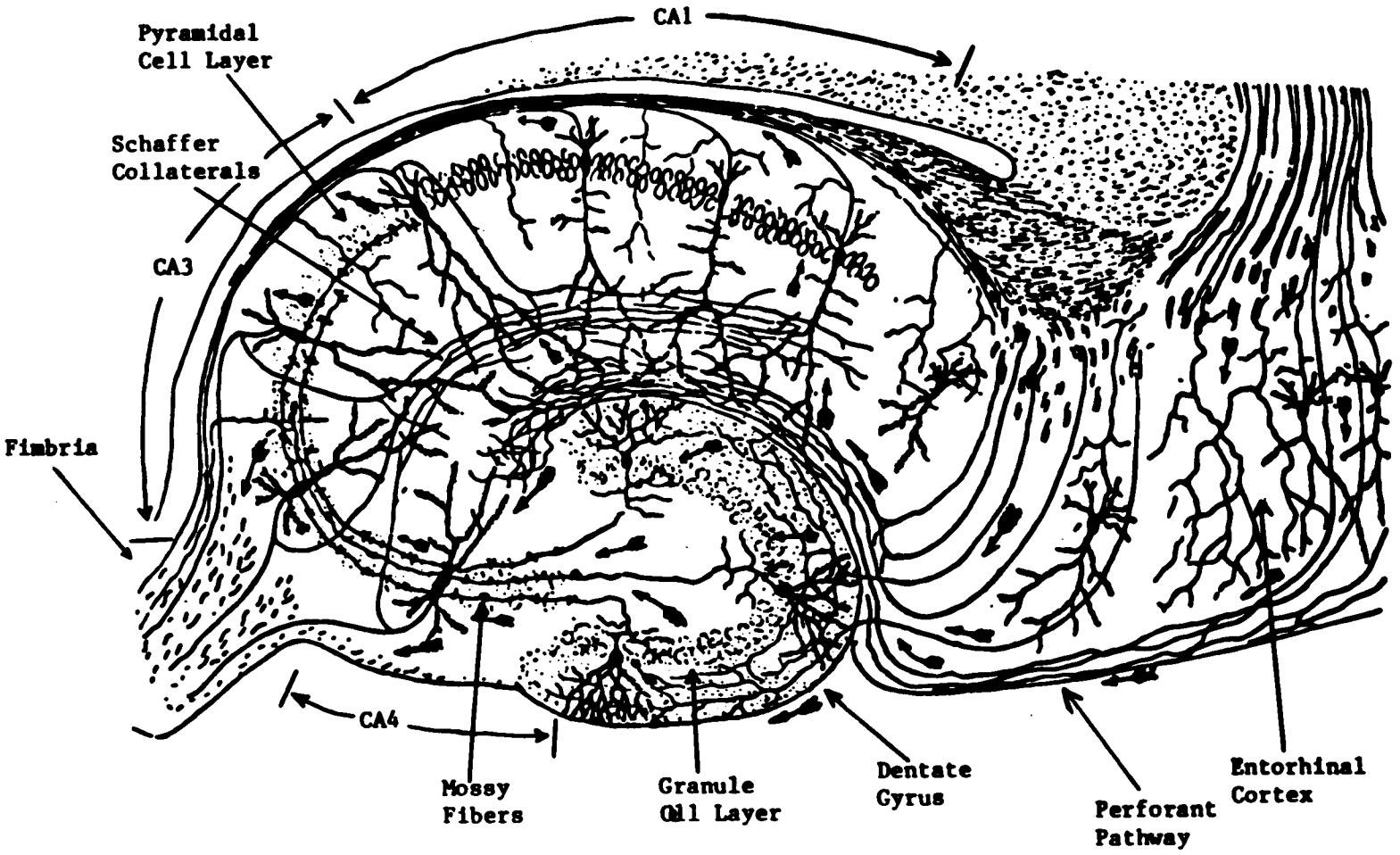
The original work on both the intrinsic and extrinsic interconnections to the hippocampus have been described by Ramon y Cajal (Ramon y Cajal, 1911) and subsequently by Lorente de No (1934). Although more modern electrophysiological, biochemical and

histochemical techniques have added further details concerning these interconnections, the original findings are, to a large extent, still appropriate. In the cat (Room and Groenewegen, 1986a,b; Witter and Groenewegen, 1986a,b; Witter, et al., 1986) and monkey, and to a lesser extent the rat, the parahippocampal (perirhinal and entorhinal) cortex that lies adjacent to the hippocampus forms an important way station for both afferent and efferent inputs to the hippocampus. Uni- and multimodal sensory inputs synapse either directly (Amaral, et al., 1983) or indirectly (cat and monkey; by way of perirhinal cortex) on the entorhinal cortex, the major output of which projects to the hippocampus through the massive perforant pathway. The parahippocampal cortex also makes reciprocal connections with a number of subcortical structures including the amygdala, the septum, the thalamus, hypothalamus, as well as a number of brainstem nuclei.

As can be seen in Figure 1 (from Ramon y Cajal, 1911), the major input to the hippocampus comes from the entorhinal cortex through the perforant pathway (PP) which is subdivided into lateral and medial components. The lateral PP projects to the outer parts of the stratum moleculare of both the DG and the hippocampus, while the medial portion projects to the mid-molecular layer of the dentate gyrus granule cells (these being the principal cell group of the DG; Andersen, et al., 1966; Raisman, et al. 1966; Steward, 1976). The axons of the granule cells (the mossy fibers) activate the CA3 pyramidal cells of the hippocampus which in turn project, through the Schaffer collaterals and the commissural pathway, to the CA1 field pyramidal cells of the hippocampus. Besides this output, the CA3

Figure 1. Schematic drawing by Ramon y Cajal (1911) of the hippocampal formation. The major subclasses of cell groups along with their primary afferents, intrinsic interconnections and major efferents are represented. The arrows represent the flow of impulses within this structure. All major subdivisions are labelled on the drawing.

Ammon's Horn


CA1
Pyramidal
Cell Layer
Schaffer
Collaterals
CA3
Fimbria
CA4
Mossy
Fibers
Granule
Cell Layer
Dentate
Gyrus
Perforant
Pathway
Entorhinal
Cortex

cells also project to the lateral septum bilaterally (Meibach and Siegel, 1977), as well as back to the EC directly (Hjorth-Simonsen, 1972). The CA1 pyramidal cells project back to the entorhinal cortex through the subicular complex (Irle and Markowitsch, 1982; Meibach and Siegel, 1977; Swanson and Cowan, 1975; 1977; Van Hoesen, 1982). That the hippocampus almost exclusively projects through the subicular complex disagrees with Cajal's original contention of direct connections to cortical and subcortical structures (for review see Andersen, 1975). A direct although sparse projection has been discovered between the CA1 and the entorhinal cortex (Hjorth-Simonsen, 1971). The CA1 field also projects sparsely through the fimbria and precommissural fornix to the ipsilateral lateral septum in a topographically arranged manner (Swanson, 1978). The lateral septum, in turn, makes connections both with the medial septum and the nucleus of the diagonal band, which projects back to the CA3 and CA4 hippocampal fields and the dentate gyrus, the subicular complex and the entorhinal cortex (Segal and Landis, 1974; Siegel and Tassoni, 1971). The lateral septum also sends afferents to the lateral hypothalamus and the mammillary complex. Connections are also made between the subicular complex and various other subcortical structures, which have been considered to be part of the circuit of Papez, including the anterior thalamic nuclei, the mammillary bodies, and the ventromedial and arcuate nuclei of the hypothalamus by way of the post-commissural fornix (Swanson, 1978; Amaral and Kurz, 1985). The amygdala, which is also considered to be part of the limbic circuit has an indirect connection with the hippocampus via the EC

(Krettek and Price, 1974), and recently there is some evidence to show that a direct connection exists in the monkey (Amaral and Cowan, 1980).

Besides the major input from the medial entorhinal cortex to the dentate gyrus through the perforant pathway, there is a direct projection from the entorhinal cortex to the CA1 subfield (Steward, 1976; Steward and Scoville, 1976).

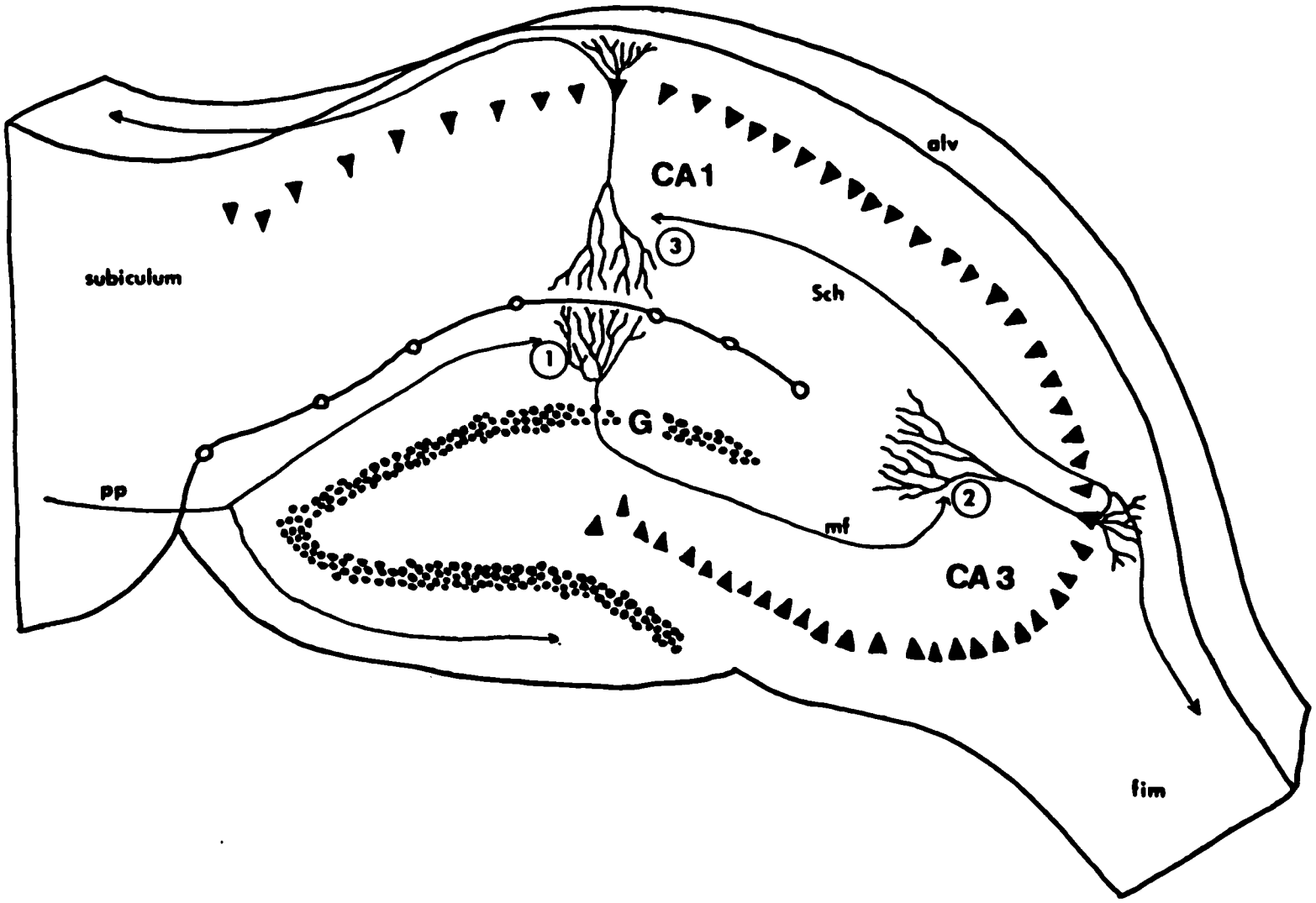
The hippocampal formation is also interconnected with a number of mid- and hind-brain structures. Through the use of horseradish peroxidase (HRP) and autoradiographic techniques it was demonstrated that the nucleus locus coeruleus (especially the anterior pole) makes prominent connections with both the dorsal and ventral hippocampus (Segal and Landis, 1974; Jones and Moore, 1977; Mason and Fibiger, 1979). There is also electrophysiological evidence to suggest that such innervation exists. Segal and Bloom (1974) stimulated the LC and found lasting inhibition as evidenced by the cessation of hippocampal cell firing.

Various brainstem nuclei have also been shown to make projections with the hippocampus (Room and Groenewegen, 1986). A number of investigators (Vertes, 1980; Robinson and Vanderwolf, 1978; and others) have reported that stimulation over a wide range of brainstem nuclei (i.e. nucleus pontis oralis, pontis caudalis, median raphe) produces hippocampal theta rhythm (see Vertes, 1980, for details).

Finally, besides the extrinsic connections, the two hippocampi are tightly interconnected by the commissural fibers (Fricke and Cowan, 1978).

There are a number of concepts to be considered in examining hippocampal organization. First, the hippocampus is organized in a trisynaptic circuit, consisting of (1) the perforant path to the DG synapse, (2) the mossy fiber to the CA3 synapse and (3) the Schaffer collateral to the CA1 synapse (see Figure 2). This represents a potential feedback circuit consisting of multimodal higher order sensory information originating in primary, secondary and higher order neocortical areas, traversing through the EC, coursing through the hippocampus and ending back in EC and the other neocortical areas from where it had originated. A second part of this circuit projects to the rest of the limbic system which then projects to the neocortex via anterior thalamus and cingulate cortex. A second organizational principle, unique to the hippocampal formation, is the fact that both the PP fibers, the mossy fibers and the Schaffer collateral fibers make en passage type connections with the cells that they innervate. Thus a single volley may influence or stimulate more than one cell simultaneously. Finally, all of the hippocampal stages seem to be arranged generally in a lamellar organization, that is, if the hippocampus is removed and cut in roughly sagittal slices 400 um in diameter, that slice was shown by Lomo (1971) and Andersen, et al. (1971) to constitute a functional unit. Thus, stimulation of a small bundle within the PP can activate one particular slice or lamella. Adjacent PP stimulation activates the lamella adjacent to the first,

Figure 2. Schematic representation of the trisynaptic circuit. The major afferents, originating from the entorhinal cortex, form the perforant pathway (PP) which synapses on the dentate gyrus granule cells (G) to form the first stage of the trisynaptic circuit. The DG granule cells give rise to the mossy fibers (mf) which synapse on the CA3 pyramidal cells (stage 2). The CA3 pyramidal cells, in turn, project through the Schaffer collaterals (Sch) and the commissural pathway (not shown here) to the final stage of the trisynaptic circuit, the CA1 pyramidal cells. (Abbrev. Alv- Alveus; Fim- Fimbria)


alv
CA1
3
subiculum
Sch
1
G
pp
mf
2
CA 3
fim

and so on. Lamella are arranged in parallel fashion, each one containing a complete trisynaptic circuit which may also be joined by a longitudinal fiber tract (for review see Andersen, 1975). The precise orientation and innervation of the lamella along the various subfields of the hippocampus is rather complex and not as well organized as originally believed. By and large, however, it appears that for the septal one-third of the hippocampus, if the brain is cut at a 45 degree angle the lamella appear to be perpendicular to the cut. For the more temporal part of the hippocampus the lamella appear to take a more traverse course (see Swanson, et al., 1978).

C- Hippocampal Evoked Potentials

Under the appropriate conditions a single electrical pulse applied to the PP activates the granule cell layer of the dentate gyrus (Andersen, et al., 1966). Representative field potentials can be recorded at each of the hippocampal fields following a single stimulus to the PP. Winson and Abzug (1978) discovered the phenomenon of neuronal gating in the hippocampus. They demonstrated that transmission through the three stages of the hippocampal circuit is dependent on the behavioral state of the animal. Thus, neuronal transmission, as measured by field potential analysis, is selectively restricted at the first stage of the trisynaptic circuit, during still alert behavior. In contrast, transmission is unrestricted in all three stages during slow wave sleep. Transmission through the DG and the CA3 field of the rat was shown to be variable during the two theta

states- voluntary movement and rapid eye movement sleep. Having demonstrated the gating phenomenon, with field potential analysis, a question that arises is what are the normal firing characteristics of hippocampal units during the different behavioral states. The unit analysis to be performed for this dissertation will be an attempt to answer this question.

D- Long Term Potentiation in the Hippocampal Formation.

The hypothesis that the hippocampal formation plays a crucial role in learning and memory is further supported by the finding of the phenomenon of long term potentiation (LTP) which (at least as described below) appears to be unique to this structure. Long term potentiation is the long-term enhancement (hours to days) of synaptic facilitation by application of brief electrical stimulation to the PP at tetanic frequencies (10-400 Hz). LTP has been demonstrated in all three fields of the trisynaptic chain (Bliss, 1979). The most effective frequency to produce LTP (one that requires the least amount of current) is 400 Hz (Winson and Dahl, 1986). Although it is possible for hippocampal complex spike cells to fire at such high frequencies (Ranck, 1973; O'Keefe, 1976), the appearance of bursts with 2.5 msec. (400 Hz) interspike intervals is low (present observations). Recently more physiological tetanic frequencies have been used in an attempt to establish whether or not LTP is indeed a physiological phenomenon. Winson and Dahl (1986) indicate that stimuli at lower frequencies, if applied asynchronously but in combination so as to add to 400 Hz net

input (as seen by the DG granule cell dendrites) are particularly effective in producing LTP. Although these lower frequencies may be closer to physiological parameters, insufficient data are available concerning the normal firing characteristics of hippocampal cells. If indeed long term potentiation is a physiological phenomenon, it must depend on the firing characteristics of entorhinal and hippocampal cell. However, little information is available, to date, as to the behavioral state under which LTP may be most efficacious, or for that matter as to the precise firing characteristics of hippocampal cells during different behaviors. One part of this study will be an attempt at a detailed analysis of hippocampal unit firing during different behaviors.

E- Hippocampal Unit Recording

1- Preliminary Findings

Another approach to the study of hippocampal function has been the electrophysiological recording of single units. O'Keefe and Dostrovsky (1971) initially reported that units in the hippocampus increase their firing rate as a function of the animal's position in space. A small number (10%) of the hippocampal units fired maximally, when the rat was situated in a particular part of the testing platform. These investigators also noticed that while half of these units increased their firing simply in response to the animal's position in space, the rest increased their firing as a function of

both location in space and presentation of an 'appropriate' stimulus (i.e. tactile, visual, etc.). Most attempts to alter the unit's increased firing to a particular place field (turning all sound off, blocking part of the animal's visual field, etc.) seemed to have no effect. The firing rates of a small number of units changed only after drastic changes were made to the environment. These units were said to be very different from the cortical units reported by Hubel and Wiesel (1959), which responded to various visual cues (i.e. lines of a particular size and orientation, moving in a particular direction). The sensory correlates of hippocampal cells are much more complex than the visual units.

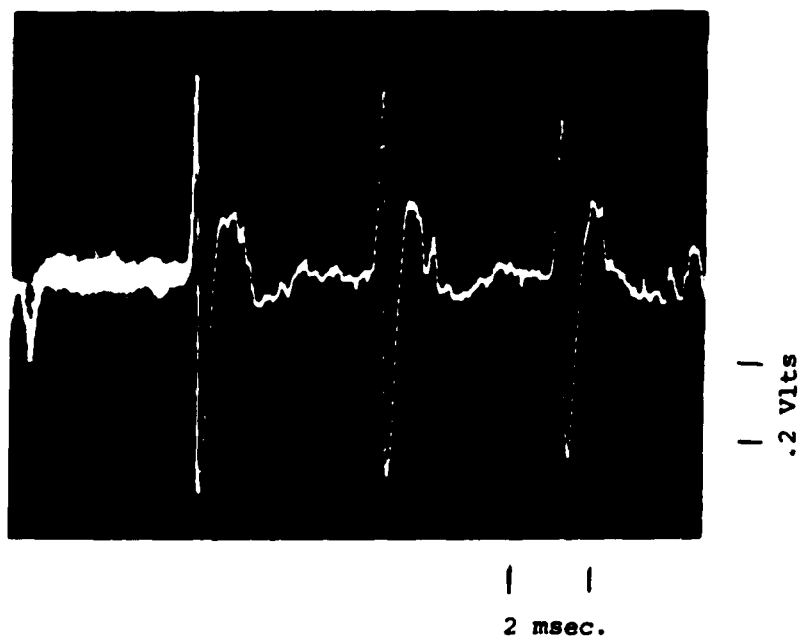
From these observations, O'Keefe and Dostrovsky (1971) concluded that the hippocampus of the rat plays a crucial role in the processing of spatial information and further postulated that adjacent units probably fire to adjacent place fields. From these and later evidence, O'Keefe and Nadel (1979) theorized that the hippocampus of rats functions as a 'cognitive' or 'spatial' map. Although the finding of place cells has been confirmed by many investigators, the hypothesis that adjacent units fire to adjacent place fields has not been shown to be true and if indeed if such a spatial map exists it must be configured in a rather complex manner.

2- Hippocampal Cell Types

Ranck (1973) reported the first quantitative and extensive study of hippocampal unit firing of rats engaged in various waking and

sleeping behaviors. Based on behavioral, firing, and spike characteristics, hippocampal units were subdivided into two distinct categories. The first of these was the 'theta' cell while the second was the 'complex spike' cell. Theta cells and complex spike cells differ on the basis of a number of criteria. Theta cells fire with shorter duration action potentials than complex spike cells (Ranck, 1973; Rose, et al., 1983; Suzuki and Smith, 1985) and, generally speaking, have significantly smaller peak-to-peak amplitudes than complex spike cells (Suzuki and Smith, 1985). A second and perhaps more important defining characteristic of these two cell types is their firing mode. Theta cells fire at much higher rates than complex spike cells and fire exclusively in single spike action potentials. Their firing increases only in the presence of the extracellularly recorded slow-wave (4-8 Hz) theta rhythm (Ranck, 1973; Rose, 1983; Kuperstein, et al., 1986). Their firing has also been shown to be phase locked to the theta rhythm. On the other hand, complex spike cells fire both in single spikes as well as complex spikes (thus the name complex spike cells). Complex spikes or bursts are defined as two or more (up to seven) spikes with short interspike intervals with each of the succeeding spikes showing a declining amplitude (see Figure 3). Although complex cells fire both in single spikes and bursts, most of the unit analysis done so far have considered the burst as a single event. As part of the dissertation, a detailed analysis of the bursting characteristics of these cells under different behaviors will be performed. Perhaps the most significant defining characteristic of theta and complex spike cells has been their behavioral correlates.

Figure 3. Oscilloscope trace of an extracellularly recorded complex spike. Note the evenly spaced action potentials and decreasing amplitude of successive spikes. Calibrations: .2 mv, 2 msec. (trace has been reversed to represent top as negative). Primary filters- Grass amplifier (model 7P511) bandpass set between 3 Hz - 3 KHz. Further filtration, to eliminate high frequency noise and to detect unit activity, was achieved with adjustable high pass active filters (1 KHz - 1 MHz, 24 dB/octive ; custom built, Rockefeller University electronics laboratory), which were set at either 3 or 5 KHz.


.2 Vlts
2 msec.

While the most important correlate of theta cells appears to be theta rhythm, the most important correlate of CS cells, in freely behaving rats, is place field.

Besides the high correlations of complex spike cell firing with an animal's position in space, these cells have also been shown to increase their firing in correlation with performance on a number of discrimination tasks including odor-discrimination (Eichenbaum, et al., 1987), tone-discrimination (Christian and Deadwhyler, 1986) as well as delayed match-to-sample tasks (Wible, et al., 1986). In a large number of experiments, Thompson and his associates have demonstrated that hippocampal complex cells also respond in a classical conditioning paradigm (for review see Berger, et al., 1986).

3- Neuroanatomical Identification and Distribution of CS and Theta Cells

Theta cells were originally hypothesized to be interneurons while CS cells were said to be projection (pyramidal and granule) cells (Ranck, 1973). This categorization was based strictly on behavioral criteria. Later studies (Fox and Ranck 1975; 1981; Christian and Deadwhyler, 1986), using electrophysiological, neurophysiological, as well as behavioral criteria came to similar conclusions. Two major criteria for cell identification were used in these studies. First, the distributions of CS cells closely overlap the known distributions of pyramidal and granule cells, while the distribution of theta cells

overlap with the distributions of interneurons. The second criterion used was based on the fact that CS cells could be antidromically activated from known projection areas of pyramidal and granule cells. Conversely, none of the theta cells could be antidromically activated. A different conclusion was reached by Rose and his associates (Rose et al., 1983; Rose, 1983). These authors recorded from dentate gyrus granule cells in anesthetized rats. A high percentage of the supposed granule cells from which they had recorded displayed theta cell characteristics (i.e. single spike action potentials, high rates of firing in phase with theta rhythm). Furthermore, it was demonstrated that a small number of these cells could be activated both antidromically as well as orthodromically with stimulation of the PP. Based on these findings, these authors concluded that granule cells were identical to Ranck's (1973) theta cells. Recently, Ranck has also recorded from at a few cells that could be activated with orthodromic stimulation (personal communication with Ranck), supporting the idea that at least some of the theta cells may be granule cells. Since all of these recordings have been done extracellularly, however, the exact identification of these two types of cells remains an open question. Regardless of the difficulties of cell identification, Ranck's classification of theta and CS cells is generally accepted.

The distribution of theta and complex spike cells in the hippocampal formation is quite wide. CS cells with place field characteristics have been reported in all hippocampal subfields (O'Keefe, 1976; O'Keefe and Conway, 1978; Olton et al., 1978; Miller and Best, 1980; Hill, 1976; 1978; Kubie and Ranck, 1983; see O'Keefe,

1979 for review) as well as in the dentate gyrus (Olton et al. 1978; Rose, 1983). Place cells have also been reported in the medial entorhinal cortex (Mitchell and Ranck, 1977; Quirk and Ranck, 1986). Some small differences in the spatial characteristics of these cells have been reported.

4- Behavioral Characteristics

Behaviorally, the firing characteristics of theta cells are thought to be rather simple. As mentioned previously, these cells fire at high rates most of the time and increase their rates only in correlation with theta rhythm- present in the awake states during locomotion and in the sleeping animal during REM sleep (Vanderwolf, 1969). Their rates of firing in the awake, still alert state, and during SWS are lower than their rates during the theta states (Ranck, 1973; Feder and Ranck, 1973).

In contrast to theta cells, the behavioral characteristics of complex spike cells are much more elaborate. To begin with, although the firing rates of individual complex spike cells vary to a large extent, their rates are nonetheless much lower (maximum rate of firing, most less than 20 spikes/sec) than those of theta cells (29-147 spikes/sec.; Ranck, 1973). In the tonic mode of firing, these cells show very low rates in still alert and exploration states. Low rates are also seen in REM sleep (Ranck, 1973; Suzuki and Smith, 1985). In comparison, their rates of discharge are relatively high in

the SWS state. The phasic mode of firing, in the majority of these cells during the awake state of the freely behaving rat, is believed to be spatially related. The percentage of cells that seem to function as place cells has been placed in the order of approximately 50 to 95 % (O'Keefe, 1979). A second category of complex spike cells, that do not appear to have place correlates, have been reported first by Ranck (1973) and later confirmed by O'Keefe (1979). These are the 'misplace' cells. The existence of these cells, however, is recently in doubt (personal communication with Ranck), and they will not be dealt with in this dissertation.

Since the original findings of 'place cells' a number of further observations concerning their firing modes have been made. For example, of primary interest has been the question of what are the sensory stimuli that influence place field firing. O'keefe and Conway (1978) studied complex spike cells with spatial characteristics in a cue controlled environment. It was determined that place fields are mostly influenced by distal cues in that rotation of the maze left the same spatial field intact, while rotation of the cues also rotated the place field in a similar direction. Systematic manipulation of particular cues had very little effect on the place field in the majority of cells recorded. For a place field to change or disappear, most of the distal cues had to be changed or eliminated and even then only a small percent of the cells ceased to fire to the particular place field. On the other hand, alteration of the proximal intramaze cues had very little effect on place field firing.

Somewhat more complex findings were reported by other investigators. Kubie and Ranck (1983) recorded from place cells in three different environments- an eight arm maze, a home cage and an operant chamber. They reported that a small number of the cells that they investigated displayed multiple place fields in all three environments. The majority of the cells, however, had place fields in a single environment. For most of the cells that they studied in the maze, the strongest factor determining place field was distal cues. Extra-maze cues, however, seemed to be of little importance in determining place fields in the other two environments that were investigated (home cage and operant chamber). Furthermore, knowing the place fields in one or two environments was of no predictive value in determining the place fields in a third environment. Kubie and Ranck (1983) also recorded from place cells in animals placed in a cylindrical enclosure. The distal cues in this environment were limited to a white cardboard on the side of the cylinder. Rotation of the cardboard also rotated the place field in the same direction. More interesting was the observation that if the cylinder and the white cardboard were replaced with larger ones, the place field of the unit also enlarged. Currently, it is believed that place fields are determined by complex, difficult to determine, environmental stimuli. It appears that in an open environment the most important determinant of place field firing, in the majority of place cells, is extra-maze, distal cues (for review see O'Keefe, 1979). Although this is the case, however, it is very difficult to determine which cues may be responsible for the formation of a particular place field.

Various other aspects of place cell firing have also been investigated. For example, place cells have been followed and recorded from in different environments and in many cases over many days. In terms of different environments, Kubie and Ranck (1983) reported that a few of these cells display place fields in more than one environment. Similar results were also reported by O'Keefe and Conway (1978). The place fields varied tremendously from one environment to the next, however, and no consistencies could be found between the different environments. On the other hand, the vast majority of cells displayed almost identical place fields across testing days. In an attempt to test the time course of place field development, Hill (1978) found that 10 of the 12 cells studied displayed place field firing almost immediately upon exposure to a new environment. Many of the units reported also showed multiple environment place fields.

Other aspects of place field firing have been looked at in closer detail. It has been repeatedly demonstrated that place field firing is very consistent with repeated visits of the animal to the cell's place field. Furthermore, with continued place field occupation by the animal only a slight habituation in firing rate of the cell is observed. In other words, place field firing remains relatively high for as long as the animal remains in the place field (O'Keefe, 1979). Other factors that may influence place field firing are directionality and velocity of an animal's movement within a place field (McNaughton, et al., 1983) as well as various other aspects of an animal's behavior within that field.

In summary, the picture that emerges so far concerning place cells is that these cells increase their rate of firing, over spontaneous rates, consistently in specific parts of an animal's environment. Cells may have multiple place fields and may also have place fields in multiple environments. In rats behaving freely in an open environment, the majority of place cells the determinants of place fields are most likely distal, extra-maze visual cues. In the few studies that measured tonic CS firing in different behavioral states, it was reported that CS cells display rather low firing rates in awake states, with the exception of in-place-field firing which is very high. High rates of firing are also observed in SWS while in REM sleep very low firing rates have been reported (Ranck, 1973; Suzuki and Smith, 1985; Olmstead, et al., 1973).

F- Overview of the Dissertation: Hippocampal Single Unit Firing Characteristics in Waking and Sleeping Behaviors.

Thus, it appears that the hippocampal formation plays a crucial role in information processing and more specifically in memory processes. Lesion studies, both clinical (human) and experimental (animal) have pointed to the role of the hippocampus in memory processes. Supporting evidence for such a hypothesis has also been derived from work on both LTP as well as electrophysiological recordings both of field potentials and single unit firing. The anatomical work points to the fact that the hippocampus is ideally situated for receiving higher order multimodal information.

Furthermore, the phenomenon of LTP may point to a possible mechanism by which memories may be processed and stored for long periods of time. The field potential and the single unit studies have suggested that information processing may occur in a behaviorally dependent manner. Although all of these studies are suggestive of information processing taking place in the hippocampal formation, none of them (with the exception of single unit recording) deal with the physiological mechanisms of how the brain may process information.

Experiment 1.

First, an experiment was performed in an attempt to answer the following questions:

1. If indeed the hippocampus is necessary for the formation and storage of memories, under what circumstances or behavioral states might information processing take place ?

2. Can any attributes of the firing characteristics of complex spike (place) cells be detected that may suggest information processing taking place in the hippocampal formation (i.e. rate of firing, bursting characteristics, etc.). And if so, is there behavioral specificity in the firing of these units ?

Hippocampal place cells with discrete non overlapping place fields were recorded in pairs. Having determined their place fields, the units were isolated from them overnight. The next day, following

confirmation of the place fields, baseline (off place-field) recording was made. Subsequently, one of the cells was exposed to its place field (while the second one was restricted from it), followed by continuous recording (for at least two hours) in waking and sleeping behaviors. Recording took place on the center of the maze which did not contain place fields for either unit. The second cell was then exposed to its place field and the procedure was repeated.

Experiment 2.

At the completion of the first experiment, a detailed descriptive analysis of the firing characteristics of hippocampal complex spike cells was performed. Most of the studies concerned with place field firing so far have dealt with the close analysis and description of place field firing. In very few of these studies have the tonic firing rates of CS cells been investigated in different behavioral states. Most frequently, the main unit of analysis used to describe the firing characteristics has been the average firing rate (single spikes and bursts counted as a single event). This is the case despite the fact that, as previously mentioned, these units fire both in single and complex spike action potentials. Furthermore, in all of the previous studies only short samples of unit firing, in the different behaviors, have been analyzed and presented as representative data.

Complete unit analysis was performed on the firing characteristics of hippocampal complex spike (place cells), in rats engaged in both waking and sleeping behaviors. The unit analysis

included, besides the mean firing rate, a complete analysis of the spiking characteristics (i.e. number of single spikes as opposed to bursts, interspike intervals, interburst intervals, etc.). This type of analysis was performed continuously over a long period of time and covering a number of waking (i.e. still alert, exploration, in-place-field) and sleeping behaviors (i.e. quiet-awake, slow wave sleep, pre-rapid-eye-movement sleep and rapid-eye-movement sleep).

CHAPTER 2

General Methods

A. Subjects.

Male hooded rats (300- 350 g: Charles River Labs) were used. The animals were housed in colony cages with littermates until shortly before surgery. Subsequently, each animal was housed individually in a plexiglass cage, lined with Purina bedding material. Ad libitum Purina rat pellets and water were available except on test days at which time the food was removed the night before and re-introduced following testing. The animals were rewarded randomly, on all parts of the testing box and also on all arms of the maze with Noyes precision food pellets. In some of the experiments, the animals were rewarded in the place field of a particular complex-spike unit with chocolate-chip cookies.

B. Apparatus.

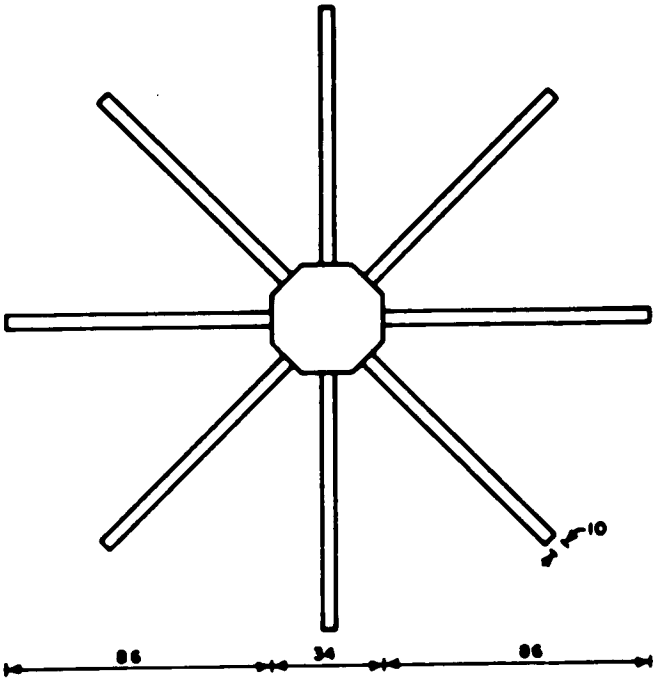
A small number of the animals was tested on a rectangular (112 X 65 X 112 cm) box in which one of the sides was open and a drop door was available that could fully enclose the box. A light, with a frosted glass, was placed on the ceiling of the box. An exhaust fan, at the side of the box, operated at all times. The fan not only

provided sufficient air circulation but also produced white noise so that the animal's sleep was not disturbed by external noises. Constant monitoring of the animal's behavior was performed via a closed circuit TV system. Visual monitoring of the animal was done through a one-way mirror which was placed on the door. It was possible to subdivide this testing cage into smaller compartments with a transparent plexiglass partition. Food and water was available in this testing apparatus when the animal was kept in the box overnight. A second group of animals was tested on an eight arm maze (see Figure 4; from Olton, 1983). Briefly, the maze consisted of eight, equally spaced arms (10 X 60 cm) which were attached to a center platform (34 cm wide). The arms radiated from a center platform, which was raised 85 cm off the floor. Easy detachment of each of the arms was possible. Testing took place with the maze located at the center of an approximately square room (3 long by 2.75 meters wide). The room had a window, a desk, book cases with books, the testing equipment, and the experimenter. The animal's view of the environment was not obstructed in any way.

C. Electrodes.

The electrodes were made of electroloy (60% nickel, 15% chrome, and 25% iron) 22.5 um in diameter, HiMOL insulated wire (Molecu-wire, Corp., Wall Township, N.J.). Multi-microwire electrodes were used for recording (Kubie, 1984). Ten separate microwires were threaded into a 26 gauge syringe which was attached to a center post of an

Figure 4. Eight arm radial maze used as testing apparatus for the first study (see text for description). Adapted from W. Seifert (Ed.). The Neurobiology of the hippocampus. Academic press, 1983. p. 341.

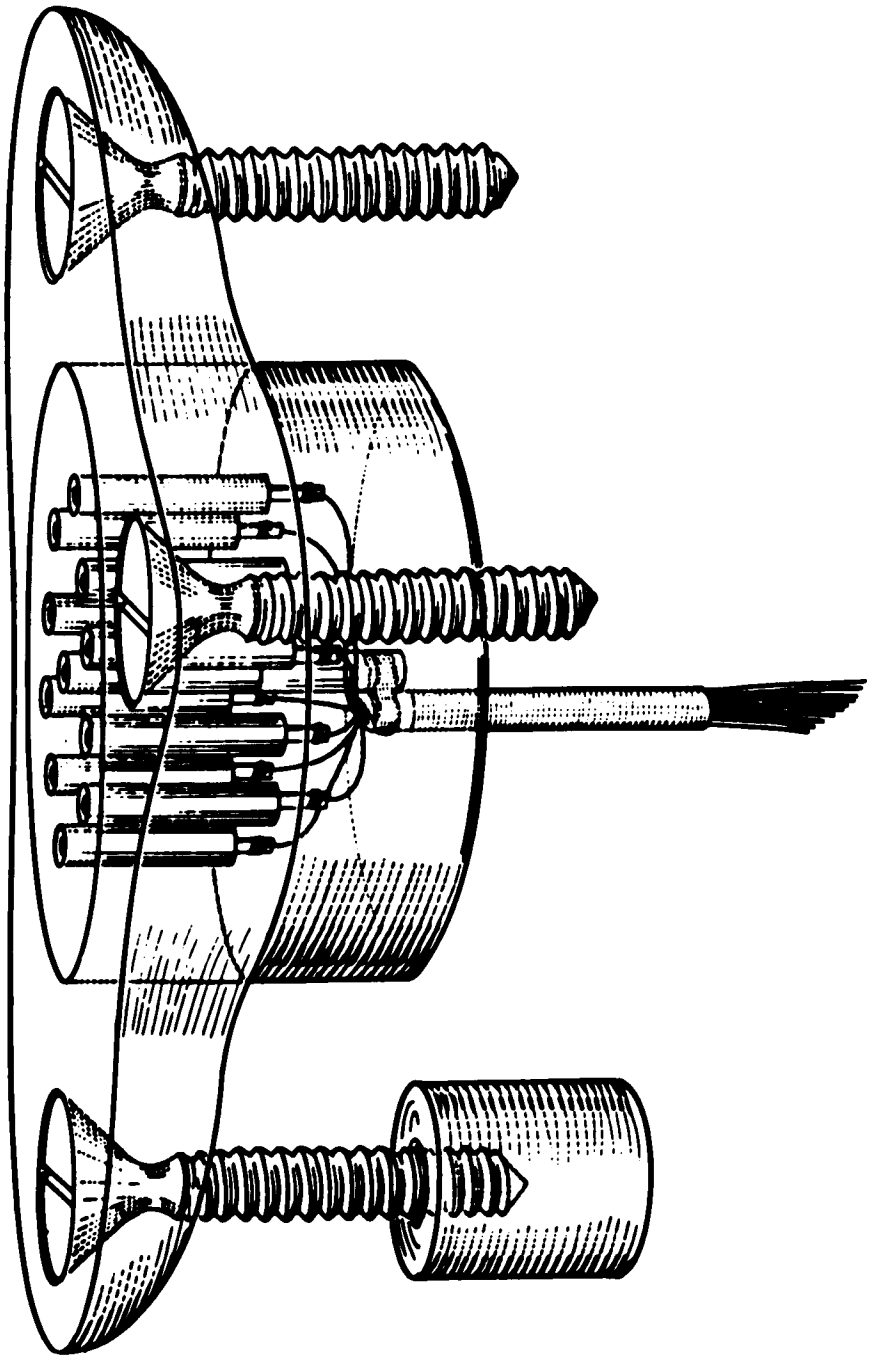


The radial arm maze

augat connector (Augat- 8058 IG34, LACO Electronics, Elmstead, N.Y.). Each of the microwires was then connected to one of the 10 pins on the augat connector. In order to guarantee electrical continuity, silver paint (Pelco colloidal silver liquid, Ted Pella, Inc., Tustin, CA 92680) was placed on the connections and the electrode was left to air dry for an hour. Finally, the electrode assembly, along with three supporting screws, were embedded in dental acrylic (see Figure 5). After the ten wire bundle was assembled the microwires were cut (2.5 mm from the syringe tubing) at a 10 degree angle. The 10 degree angle cut separated the ten electrodes a total of 100 um. This was essential, since the experimental paradigm required that at least two CA1 or CA3 pyramidal cells be recorded simultaneously. The CA1 pyramidal cell layer is approximately 50 um in width and, therefore, simultaneous recording of two units would be very difficult if the vertical microelectrode spacing exceeds 50 um. Although more than one unit could be recorded from the same electrode it was desirable that the units be recorded from separate electrodes since this made it easier to isolate the units. At the completion of all recording in a particular animal, the animal was decapitated (under deep nembutal anesthesia) the electrode assembly was removed, dipped in acetone, and most of the electrode assembly components, excluding the microwires, were cleaned and re-used.

Two screw electrodes were also used. These consisted of stainless steel screws fastened to nuts (Small Parts, Inc., Miami, Fla.,) and connected with a wire to a female Amphenol connector. After the electrodes were fixed on the animal's skull, these two electrodes were

Figure 5. Electrode assembly. Microwires are threaded through a 26 g. syringe and are individually wrapped to the 10 posts of the Augat connector. Three screws are then attached to the assembly with dental acrylic. The screw posts (seen on one of the screws) are fastened to the animal's skull. Clockwise rotation of the screws advances the electrodes into the animal's brain. (courtesy of Dr. J. Kubie, Downstate Medical Center).



inserted into a female connector that was implanted on the animal's headstage. These were used for recording the animal's hippocampal electroencephalogram (EEG) and for grounding purposes.

D. Surgery.

Animals were anesthetized with a 40 mg/kg, i.p. injection of chloral hydrate-pentoborbital (Chloropent; Fort Dodge Labs, Fort Dodge, Iowa; with supplemental doses as required) and stereotaxically (Kopf) implanted with the electrode assembly. The skin on the skull was opened and the bone was thoroughly scraped and cleaned. The skull was set level (on the bregma-lambda plane). Four small holes were drilled, two just anterior to bregma an either side of the midsagital suture, and two just anterior to lambda line, on either side of the midsagital suture. The two screw electrodes were fastened to the two anterior holes. On the posterior holes, screws were fastened firmly and they were used as anchors to which the electrode assembly was cemented to the skull. One of the two screw electrodes, placed above the frontal bones, served as an indifferent for recording the animal's EEG while the second was used to ground the animal to the equipment. The two female Amphenols were inserted into a three pin female connector. A semi-flexible copper wire was inserted into the third hole of the female connector. This wire was used to fasten the female Amphenol connector on the animal's head to the male connector used to plug the animal to the equipment. This last step prevented the animal from being disconnected from the equipment during movement. A fifth

hole was drilled on the skull (coordinates: 4.1 mm posterior to bregma, 2.6 mm lateral to the midsagital suture (left hemisphere), and 2.1 mm ventral to skull surface) where the hippocampal unit recording electrodes were inserted. The dura matter was severed and the ten microelectrode assembly was lowered 2.1 mm ventral to the skull surface. This initial electrode placement located the electrodes approximately .3 mm above the CA1 pyramidal field of the hippocampus. Following surgery, the animals were given a local antibiotic (Panolog ointment), to prevent infections and were allowed to recover for approximately a week before testing began. Following post operative recovery, the animals were placed on either of the two testing apparatus daily, for two or three days in order to become acclimated to the testing environment. The animals were food deprived the night before and were food rewarded on the testing apparatus. This was done to entice the animals in exploring the environment and thus allow the detection of place fields.

E. Unit Recording Procedure

Following post-operative recovery, the animals were placed on the testing apparatus and recording proceeded as follows: A male augat, fitted with field effect transistors (FET; source-follower configuration), in order to eliminate movement and other recording artifacts, was attached to the headstage of the animal. Three shielded, triple conductor cables (Cooner Wire, Co., Clatsworth, CA) on the other side of the connector led the signal from the animal to

an eight pin commutator (Micco, 1977). The commutator was fixed to the ceiling directly above the center of the testing box or the eight arm maze. This allowed the animals to move freely or sleep on the two testing apparatus without entangling the wires (see Figure 6). The unit signals were then led to a Grass amplifier (Model 7P511) with the filters set between 3 Hz - 3 KHz. Further filtration, to eliminate high frequency noise and to detect unit activity, was achieved with adjustable high pass active filters (1 KHz - 1 MHz, 24 dB/octive ; custom built, Rockefeller University electronics laboratory), which were set at 3 or 5 KHz. The signals were recorded on the two audio channels of a video cassette recorder (Sony Beta I) for later analysis. The signals were also led simultaneously to a window discriminator for unit discrimination, and finally to a digital oscilloscope (Tektronix, model 5223) for on-line viewing.

Besides recording from one or more hippocampal units simultaneously, the hippocampal EEG was also recorded differentially between one of the hippocampal electrodes and the skull screw indifferent. The signal was fed to a Grass amplifier (model and filtered between 3 and 20 Hz (24 dB/octive), for clear detection of the various behavioral states (to be discussed below). The EEG signal was viewed on one of the three oscilloscope channels available and was also recorded with a video camera (RCA, model TC2011, with 16 mm lens) from one of the two oscilloscope channels for off-line analysis. This was achieved by monitoring the oscilloscope screen which was displaying the firing of two units and the EEG trace.

Figure 6. Photograph of rat in the test box with FET source follower attached to the animal's headstage. The recording lead was hooked to a counterbalanced, rotating arm mounted on the center of the testing apparatus (not shown here) which minimally limits on the animal's behavior.

The animal's behavior was also recorded with a second video camera (RCA, model TC2011/U ultracom) equipped with a manual zoom lens (12.5-75 mm) for close observation of the animal's behavior. This camera rested on a tripod on the side of the testing box or the side of the eight arm maze. The two video signals were fed into a splitter-inserter (RCA TC1417A) and were then fed into the single video input of the Sony Beta system (SLO-323), and recorded on Sony Dynamicron, L-500 or L-750 video cassette tapes. The display of the video monitor (RCA, 35 cm B/W monitor, model TC1214) was split into two displays, one of these showing the animal's behavior while the second was showing the signals of two units and the hippocampal EEG. An RCA date time generator (TC1440B) displayed the date and time while recording was taking place, and on play-back. The electrode assembly was lowered at 25 um steps by screwing the three screws on the top of the electrode assembly in a clock-wise direction (1/16 turn = 25 um).

F. Testing Procedures.

Following post operative recovery, each of the electrodes was checked for its ability to record and the availability of units. In the event that no units were located on any of the ten electrodes, the whole electrode assembly was lowered by 25 um and, after approximately two hours (allowed for the electrodes to settle in the brain), all the electrodes were tested again for the availability of place cells. All testing for place cells and subsequent sleep recording took place in a single testing apparatus (i.e. either the box or the eight arm maze).

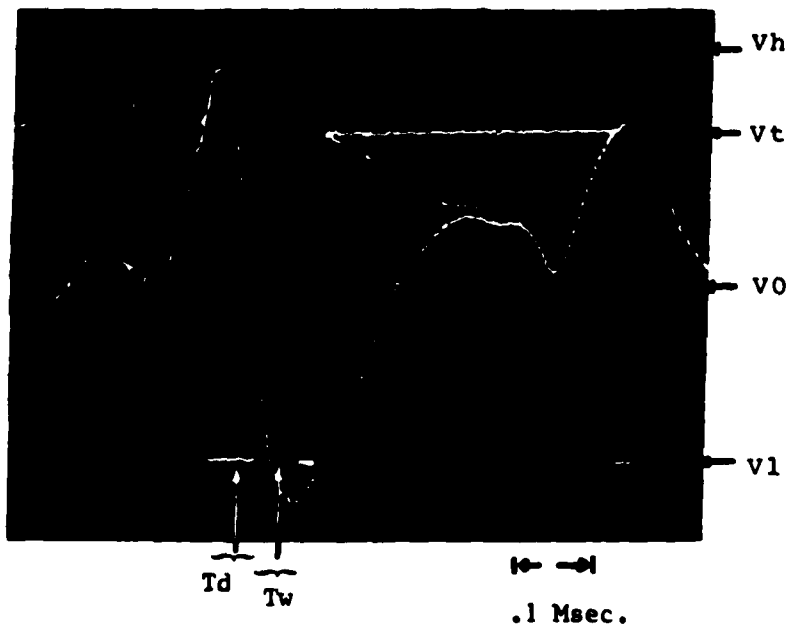
Non of the animals were tested in both of these environments. An exact record of the electrode movement was recorded in order to allow the calculation of the exact location of the electrodes at the completion of the experiment. This procedure was repeated until two well discriminable units (signal to noise ratio of over 3:1) with different place fields were encountered. In the event that this was accomplished, actual recording began. The exact experimental paradigm will be discussed in more detail for each of the experiments performed.

G. Unit Discrimination and Data Analysis.

Unit discrimination was achieved through the use of a custom built, dual digital window discriminator (Rockefeller University electronics laboratory). Available on the discriminator were three adjustable voltage windows and two adjustable time windows. The signal to be discriminated had to fulfill all of the specified window restrictions to register as a spike. The three voltage windows were, a voltage trigger (Vt), through which the signal had to cross; a voltage high (Vh) through which the signal could not cross; and a voltage low (Vl) window through which the signal had to cross for a spike to register. If all the parameters were met, the discriminator produced both a .4 usec TTL and a +15 volt output. A digital display of the output was also available on the discriminator. The time windows consisted of a time width (Tw) and a time delay (Td) parameter (see Figure 7).

Figure 7. Oscilloscope trace of extracellularly recorded action potential along with markers showing adjustable discriminator settings. Marked on the photograph are the following voltage windows: voltage trigger level (Vt) through which the signal has to pass, the voltage high level (Vh) which must not be exceeded, and the voltage low through which the signal must pass. The voltage zero (V0) or baseline is also marked. Also marked are a number of time windows including the time delay (Td), and the time width window (Tw). In order for a signal to register it had to satisfy all of the set parameters. Note that the signal has been reversed to show negative up.

Figure 7



Data analysis was performed as follows: the video tapes were played back and the animal's behavior along with the EEG was displayed on the video monitor. The unit signals were fed back into the window discriminator, the settings of which had been arranged so that only a single unit would register on the discriminator. At times, unit discrimination did not turn out to be such a trivial task. Unit discriminator difficulties arose for a number of reasons. For one, most of the time a single electrode would record more than one unit, due to the compact arrangement of hippocampal pyramidal cells. Secondly, hippocampal complex spike cells fire in both single spikes and in bursts (see Figure 3, page 21; Ranck, 1973; Kandel and Spencer, 1961). A burst consists of a variable number of spikes of decreasing amplitude. Since in this study the firing characteristics of all spikes within a burst were analyzed, the discriminator parameters were set wide enough to include all of the spikes within the burst while excluding the spikes of different cells. This was accomplished by first choosing only units with a high signal to noise ratio and also recording only from cells that were sufficiently isolated from other units. If more than one units were available, their wave forms had to be sufficiently different to allow easy differentiation from each other.

The TTL output of the discriminator was fed into a custom built (Dejong, 1982; input/output (I/O) card that was installed into an Apple IIe computer. The I/O card consisted of dual precision timers (6522 microprocessor) which in combination with custom written software performed the task of analyzing the bursting characteristics

of the units for this dissertation. Briefly, a data acquisition program (written in machine language to permit various calculations that had to be performed in microseconds) with adjustable parameters, was started simultaneously with the Sony tape recorder. Once an input was detected by the I/O card, the timer began counting. If another spike appeared within a certain period of time (10 or 15 msec.- present at the beginning of the data analysis) then that spike was counted as part of the same burst. This was continued until the spike following the previous one exceeded the 10 or 15 msec. interval. In this case, a number of calculations pertaining to the burst were performed (i.e. Inter-spike interval (ISI), Inter-burst interval (IBI), number of spikes within the burst, etc.), the averages of these calculations were kept in random access memory, the timer was cleared and reactivated to detect the next spike or burst. This analysis continued either until the animal changed his behavioral state (as could be determined by the video tape and in sleep behaviors also aided by the animal's hippocampal EEG), or if a particular behavior was maintained for more than five minutes, the data analysis was terminated, the data was stored on floppy-disks and the analysis continued.

The animal's behavior was subdivided into eight different categories. These included a number of awake behaviors: exploration (XPL); still alert (SAL); in-place field (IPF); out of place field (OPF), as well as a number of sleep behaviors. These included: quiet-awake (QA)- animal standing still with a small increase showing in the EEG amplitude (it should be emphasized that based on both the animal's

EEG- increase in amplitude and the appearance of slow wave spikes, as well as his behavioral state- lying still, that this should be considered to be a drowsy as opposed to an awake state; slow wave sleep (SWS)- large increase in EEG amplitude and the appearance of large irregular slow wave spikes; Pre rapid-eye-movement (PreREM; EEG began to synchronize, the animal's breathing became heavier, whisker movements became apparent, however, the animal either became alert after a few seconds or it entered into rapid-eye-movement sleep (REM). REM was evidenced by theta rhythm (4-8 Hz synchronized EEG), eye and whisker movements, heavy, irregular breathing and head collapsing to the floor of the maze or box. Each particular sleep state was determined by observing an animal's behavior in the video display monitor and also observing the animal's EEG. All of the data analysis done for these experiments were performed by a single experimenter. However, a number of tapes were also scored by an individual that was blind to the experimental condition or design. This was done to eliminate any experimenter effects.

Statistical analysis was performed on all the unit firing data, using a number of standard statistical procedures which will be described in greater detail in the results section.

H. Histology.

At the completion of all recording in an animal, the most ventral location of the recording electrodes in the hippocampus was localized

histologically. The exact location of a particular microelectrode was determined by the Prussian Blue method. The animals were deeply anesthetized with an i.p. injection of either chloropent (60 mg/kg) or a 2 ml injection of sodium pentobarbital (Nembutal; Abbott laboratories) and a 10 uA current for 10 seconds was passed between one of the recording electrodes (positive) and the skull indifferent (negative). The animals were then perfused transcardially with a 9% saline solution mixed with 10% formaline, 4% potassium ferrocyanide, and 4% acetic acid. The brains were removed and stored in a 10% formaline, 40% sucrose solution for two days. They were then cut by the freezing method, at 40 um section thickness, mounted on slides and stained using the Kluver-Barrera method.

CHAPTER 3

Experiment 1: Simultaneous, Dual 'Place Cell' Recording.

To reiterate, the objective of the first study was to test the hypothesis that complex spike cell firing, in the various sleep states tested, would change following increased firing of the cell during the animal's awake state. More specifically, it was predicted that if a 'place cell' was exposed to its place field and was allowed to fire extensively there, an increase in its firing characteristics would become apparent in the subsequent sleep episodes. The design used to test this hypothesis was to record from two place cells simultaneously, each with a place field in a different part of the testing apparatus. This way, each of the 'place cells' was exposed to its respective place field at different times in a counterbalanced manner, followed by recording of both units during sleep.

Methods

Procedure

Over two hundred units were observed in both the dorsal and ventral part of the hippocampus as well as in the dentate gyrus. However, before units were included in the study, the following criteria had to be met: (i) the unit had to display complex spikes during the initial course of testing; (ii) units had to have a signal to noise ratio of at least 3-to-1; (iii) the cells had to display clear and discrete place fields; (iv) at least two units with the above mentioned characteristics had to be present simultaneously and to display disperse, non-overlapping place fields. It was also preferable that the two units be recorded from different electrodes; (v) finally, for the units to be included in the study, they had to be exposed to their respective place fields independently of each other and in a counterbalanced manner. Seven pairs of units recorded met the criteria set for the first study. Of these, two pairs (four cells) were recorded simultaneously. One of the four cells within this group, however, displayed extremely high rates of firing most of the time and was excluded from the analysis. The other five pairs were recorded separately and on different experimental days.

On the day preceding recording, hippocampal complex spike cells, meeting the above criteria, were checked thoroughly for place fields on the maze. These were carefully mapped and documented. The animal

was then returned to his home cage which was placed on the center of the maze. At this time the home cage was also checked for place fields in order to ascertain that none existed. On the following day, the home cage was checked again for place fields. In the event that none existed, the animal was placed on the eight-arm maze and all the arms were checked briefly for place fields. This was done to ensure that the place fields of the previous day were still intact and that no new ones had developed. If no major complications arose and the old place fields were still intact, baseline recording for at least one hour commenced. Following baseline recording, one of the cells was exposed to the place field for approximately 10-15 minutes following which the animal was isolated on the center of the maze. Continuous recording followed for at least two hours or until the animal had undergone a number of complete SWS and REM episodes (in all cases recording lasted for at least two hours after a cell had been exposed to the place field and while the animal was restricted to the center of the maze and was engaging in sleep). In a small number of cases (approximately 15%), the animal failed to go to sleep for a long period of time (30-45 min.) after exposure. When this happened recording was discontinued temporarily and resumed when the animal settled down for sleep. Since, in later off-line analysis, the firing characteristics of these cells did not seem to differ from the rest of the cells, these units were not analyzed separately. At the completion of a number of sleep episodes the animal was exposed to the second cell's place field for approximately the same amount of time and the sequence was repeated. The order of cell exposure was arbitrary. At the end of

the experiment, the place fields were checked again to ensure that they were still present. All recording took place between 10:00 am and 6:00 pm.

Determination of the animal's behavioral state was done by observing both the animal's behavior and also the electroencephalogram (EEG). These two criteria were used to subdivide the animal's behavior into the seven different categories (see methods section for description). At the beginning of all data reduction an independent observer reviewed the recorded data in order to confirm the animal's behavioral state. Since there appeared to be almost complete agreement between the observers, almost all of the recordings thereafter were reduced by a single experimenter. In some cases a third person, blind to the experimental paradigm, performed the data reduction, with supervision by the primary investigator. In a few cases the data reduction was performed twice, first by the primary investigator and again by a person blind to the experimental design. This last procedure was undertaken in an attempt to discount any experimenter effect that may influence the results. Inspection of the data did not reveal any noticeable differences in the unit firing. At times, value judgements had to be made as to whether the neuronal firing observed was actually generated by the unit under investigation or the result of other units with similar spike characteristics traversing the discriminator settings. In such cases the discriminator settings were readjusted to exclude the other units. In the event that the readjustment did not produce proper results, the unit was precluded from the final analysis.

The firing characteristics of each pair of units have been analyzed in the following manner: the recorded signals were played back and the units were carefully discriminated to ensure that other units did not contaminate the analysis. The data was analyzed continuously for a particular behavioral state as determined from the animal's EEG along with the animal's behavior simultaneously being displayed on the TV monitor (see methods section for details on determination of behavioral state). The continuous data reduction lasted for the period of a particular behavioral state or at most for five minutes, which ever was shorter. For each of the parameters tested (i.e. rate, bursts, etc.) an average was achieved for each of the behavioral states. Although there was considerable loss of information in collapsing all data into a single average this measure was appropriate in answering the the main question of the hypothesis, namely, does exposure to place field influence unit firing in subsequent sleeping behaviors. For the final statistical analysis the data were subdivided into pre- and post-exposure intervals. An analysis of variance was then performed with exposure comprising one of the factors and behavioral state the second factor. When significant main effects were obtained with the analysis of variance, simple effects for behavioral state or the interaction of behavioral state by exposure to place field were analyzed with the use of the Schaffer-Welsh or the Tukey tests. These tests allow for the detection of differences between means.

The firing characteristics of these complex spike (place) cells were analyzed on a number of different parameters. These included: the

average firing rate (single spikes as well as all spikes within a burst); the average bursting rates (all complex spikes excluding single spikes); the average percent bursts (versus single spikes) as well as finer analysis of these (single spike, double spike, triple spike, etc.); the average interspike intervals (within a burst) as well as subdivisions of these into finer intervals (0-2, 2-4....8-10 msec.). These interspike intervals were chosen to span the whole range of interspike intervals observed for complex spike cells and were included in the analysis to allow the detection of changes that may take place on this parameter that may not be detected by analysis of the average ISI alone. Finally, the average interburst intervals were also examined (see Figure 8).

Results

Overall View of the Results

The major findings of this study are the following:

1. The hypotheses being tested in the study were confirmed, namely, an increase in the firing rate occurred in the subsequent sleep episodes, following exposure of place cells to their place fields. In contrast, the cell not exposed to its place field remained relatively inactive. Further, the firing rates of each of the place cells remained relatively constant in the awake states, throughout the experimental session.

Figure 8.

Histograms of spike characteristic analysis. For each behavioral episode, analysis of the rate of firing was made in 15 second episodes. Graph A (leftmost) represents the rate of firing (single spikes as well as all spikes within a burst). Each bar represents a 15 second period. The analysis lasted for the length of a behavioral state or for a maximum of 5 minutes. At the conclusion of each behavioral episode, the number of single spikes as well as bursts with 2, 3, 4, 5 and 6+ spikes were analyzed (see graph B). The average inter-spike-intervals as well as finer subdivisions of these (0-2, 2-4, 4-6, 6-8, etc.) were calculated (see graph C). Finally, subdivisions of the percent inter-burst-intervals were computed and histograms were made (see graph D). This analysis was made throughout the recording period and was used to calculate the various parameters analyzed in the present studies.

Rate/ 15 sec.
% spikes/Total Period
Subdivisions of ISI
Subdivisions of ISI
msec
msec
AVG ISI: 4.215
% ISI
% SPIKES

Figure 8

2. An increase was observed in the number of bursts following exposure. Also, the interburst intervals were lower following exposure.

3. A significantly higher number of bursts containing three or more spikes appeared in the SWS, PREM and REM states following exposure than prior to exposure. Similar increases in the number of spikes within a burst were not evident for the awake behaviors (SAL and XPL).

4. The firing rates of these units differed significantly in the different behaviors tested. In decreasing order, the rate of firing in the different behavioral states was as follows: in-place-field, slow-wave sleep, quiet-awake, pre rapid-eye-movement sleep, rapid-eye-movement sleep, exploration, and still-alert.

5. An increase was observed in the number of bursts with shorter ISI's (2-4 msec.) following exposure of the cell to its place field.

6. Rewarding an animal while exposing the cells to their place fields failed to affect the rate of firing in subsequent sleep episodes.

Detailed Findings

a. Histology

Histological analysis of the rat's brains revealed that all of the 14 units included in this study were located in the dorsal part of the hippocampus. Of these, three were located in the CA1a subfield while the remaining 11 cells were located in the CA1c hippocampal subfield (see Figures, 9a,b;10a,b).

b- Unit Firing

The firing rates in the in-place-field state increased at least 3-fold or more over all the other places, as well as other awake behavioral states, and at least 2-fold over the sleeping rates (see Table 1). All of these cells displayed distinct place fields which, within a pair of cells, were in different places on the eight-arm maze. At least half of the cells tested had multiple place fields and the area of increased firing differed from a very small area (few inches) to covering a much wider area (half an arm of the maze). In all of these cases, however, increased firing within one of the place fields was much greater than the other place fields. This observation was made at the time of recording and it was utilized to determine which of the place fields the cells were going to be exposed in.

The average firing rates (in spikes/sec.) of the fourteen cells, in all of the behavioral states tested, are presented in Table 1. The

Figure 9.

a. Coronal schematic of the rat brain at the approximate position of the recording electrodes, in animal HUR-32 (Koenig and Klippel, 1974 atlas). Abbreviations: HI- hippocampus; GD- dentate gyrus.

b. Coronal photomicrographs of the histological sections containing the recording electrodes in animal HUR-32. Note that while some of the electrodes advanced perpendicular to the hippocampus, one electrode diverged an on approximately 45 degree angle to the rest of the bundle. At the completion of the recording in an animal, the final position of the electrodes were marked by the Prussian blue method (see methods section). In this and the remaining photomicrographs, the placement of the electrode does not necessarily imply that the units were recorded at this final position. Note that the recording site was in the CAla pyramidal cell layer. (Abbrev. CC- Corpus Callosum; Cx- Cortex; DG- Dentate gyrus; Pyr- pyramidal cell layer).

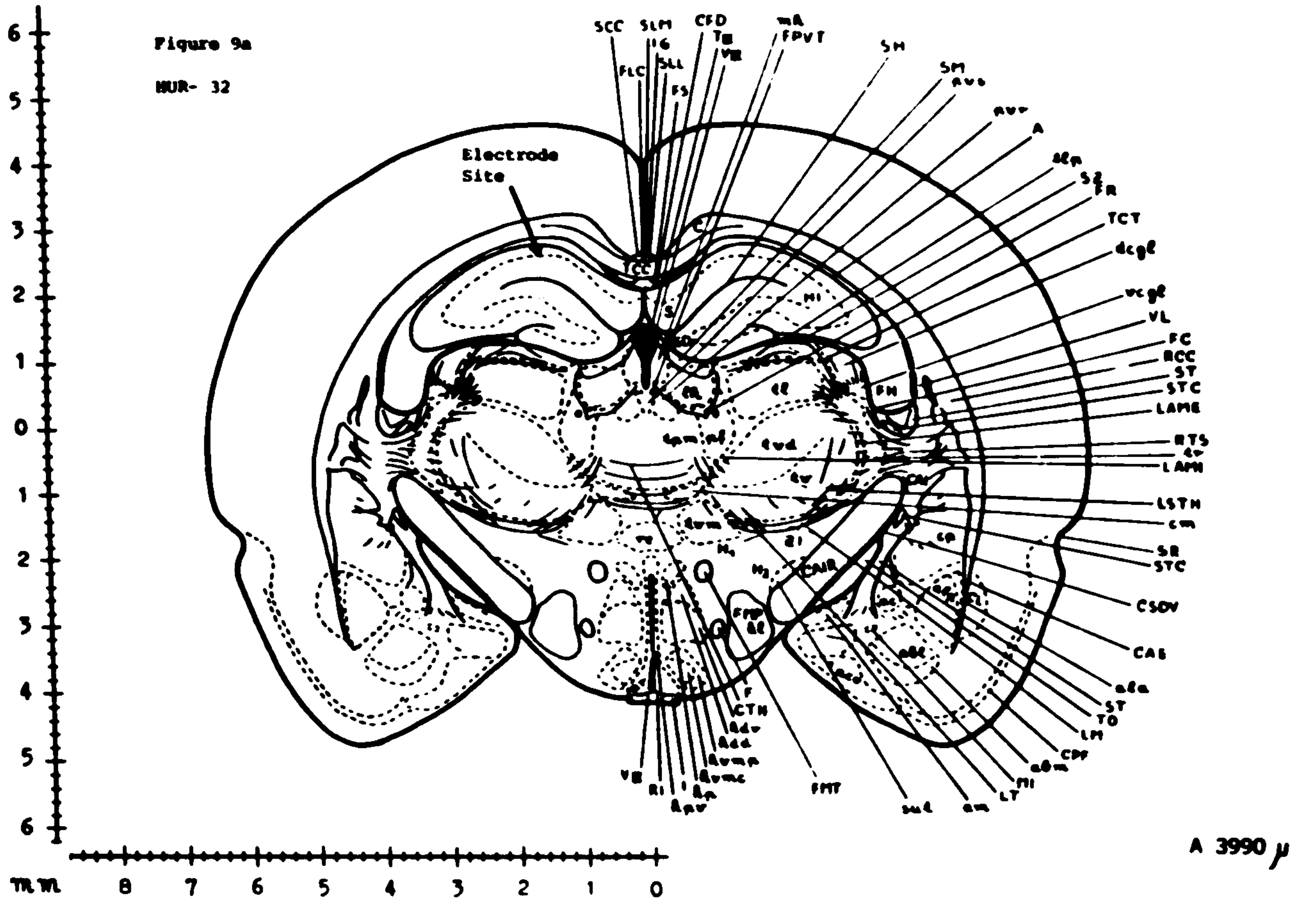


Figure 9a
HUR- 32
Electrode Site
SCC
SLM
CFD
mA
FPVT
SM
A
TCT
VL
FC
RCC
ST
STC
LAME
RTS
LAMH
LSTH
SR
STC
CSDV
CAS
TO
LM
CPF
LT
FMT
VE
CTH
A 3990 μ
mm
8
7
6
5
4
3
2
1
0

Figure 9b

Figure 10.

a. Coronal schematic of the rat brain at the approximate position of the recording electrodes, in animal HUR-38 (Koenig and Klippel, 1974 atlas). Abbreviations: HI- hippocampus; GD- dentate gyrus.

b. Coronal photomicrographs of the histological sections containing the recording electrodes in animal HUR-38. On the right, upper corner, the electrode tract and the prussian blue stain (marking the final position of the electrodes) could be seen. The position of these electrodes were in the CA1c pyramidal cell layer. (Abbrev. CC- Corpus callosum; DG- Dentate gyrus; Pyr- Pyramidal cell layer).

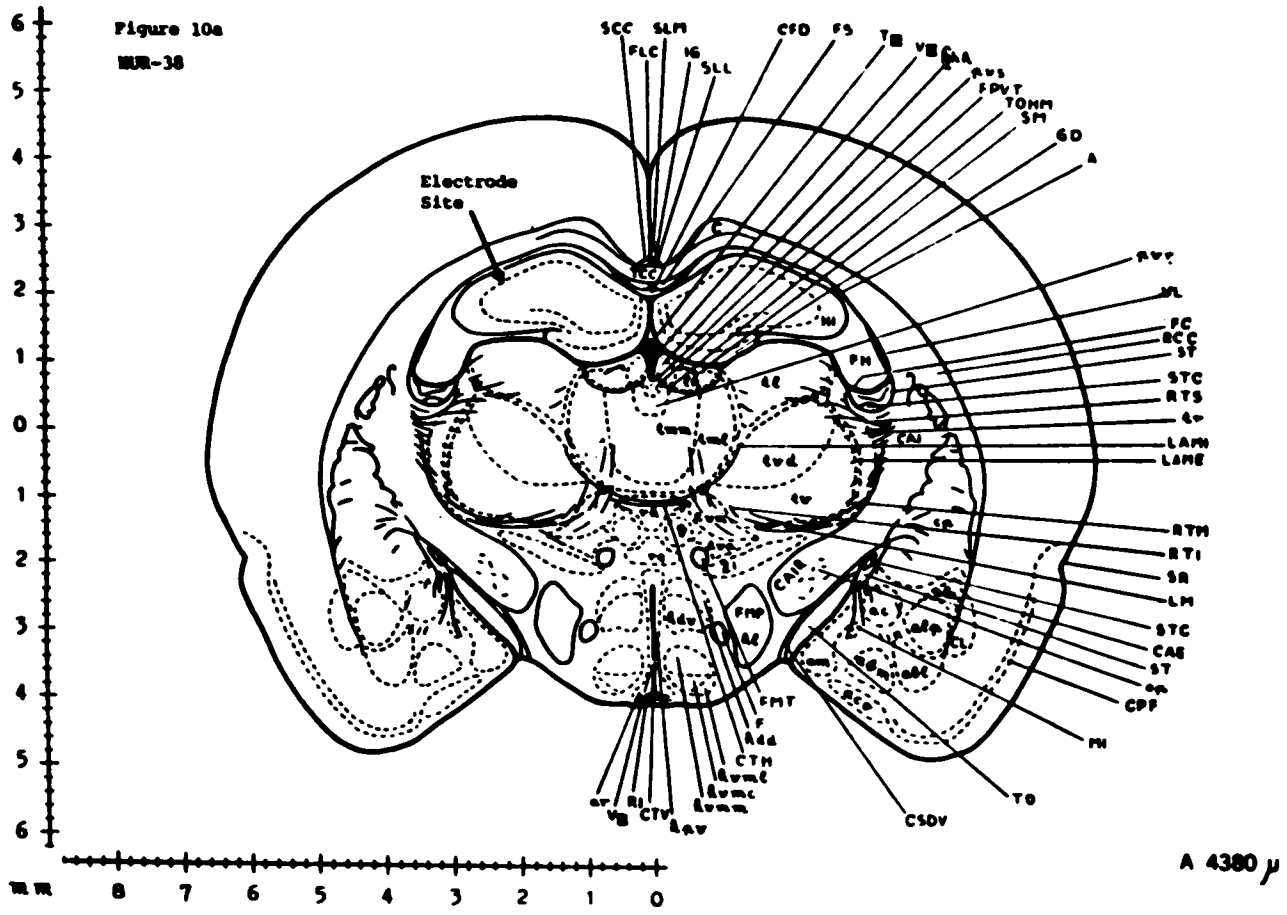


Figure 10a
MUR-38
Electrode Site
SCC
SLM
FLC
IG
SLL
CFD
FS
TB
VB
AA
AVS
FPVT
TOHM
SM
GD
A
AVT
AL
FC
BCC
ST
STC
RTS
LAM
LAME
RTM
RTI
SR
LM
STC
CAE
ST
CPP
PM
TO
CSDV
FMP
FMT
CTM
CTV
MR
8
7
6
5
4
3
2
1
0
A 4380 μ

Figure 10b

Table 1.

This table presents the rate of firing (spikes/sec., including all spikes within a burst measured separately) of the 13 complex spike (place cells) included in the counterbalanced study. The units were recorded in pairs, and they were all found in the CA1 hippocampal field. The firing rates are separated in pre- and post- exposure means and calculated separately for each behavioral state.

The highest rates of firing are seen in the in-place-field (IPF) state. The lowest firing is seen in the out-of-place field awake states. There do not appear to be pre- post in the firing rates in the awake states. In comparison, significant pre- post differences in the firing rate was observed in all of the sleep states. The large standard deviations reflect the wide range of firing rates among the individual units.

Table 1

Rate (spikes/sec.)

	XPL		SAL		IPF	QA		SWS		PREM		REM	
	pre	post	pre	post		pre	post	pre	post	pre	post	pre	post
Mean	.14	.13	.15	.19	2.17	.27	.34	.28	.44	.27	.37	.17	.27
SD	.30	.21	.18	.18	1.62	.15	.23	.16	.28	.17	.28	.17	.22

highest firing rates were observed in the IPF state ($\bar{X}$ = 2.26) followed by SWS ($\bar{X}$ = .36), PREM ($\bar{X}$ = (.32), QA ($\bar{X}$ = .31), REM ($\bar{X}$ = .22), SAL ($\bar{X}$ = .17) and XPL ($\bar{X}$ = .15) (note that the firing rates for pre- and post-exposure to place field were collapsed into a single mean).

The counterbalanced procedure used in this experiment was tested, on the rate of cell firing, with a three way analysis of variance, with the three factors being, order of testing (pre and post), behavioral state, and time of testing. A significant interaction was achieved for the order by testing-time factors [$F(1,11) = 6.43$; $P < .05$]. The interpretation of this finding is that the counterbalancing succeeded. Thus, the unit that was exposed increased its firing in the subsequent behaviors while the unit that was not remained relatively inactive. When the second unit was later exposed to its place field, it then increased its firing rate in the sleep episodes that followed while the first unit subsided to a baseline rate (see Figures 11 & 12).

Pre- post-exposure comparisons of the units' firing rate revealed a significant effect of stage of testing [$F(1,12) = 6.57$; $P < .02$] (see Figure 13). Thus, following exposure of the units to their place fields, there was an increase in the firing rate of these cells in the subsequent behaviors. A significant interaction between stage of testing and behavior was also found [$F(5,60) = 2.40$; $P < .05$], indicating that not all of the behaviors tested increased their firing rate following exposure. Simple effects analysis of behavior by stage

Figure 11.

Histogram of rate of firing for a pair of place cells, recorded simultaneously. Unit recording took place for the following behavoiral states:

X- exploration

A- still-alert

I- in-place-field

Q- quite-awake

S- slow-wave-sleep

P- pre rapid-eye-movement sleep

R- rapid-eye-movement sleep

O- other (any behavior that did not fit into any of the above categories; i.e. feeding, chewing, grooming, etc.)

In the figure, the top of the line represents the awake behaviors while the bottom of the line represents the sleeping behaviors. At the beginning of recording each arm was tested for place fields. Each of the arms, beginning with arm one, were tested successively. For unit-1 increased firing was seen only in arm one, while for unit-2 increased firing was seen in arms two and seven. The rate of firing for arm two, however, was higher than for arm seven. Thus the unit was exposed to arm two. Each bar on the histogram represents one episode. Each episode is not necessarily of equal time length.

Figure 11 (cont.)

Following a brief place field testing, in order to verify the place fields measured on the previous night, baseline was recorded for approximately two hours. During this time the animal was restricted to the center of the maze in which these units did not have place fields. Baseline rates were not included in the analysis. At the end of baseline recording, unit-1 was exposed to its place field, on arm-1, for approximately 15 minutes, while the second unit remained unexposed. Following unit-1 exposure, recording took place for approximately two hours. The second unit was then exposed to its place field, on arm-2, and the process was repeated. At the end of the experiment the units were checked again to ensure that the same place field were still intact.

As noted in the text, similar tests carried out on 7 pairs of units revealed higher rates of firing following exposure.

Figure 11

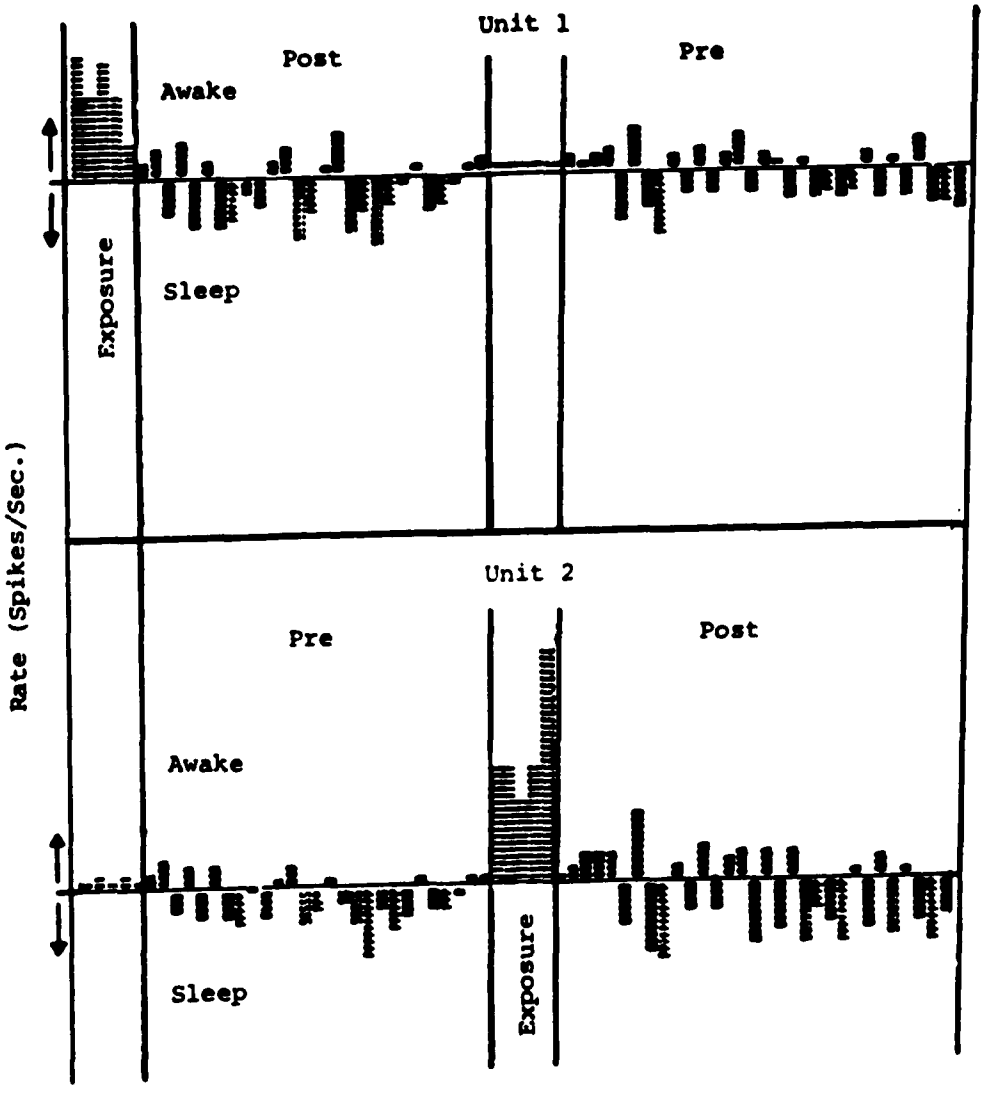

Unit 1
Post
Pre
Awake
Exposure
Sleep
Unit 2
Pre
Post
Awake
Sleep
Exposure
Rate (Spikes/Sec.)
Episodes in Serial Order

Figure 12.

Line graph of unit firing for a pair of units recorded simultaneously and in a counterbalanced manner. The four graphs show each of the four sleeping states tested (12a-QA; 12b-SWS; 12c-PREM and 12d-REM). Initially, one of the units was exposed to its place field, followed by sleep at the center of the maze. Subsequently, the other unit was exposed to its place field, followed by sleep. The figure shows that the counterbalancing procedure used in the first study succeeded. Namely, the unit that was exposed first increased its firing in the subsequent sleep episodes while the second unit's rate remained at a low rate and vice versa. Testing-order refers to the unit's order of exposure (i.e. order 1 refers to exposed-unexposed while order 2 refers to unexposed-exposed.

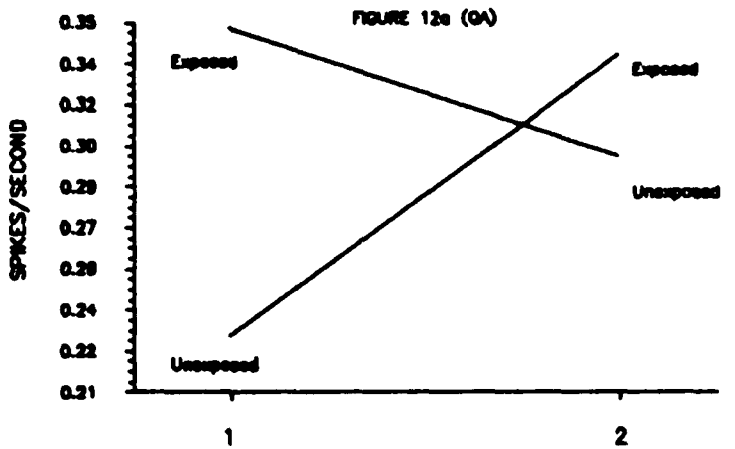


FIGURE 12a (OA)
SPIKES/SECOND
0.35
0.34
0.32
0.30
0.28
0.27
0.26
0.24
0.22
0.21
Exposed
Unexposed
Exposed
Unexposed
1
2

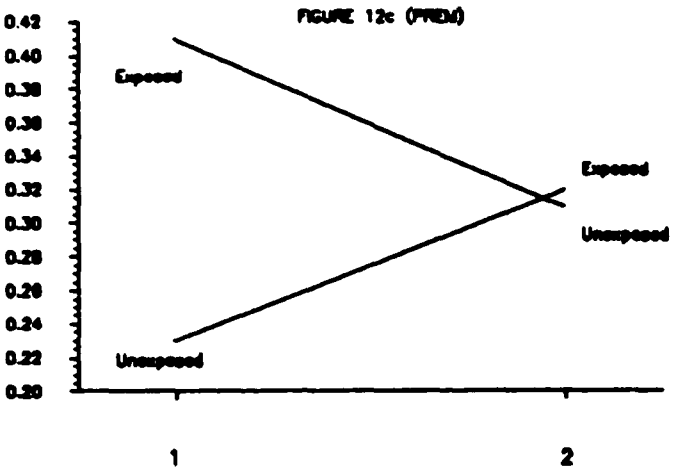


FIGURE 12c (PREM)
0.42
0.40
0.38
0.36
0.34
0.32
0.30
0.28
0.26
0.24
0.22
0.20
Exposed
Unexposed
Exposed
Unexposed
1
2

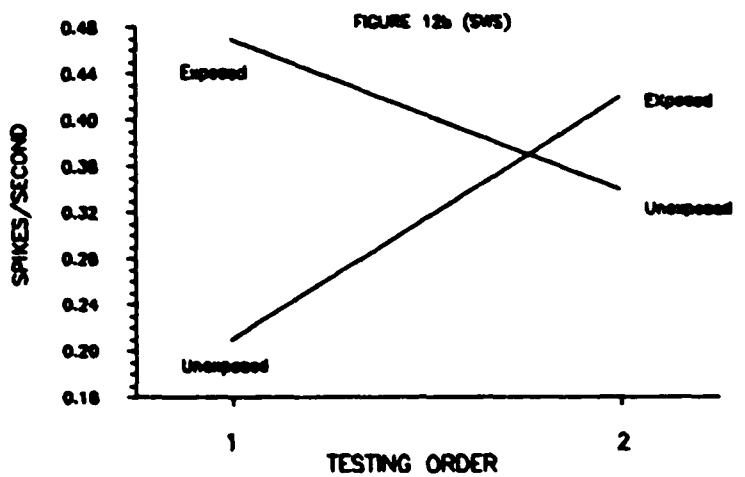


FIGURE 12b (SWS)
SPIKES/SECOND
0.48
0.44
0.40
0.36
0.32
0.28
0.24
0.20
0.16
Exposed
Unexposed
Exposed
Unexposed
1
2
TESTING ORDER

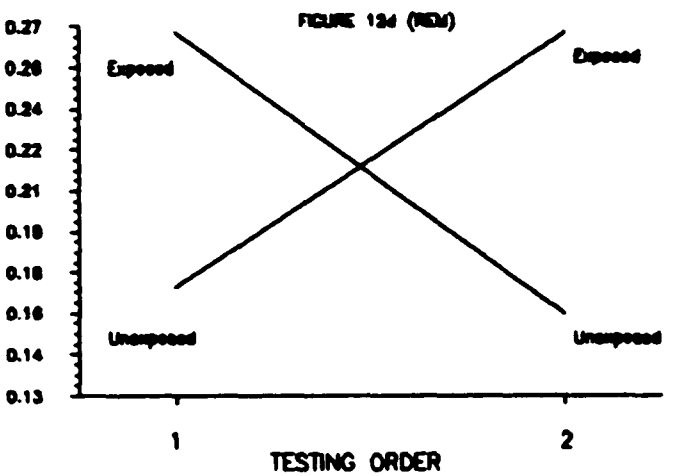
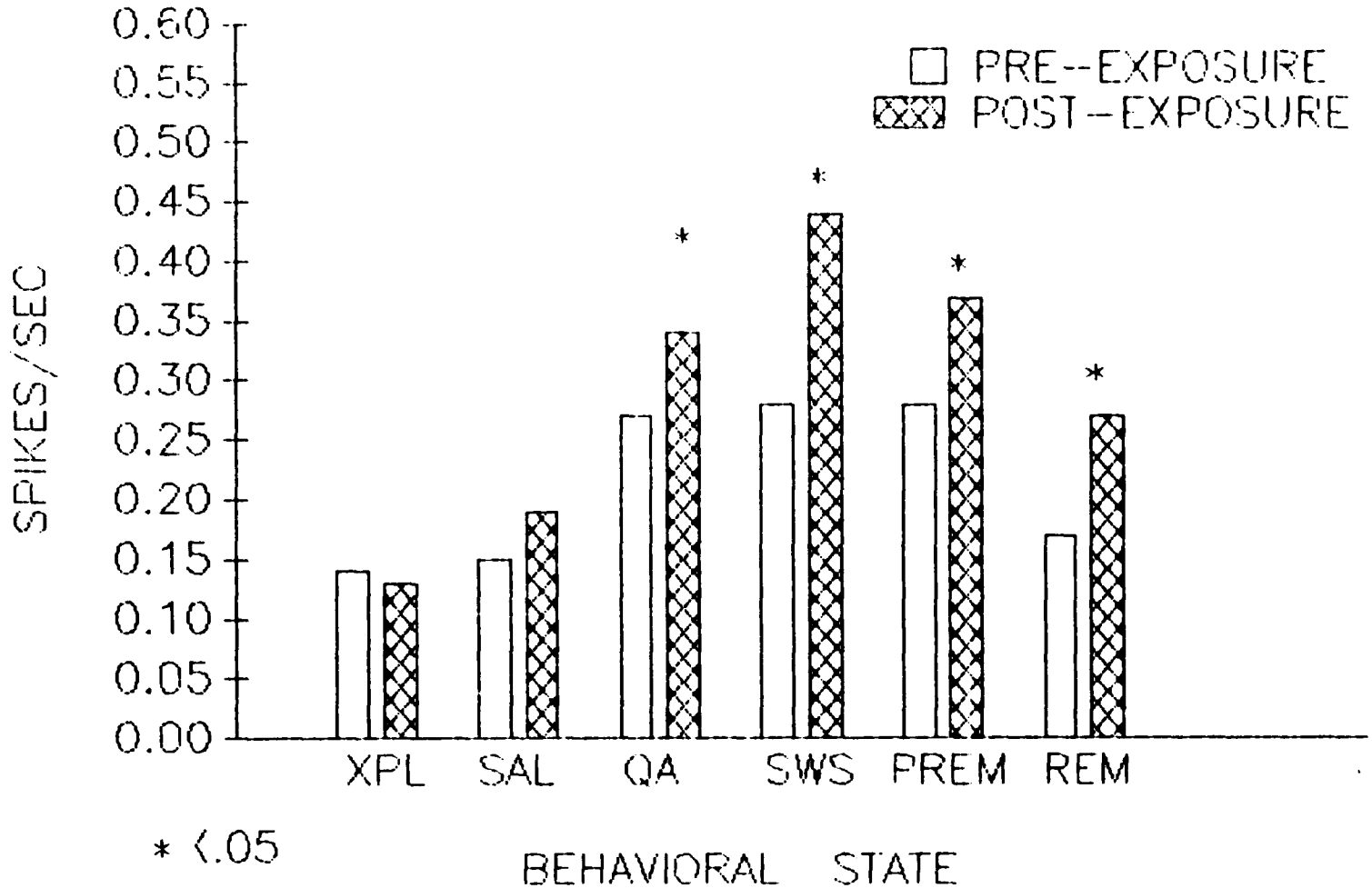


FIGURE 12d (REM)
0.27
0.26
0.24
0.22
0.21
0.18
0.18
0.16
0.14
0.13
Exposed
Unexposed
Exposed
Unexposed
1
2
TESTING ORDER

Figure 13.

Bar graph of the rate of firing, pre- and post-exposure to place field, of the 13 units included in the first study. An ANOVA revealed significant ($P < .02$) post-exposure increases in the firing rate of these cells. A significant interaction ($P < .05$) between order of testing (pre-post) and behavioral state was also obtained. Schaffer-Welsch, post-hoc comparisons revealed significant increases for all the sleep states tested but not for the awake states.

FIGURE 13


PRE--EXPOSURE
POST-EXPOSURE
SPIKES/SEC
0.60
0.55
0.50
0.45
0.40
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0.00
XPL
SAL
QA
SWS
PREM
REM
* <.05
BEHAVIORAL STATE

using Tukey's method ($a = .05$) yielded significant pre-post effects for SWS, PREM and REM. No pre-post changes were detected for the behavioral states of XPL, SAL and QA. It should be noted, however, that the rate of firing in the QA state approached the level of significance ($P = .05$). The simple effects analysis also revealed significantly higher firing rates in SWS than in REM sleep (Schaffer-Welsch test; $P < .05$). Thus, exposure of the cell to its place field, in the waking state, significantly increased its firing rate only in the sleep states that followed.

Figure 14 presents the average rate of firing of each of the individual cells pre-and post-exposure in all of the sleep states. It should be noted that an increase in the firing rate, following exposure to place field, occurred in a large majority of place cells tested, leading to the overall significant increase in the firing rate, noted above. This increased firing was especially evident in the SWS, PREM and REM states. It should also be emphasized that the increased firing occurred in a counterbalanced manner (see Figure 12).

An ANOVA performed on the bursts per second revealed similar results to those seen with the rate of firing. Increased bursting was observed following exposure [$F(1,12) = 5.07$; $P < .04$]. A significant main effect of behavioral state was also observed [$F(5,55) = 21.6$; $P < .001$], with higher bursting rates seen in SWS than in REM sleep (see Figure 15). The interaction of behavior by stage failed to achieve

Figure 14.

Line graph depicting the rate of firing, pre- and post-exposure to place field, for each of the 13 units included in the first study. Note that the great majority of units increased their firing following exposure to place field, especially in the SWS and PREM states. A few units, however, continued firing at a constant rate. These appear to be the slow firing cells, although that was not always the case. One or two cells can also be seen that decreased their firing rates following exposure (in QA and REM sleep).

Figure 14

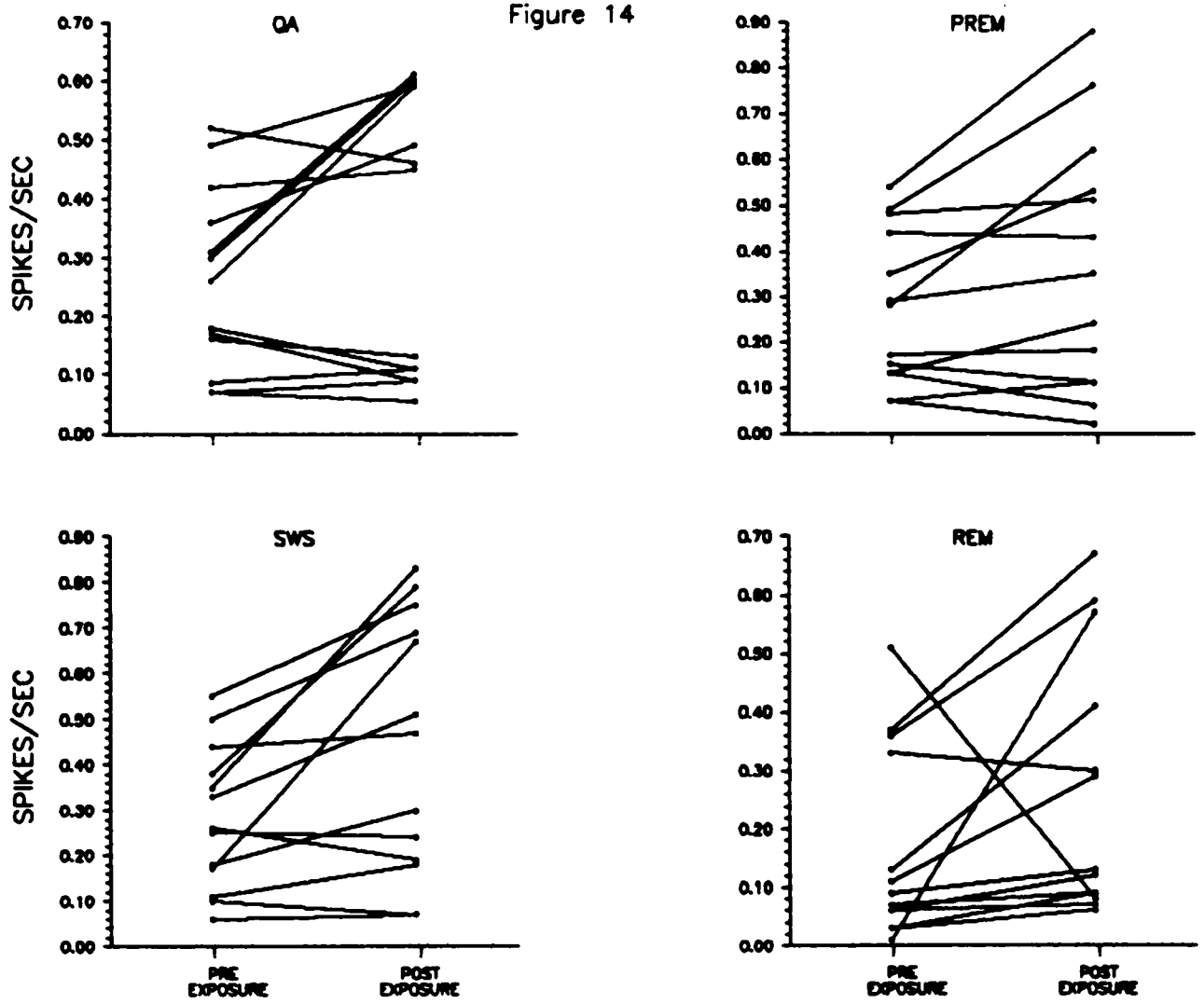
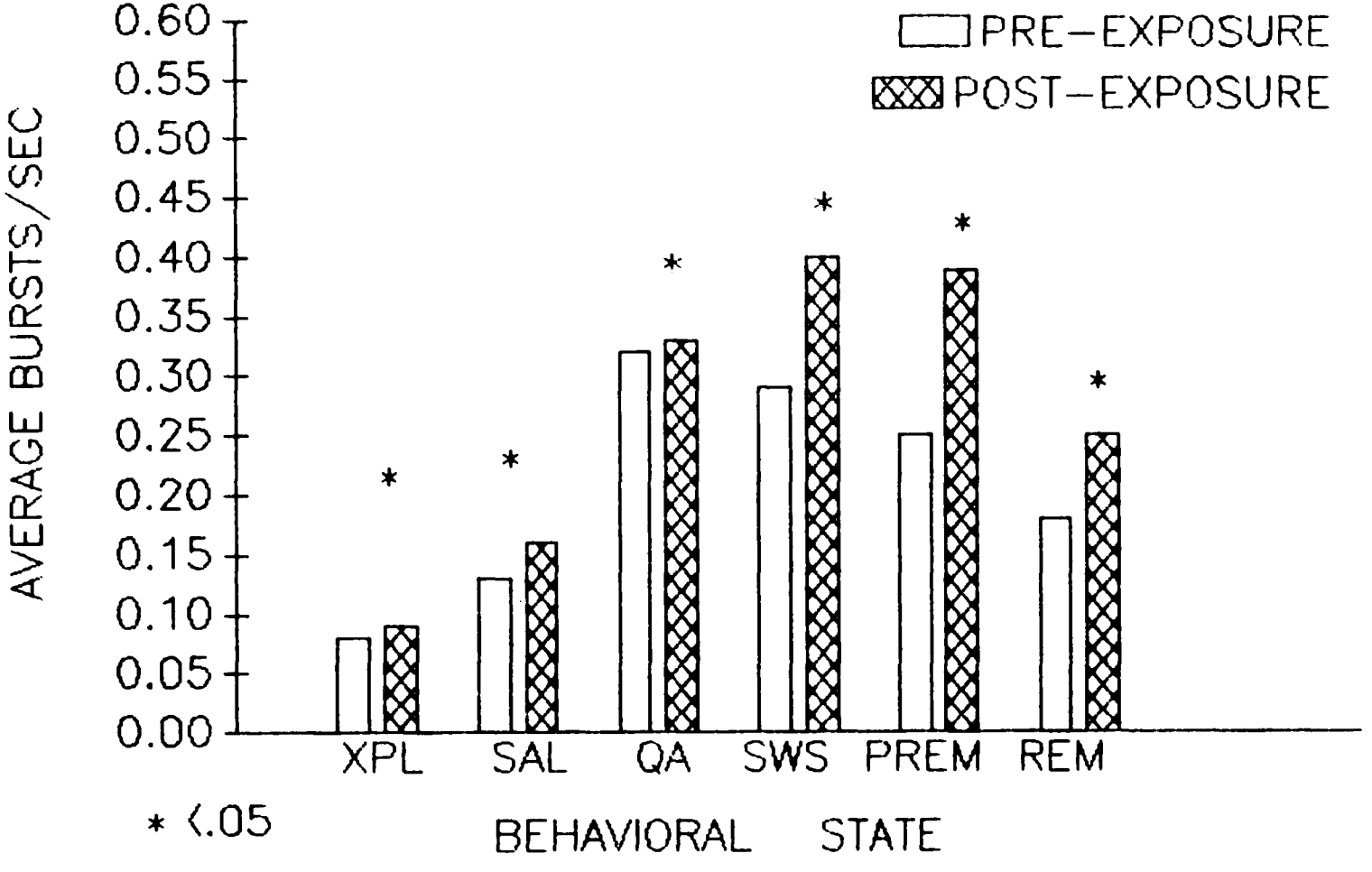

QA
PREM
SWS
REM
SPIKES/SEC
SPIKES/SEC
0.00
0.10
0.20
0.30
0.40
0.50
0.60
0.70
0.80
PRE EXPOSURE
POST EXPOSURE

Figure 15.

Bar graph of the average bursts per second, pre- and post-exposure to place field, for the thirteen units included in the first study. An analysis of variance showed significant main effect of exposure to place field ($P < .05$) but the interaction of exposure and behavioral state did not reach significance. Close observation of the graph, however, reveals that large increases in the bursting rates took place only in the SWS, PREM and REM sleep states. The failure to obtain a significant interaction is most likely due to the very large variability seen for this variable (see discussion).

FIGURE 15


PRE-EXPOSURE
POST-EXPOSURE
AVERAGE BURSTS/SEC
0.60
0.55
0.50
0.45
0.40
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0.00
XPL
SAL
QA
SWS
PREM
REM
* <.05
BEHAVIORAL STATE

significance. Note in Figure 15, however, that the only bursting rates that increased notably were those of the SWS and PREM states.

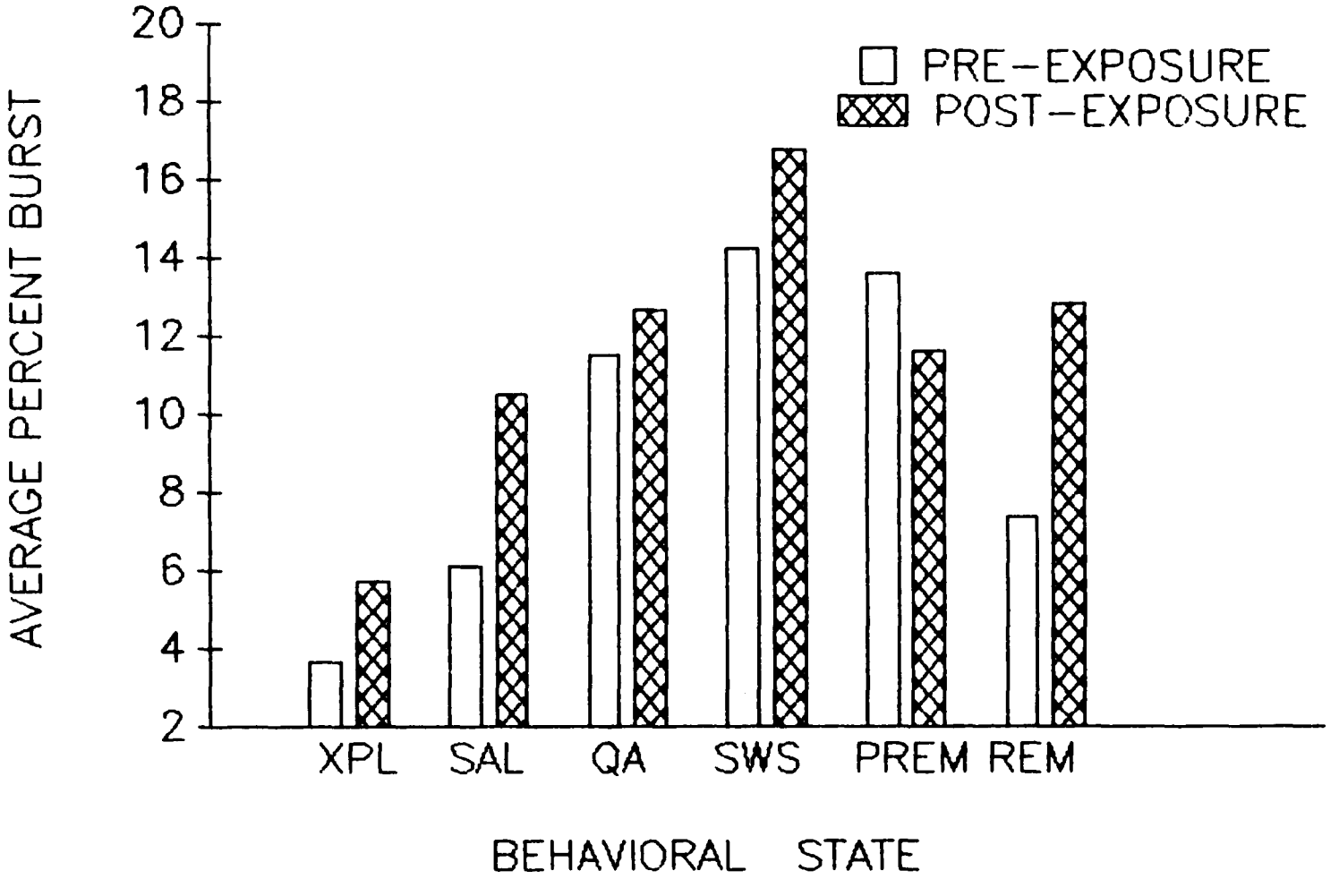
The percentage of bursts versus single spikes was also analyzed. This represents the ratio of single spikes to bursts. An analysis of variance performed on the pre- post-exposure proportion of bursts versus single spikes failed to detect significant main effects of exposure to place field [$F(1,11) = 1.8$; $P > .05$]. Thus, the ratio of single spikes as opposed to bursts apparently did not differ following exposure to place field. A significant effect, however, was found for behavioral state [$F(5,55) = 9.05$; $P < .001$]. The Schaffer-Welsch post-hoc comparison ($a = .05$) revealed significantly higher bursting occuring during the sleep states (SWS, PREM, QA) than the awake states (SAL, XPL). An interaction approaching significance ($P = .08$) was also observed between behavioral state and order of testing (see Figure 16). Due to the lack of a significant interaction post-hoc tests were not performed. As can be seen in Figure 16, however, it may be noted that the highest increase in the ratio of bursts to single spikes, following exposure, took place in REM sleep.

A further burst characteristic that was analyzed was the average number of spikes per burst. An increase has been observed in the average number of spikes within a burst, following exposure of the units to their place field [$F(1,12) = 4.61$; $P < .05$]. A significant main effect of behavioral state was also obtained [$F(5,60) = 16.00$; $P < .001$]. A Schaffer-Welsch post-hoc analysis ($a = .05$), however, revealed a significant difference only between the sleep states and

Figure 16.

Bar graph of the average percent burst versus single spikes, pre- and post-exposure to place field, for the thirteen units of the first study. An analysis of variance did not reveal a significant effect of exposure. Thus the ratio of single spikes to bursts did not change with exposure to place field (i.e. both increased with the same ratio).

FIGURE 16


AVERAGE PERCENT BURST
20
18
16
14
12
10
8
6
4
2
PRE-EXPOSURE
POST-EXPOSURE
XPL
SAL
QA
SWS
PREM
REM
BEHAVIORAL STATE

the awake states but no differences within these two categories. A significant interaction between behavioral state and stage of testing was also seen [F (5,60) = 2.62; P < .03]. A simple effects analysis (Tukey test; a = .05) of behavioral state by stage of testing interaction revealed significant increases in the number of spikes per burst following exposure in the SWS and REM states but not in other behaviors (see Figure 17).

The number of spikes within a burst were also analyzed separately (i.e. single spike (no burst), double spikes, triple spikes, etc.). ANOVA for each of the different number of spikes showed significant pre-post increases for the number of single spikes [F (1,12) = 4.44; P < .05]. A significant interaction between stage and behavior was not observed. Significant main effects, however, were seen for behavioral state [F (5,60) = 15.83; P < .001]. Post-hoc comparisons (Schaffer-Welsch test; P < .05) exhibited significantly higher rates in the occurrence of single spikes between the SWS, PREM and QA and the awake states (XPL and SAL) as well as SWS and REM sleep (see Figure 18).

The bursts composed of two spikes were also analyzed separately. An ANOVA failed to reach significance for stage of exposure, indicating that the number of bursts with two spikes did not change following exposure to place field. Significant differences, however, were seen for behavioral state [F (5,60) = 13.68; P < .001]. The following is the order of double bursts, in decreasing order of magnitude: SWS, QA, PREM, XPL, SAL, REM (see Figure 19).

Figure 17.

Bar graph of the average spikes/burst/second, pre- and post-exposure to place field, for the thirteen units included in the first study. An analysis of variance revealed a significant main effect of exposure ($P < .05$). A significant interaction of exposure and behavioral state was also obtained ($P < .02$). A simple effects analysis (Tukey test; $a = .05$) revealed significant increases in the average number of spikes within a burst for the behavioral states of SWS and REM sleep.

FIGURE 17

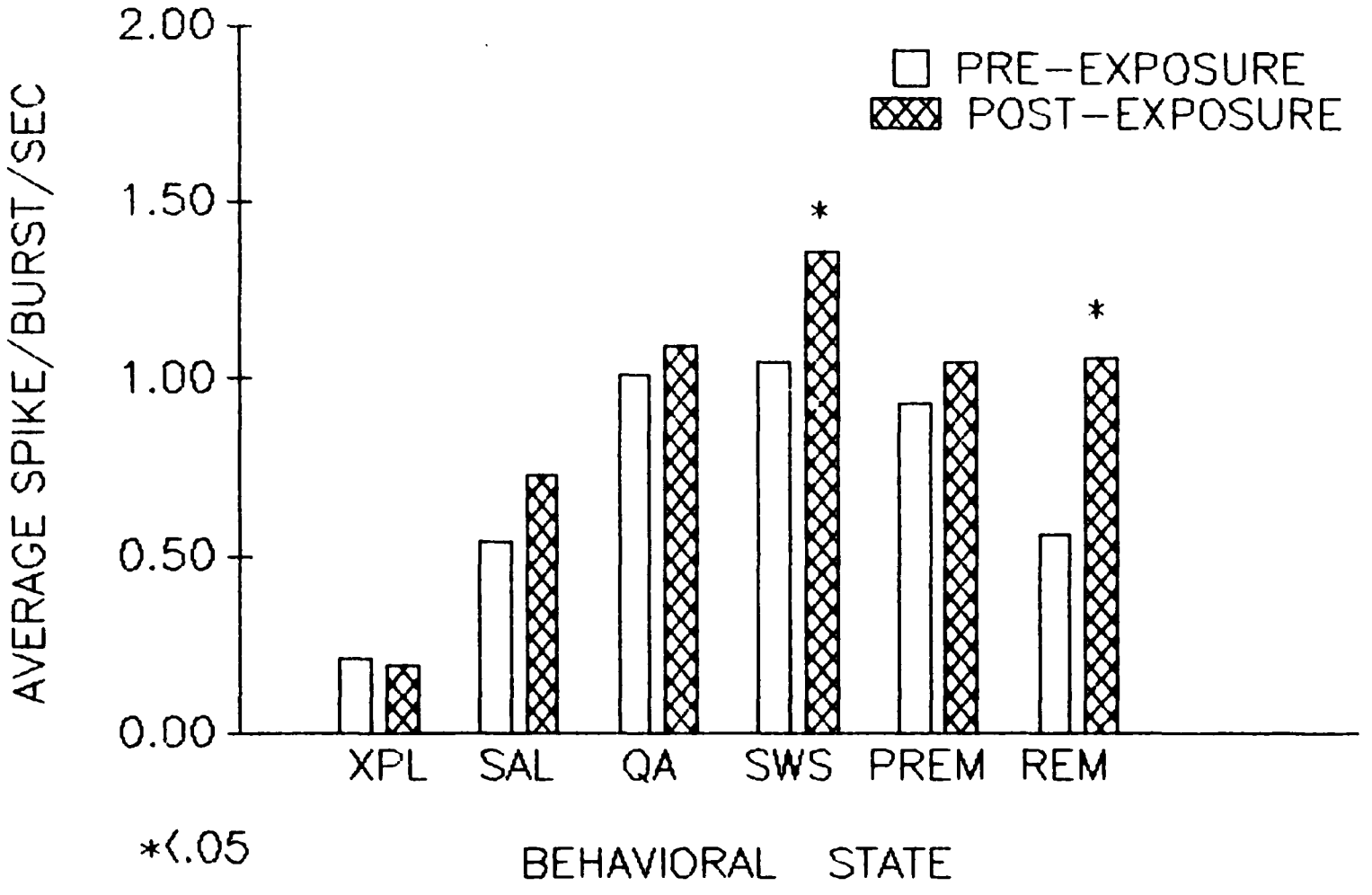

2.00
1.50
1.00
0.50
0.00
AVERAGE SPIKE/BURST/SEC
PRE-EXPOSURE
POST-EXPOSURE
*
*
XPL
SAL
QA
SWS
PREM
REM
*<.05
BEHAVIORAL STATE

Figure 18.

Bar graph of the number of single spikes, pre- and post-exposure, for the thirteen units included in the first study. An analysis of variance did not reveal a significant effect of exposure for this variable. Note, however, that the largest increase in single spikes took place in SWS.

FIGURE 18

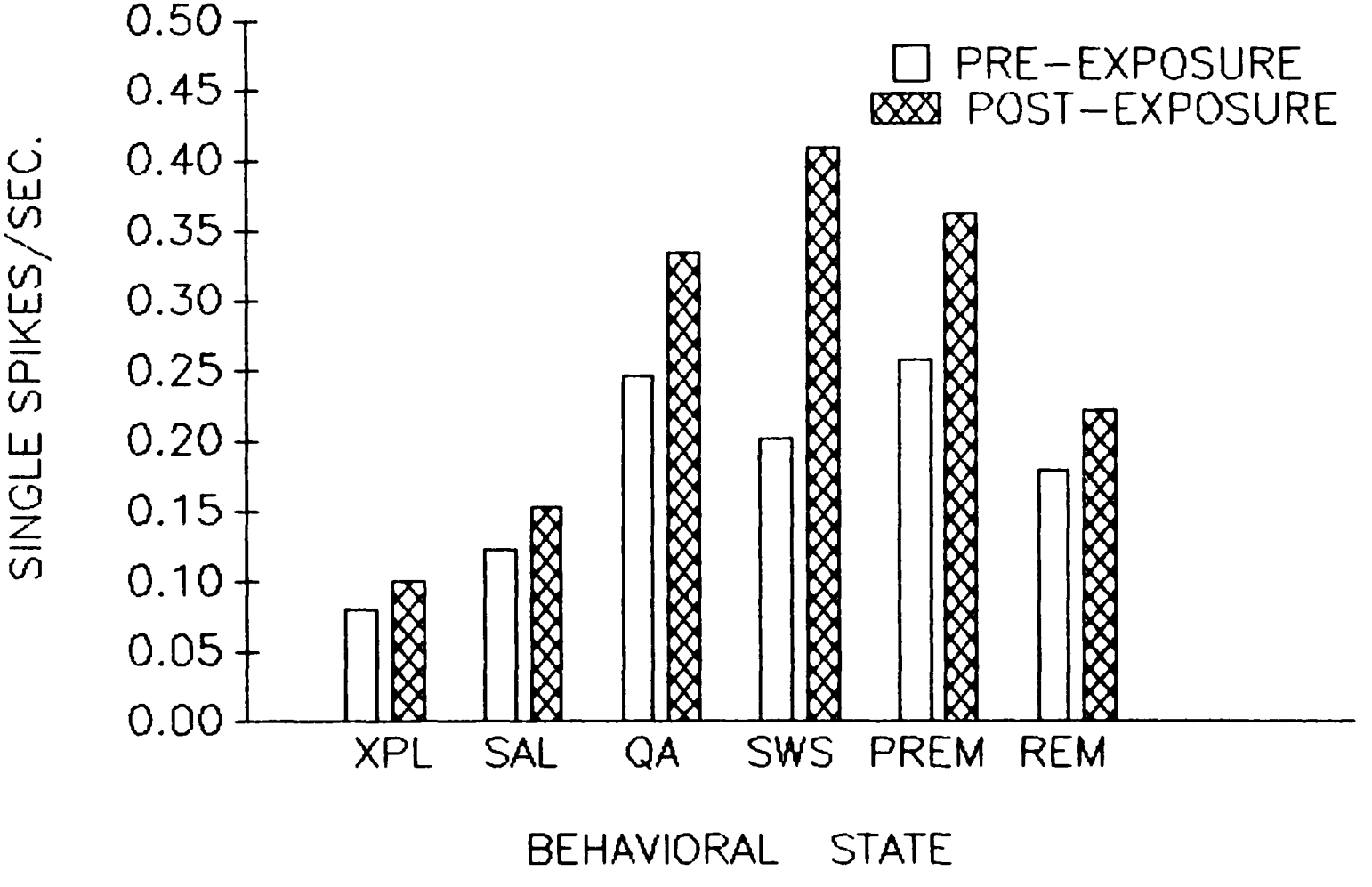
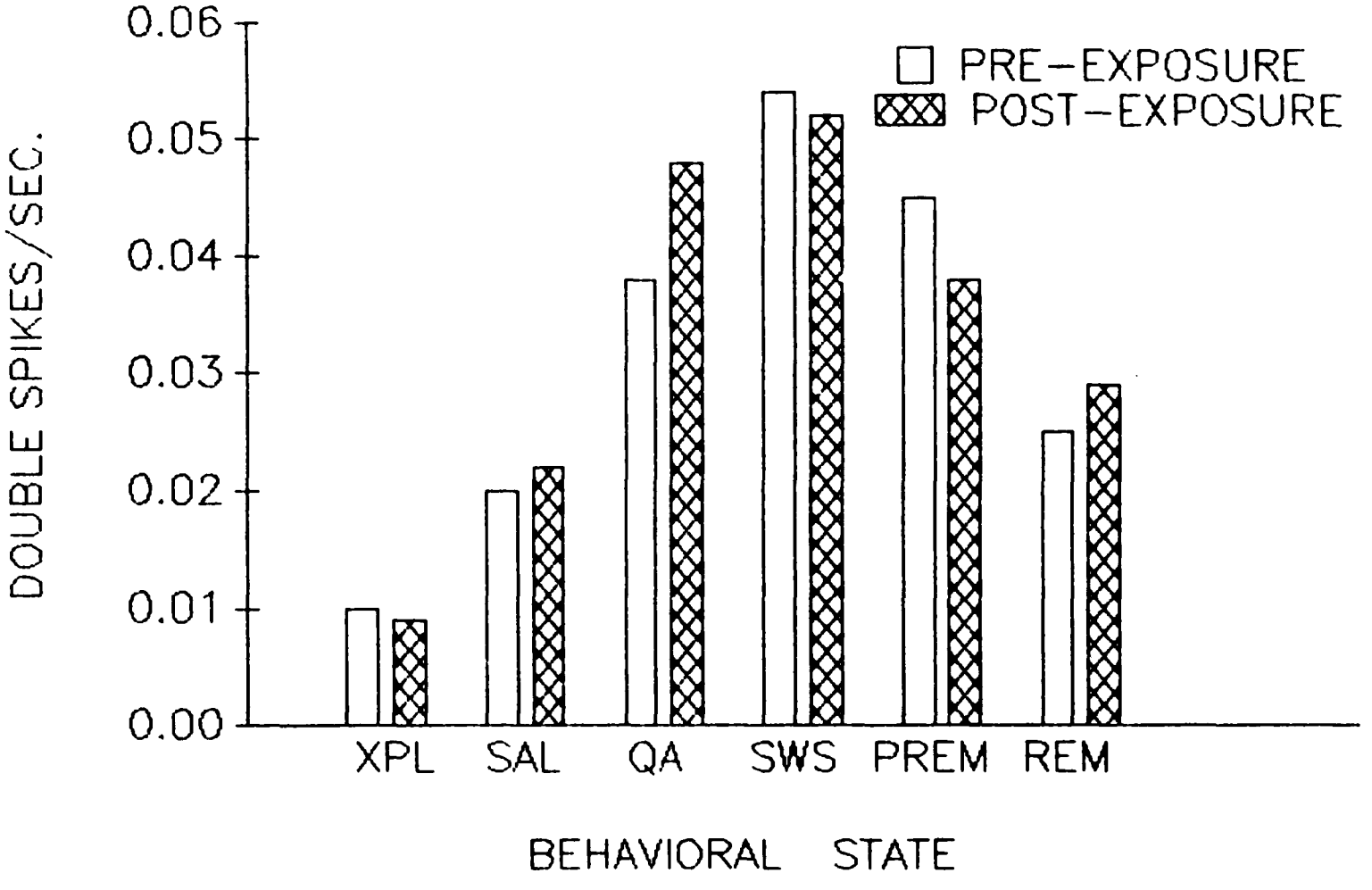

PRE-EXPOSURE
POST-EXPOSURE
SINGLE SPIKES/SEC.
0.50
0.45
0.40
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0.00
XPL
SAL
QA
SWS
PREM
REM
BEHAVIORAL STATE

Figure 19.

Bar graph of the number of bursts containing two spikes, pre- and post-exposure to place field, for the thirteen units included in the first study. An analysis of variance did not reveal a significant effect of exposure to place field. A significant effect of behavioral state was observed ($P < .001$).

FIGURE 19


0.06
0.05
0.04
0.03
0.02
0.01
0.00
DOUBLE SPIKES/SEC.
PRE-EXPOSURE
POST-EXPOSURE
XPL
SAL
QA
SWS
PREM
REM
BEHAVIORAL STATE

The number of bursts with three spikes increased following exposure [$F(1,12) = 6.00$; $P < .03$]. A significant interaction between stage and behavioral state was also achieved [$F(5,60) = 2.79$; $P < .02$]. A simple effects analysis of this interaction using Tukey's method ($a = .05$) revealed an increase in the number of bursts composed of three spikes, following exposure, in SWS, PREM and REM (see Figure 20).

Measurements were also made of the average interburst interval. An ANOVA of the average IBI pre- and post-exposure failed to achieve significance [$F(1,12) = 2.17$; $P = .16$] (see Figure 21).

Another measure taken was the average interspike interval which represents the time interval between spikes, within a burst. An ANOVA performed on the pre-post exposure of the average ISI's revealed no significant changes in this measure after exposure of the unit to its place field (see Figure 22).

The interspike intervals were also subdivided and analyzed in finer gradations (0-2, 2-4, 4-6, 6-8, 8-10, and 10-12 msec.). Although no significant increases were observed in the average ISI, significant increases in the 2-4 msec. range were detected following exposure of the cell to its place field [$F(1,12) = 8.22$; $P < .03$]. All of the other intervals failed to reach significance (see Figure 23).

For a number of the cells (5 out of 13), the animal was rewarded when it occupied the place field of a particular place cell (while the cell was being exposed). The animal was presented with a half

Figure 20.

Bar graph of the number of bursts containing three spikes, pre- and post-exposure to place field, for the thirteen units included in the first study. A significant increase in the number of triples was obtained following exposure to place field (ANOVA; $P < .03$). A significant interaction of exposure and behavioral state was also observed ($P < .02$). The Tukey test ($P = .05$) revealed significant increases for SWS, Pre-REM and REM sleep but not for the other behavioral states tested.

FIGURE 20

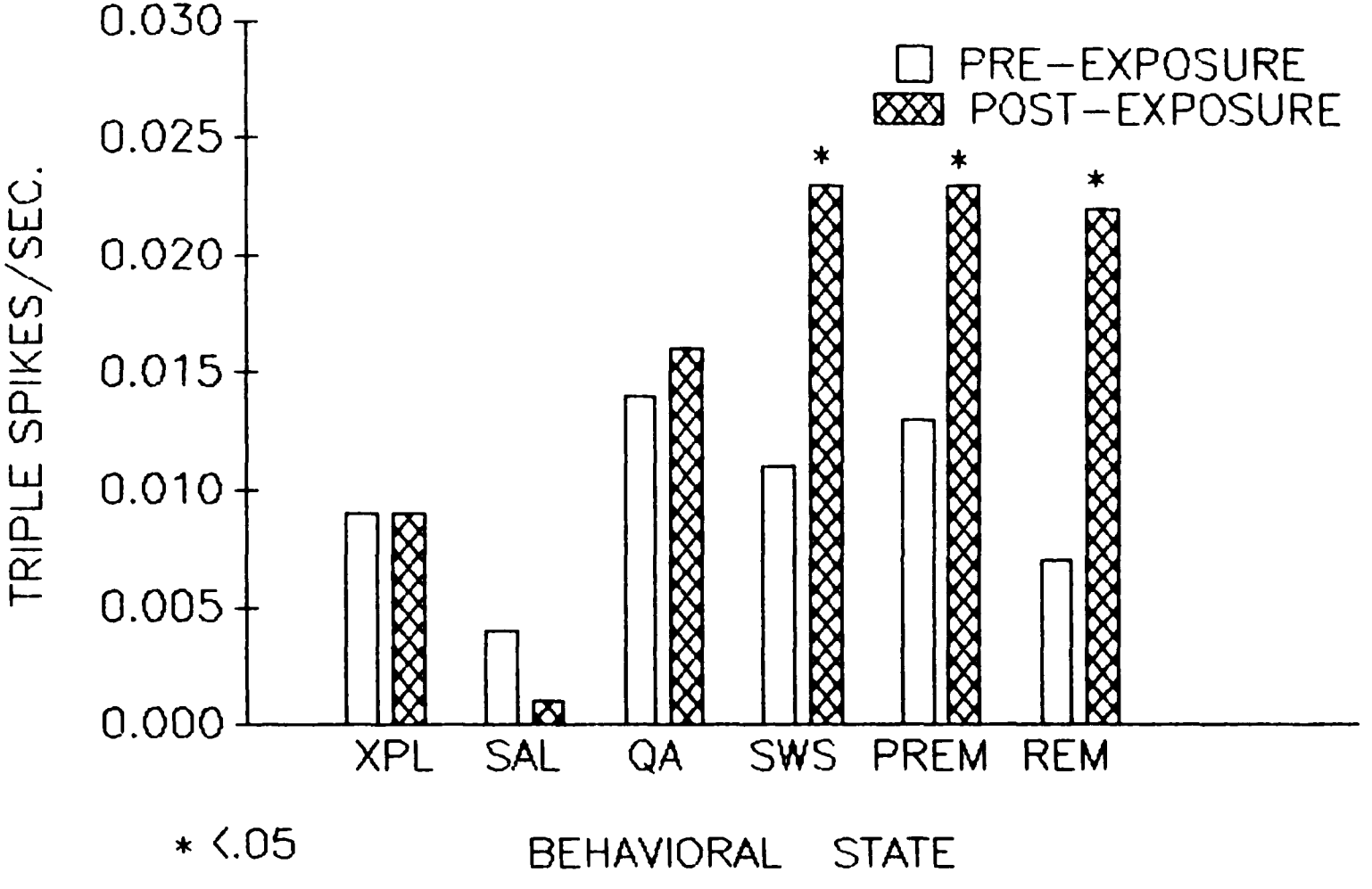

0.030
0.025
0.020
0.015
0.010
0.005
0.000
TRIPLE SPIKES/SEC.
PRE-EXPOSURE
POST-EXPOSURE
*
*
*
XPL
SAL
QA
SWS
PREM
REM
* <.05
BEHAVIORAL STATE

Figure 21.

Bar graph of the interburst intervals, pre- and post exposure to place field, for the thirtenn units of the first study. An analysis of variance did not reveal a significant effect of exposure. Close observation of the graph indicates that a small nonsignificant decrease in the IBI occurred following exposure. This is to be expected since the number of bursts increased following exposure.

FIGURE 21

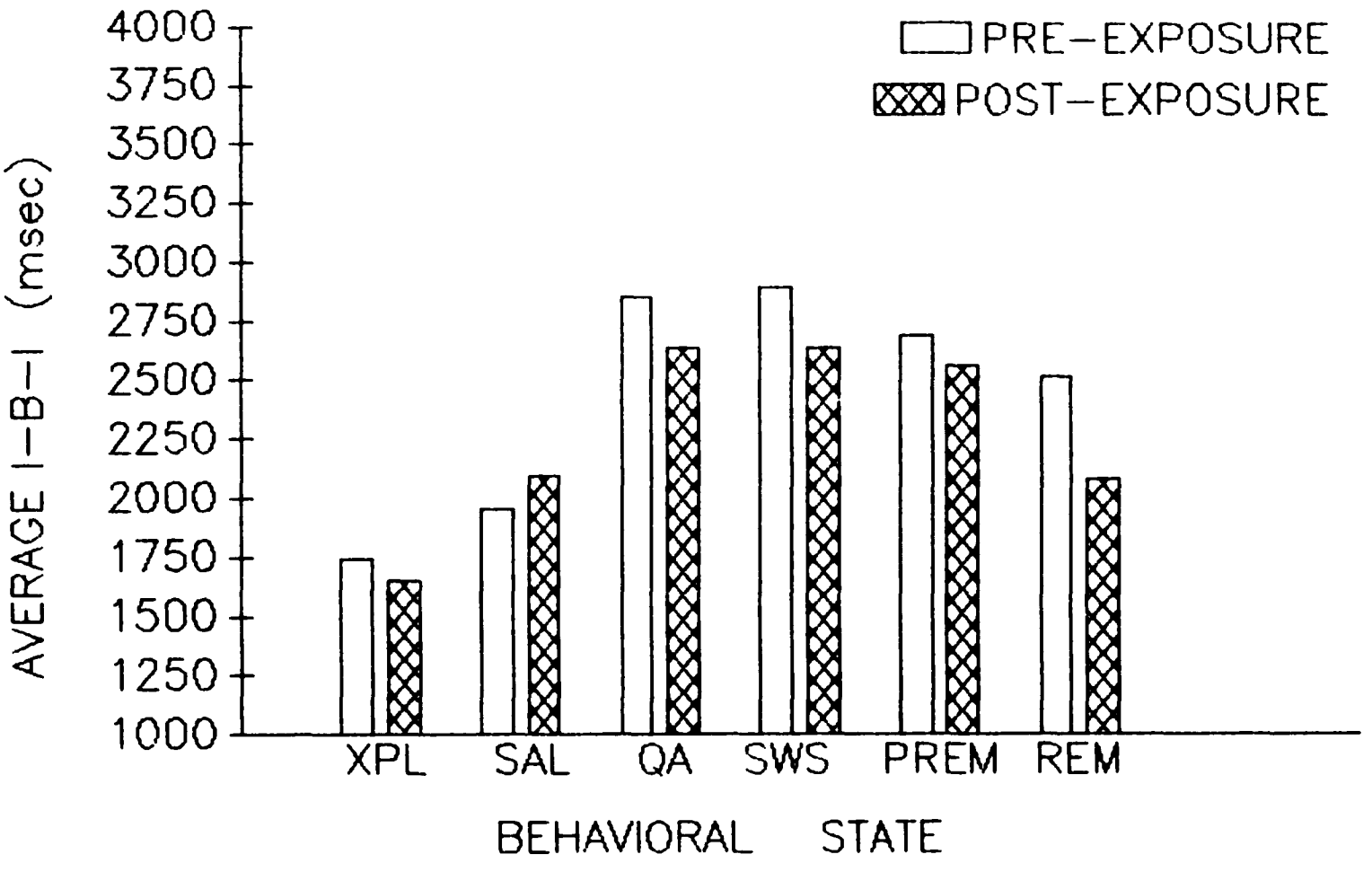

PRE-EXPOSURE
POST-EXPOSURE
AVERAGE I-B-I (msec)
4000
3750
3500
3250
3000
2750
2500
2250
2000
1750
1500
1250
1000
XPL
SAL
QA
SWS
PREM
REM
BEHAVIORAL STATE

Figure 22.

Bar graph of the interspike intervals, of the thirteen units included in the first study, pre- and post-exposure to place field. No changes were observed in this variable following exposure.

FIGURE 22

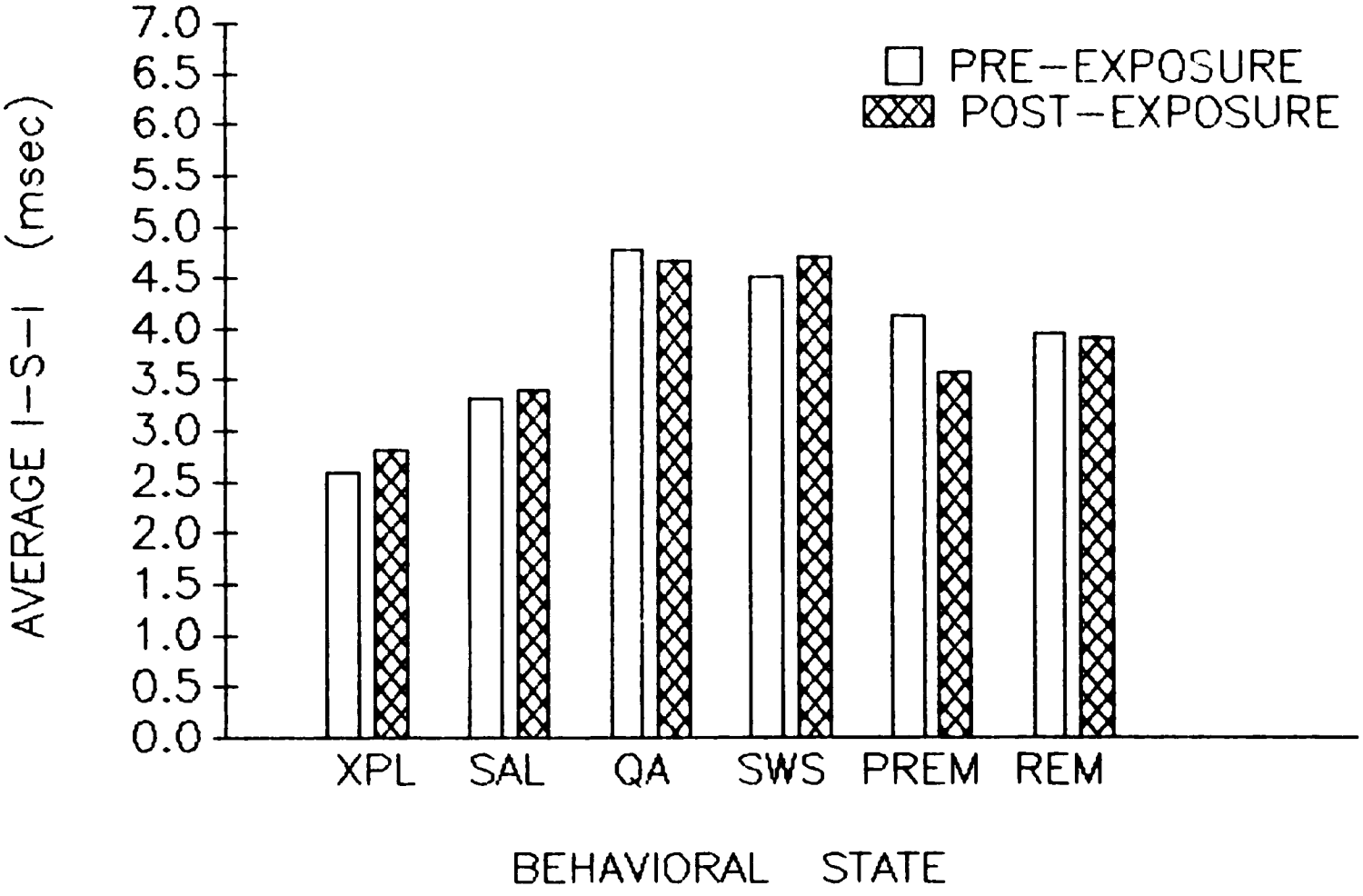
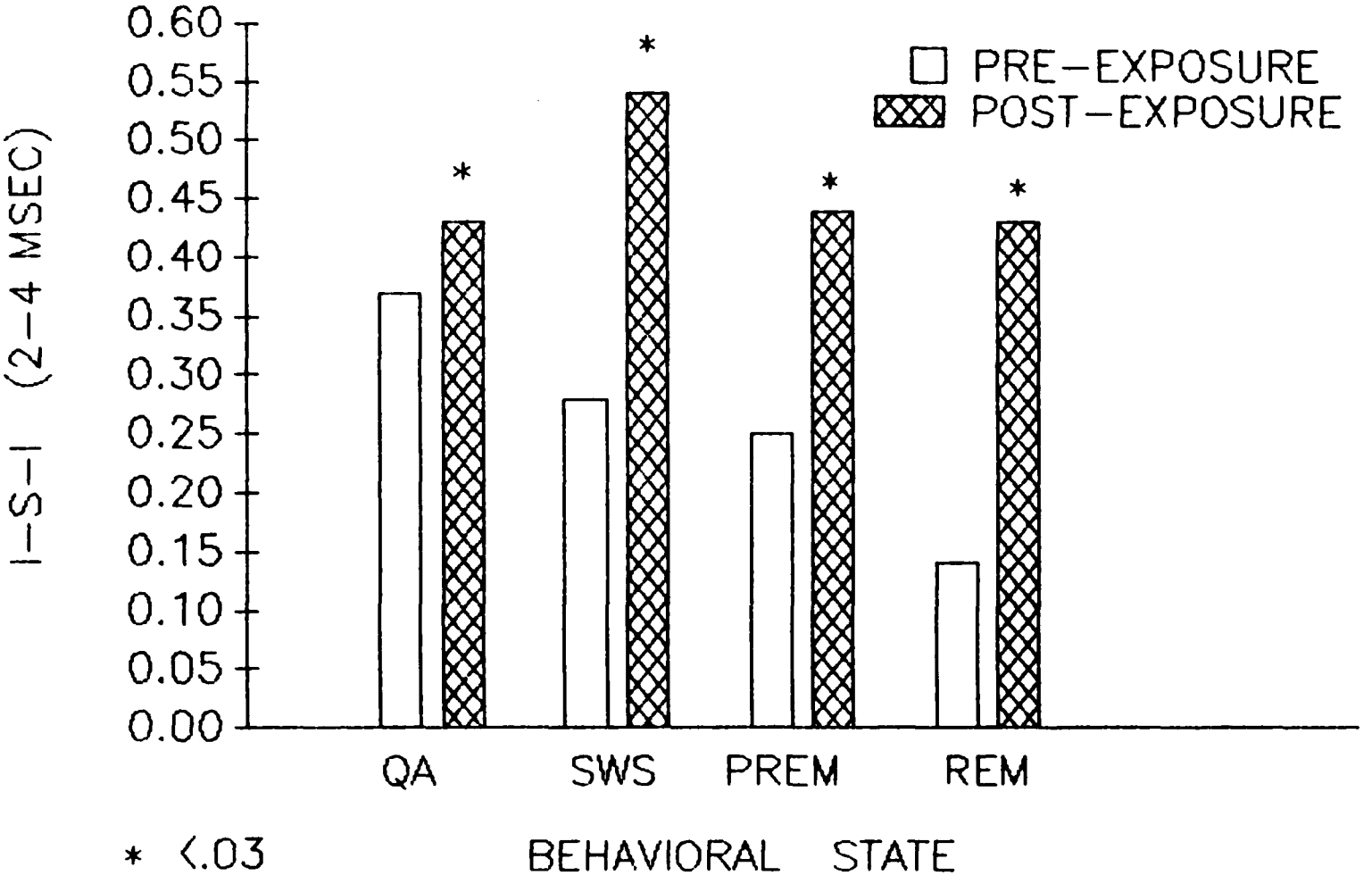

PRE-EXPOSURE
POST-EXPOSURE
AVERAGE I-S-I (msec)
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
XPL
SAL
QA
SWS
PREM
REM
BEHAVIORAL STATE

Figure 23.

Bar graph of the number of bursts with interspike intervals falling in the 2-4 millisecond range, Pre- and post-exposure of the units to their place field. An anlysis of variance revealed significant increases of bursts with 2-4 msec. ISI's following exposure to place field ($P < .03$). This increase was seen for all of the sleep states but not the awake states.

FIGURE 23


0.60
0.55
0.50
0.45
0.40
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0.00
I-S-I (2-4 MSEC)
PRE-EXPOSURE
POST-EXPOSURE
*
*
*
*
QA
SWS
PREM
REM
* <.03
BEHAVIORAL STATE

chocolate-chip cookie at the precise location of the unit's place field. In almost all cases the animal, which had been exposed to this kind of reward on previous days, consumed a large portion of the cookie during the course of the unit's exposure. The unit's firing did not seem to be affected by the animal's consumatory behavior (i.e. it remained rather high as long as the animal occupied the cell's place field. Close observation of the in-place-field firing rates, of a number of cells, failed to show noticeable changes in the firing patterns following reward in the place field. Similarly, significant effects were not detected in the firing rates of the 'rewarded cells' in the subsequent sleep episodes [$F(1,9) = 1.28$; $P > .05$]. It should be noted, however, that the animal was rewarded with Noyse food pellets throughout the eight arm maze over the extent of the experimental session and the chocolate-chips represented only a different kind of reward.

In summary, the main findings of this study are the following: (i) there was a significant increase in the rate of firing of hippocampal complex spikes (place cells) following exposure of these cells to their place field; (ii) there was a significant increase in the average number of spikes within a burst following exposure. This increase was only evident during the SWS and REM states; (iii) a significant increase in the number of single spikes was observed following exposure of these cell to their place field; (iv) there was a significant increase in the number of bursts with three spikes following exposure. Finally, there was an increase in the number of bursts with the spikes falling in the 2-4 msec. ISI interval. These

points and their relevance to information processing during sleep will be discussed in the final chapter.

Discussion

The results of the present experiment suggest that the awake unit activity of hippocampal place cells may influence the firing activity of these units in the subsequent sleep states. Thus, the increased activity of these units in their place fields also enhanced their activity when the animal engaged in sleep but not during the awake states that followed. The increased firing activity was evident for a number of the parameters tested (i.e. rate of firing, rate of bursting, the number of spikes within a burst and the interspike intervals) and was present for all of the behavioral states tested. Pre- and post-exposure firing rates are presented in Figure 14. It becomes apparent from this figure that although the majority of cells increased their firing rates in sleep following exposure, the largest increase was observed in the faster firing cells. Many of the slow firing cells did not show significant change following exposure. Furthermore, the increased firing appeared not to last beyond an approximately three hour period during which time a number of SWS-REM episodes transpired. A search (in a few units) for a systematic time course in the increased firing rate failed to reveal any such trends. For some of the cells, the increased firing occurred within the first few sleep episodes immediately following exposure, other cells increased their firing rates after a few sleep episodes had elapsed.

For most of the units, however, there appeared to be a gradual decrease in the last few intervals of the 2-3 hour recording. A systematic analysis, however, may yield such trends in the time course of increased firing. It should be noted that the unit analysis was done off-line and the decision for the length of recording was not influenced by the unit firing.

In addition to the increase in the firing rate, an increase was also seen in the rate of bursting following exposure to place field. This increased bursting took place mainly during SWS, PREM and REM (see Figure 15). Based on the absence of an interaction between exposure and behavioral state, however, it could not be determined whether the increased bursting occurred only in the sleeping behaviors. A possible reason for the lack of a significant interaction was due to the high variability among the units. It is evident from figure 15 that the only behaviors that show an increase are those of SWS, PREM and REM. It is unlikely that following exposure to place field an increase in the bursting of these cells occurs in all the behavioral states that follow. As previously stated these cells show steady activity in the awake states (at least for firing rate) over long periods of time.

The average number of spikes per burst also increased following exposure of the cell to its place field. These increases were only evident during the SWS and the REM states. Closer analysis of the number of spikes within a burst revealed that the number of bursts containing three spikes increased in the SWS, PREM and REM states that

followed exposure. It should be noted that complex spike cells have been shown to fire in bursts containing as many as seven or eight spikes of decreasing amplitude (Ranck, 1973). Besides the single-, double- and triple-spikes reported in the results, measurements were also taken of the number of bursts containing four, five and six-plus spikes. These last three categories were excluded from the final analysis, however, due to the low frequency of their occurrence. It should be noted, however, that although long bursts occurred too infrequently to include in the present analysis, bursts with many spikes (4-10+) were observed; in a small number of cells, bursts with as many as fifteen spikes were also recorded.

Finally, there was an increase in the number of bursts with interspike intervals in the 2-4 msec. range. This increase was evident in all of the sleep states tested but was especially noticeable during the SWS, PREM and REM states. This interval corresponds to approximately 400 Hz. which has been shown to be a sensitive frequency for inducing long-term potentiation. Long-term potentiation has been hypothesized to be a mechanism underlying learning and memory (to be discussed in greater detail in the final chapter).

The present findings of high SWS and lower REM sleep firing rates for CA1 complex spike cells, were also reported previously (Ranck, 1973; Olmstead, et al., 1973; Suzuki and Smith, 1985). It should be noted that in none of these studies was there an attempt to correlate awake state unit activity with subsequent sleep firing. In all of these studies awake state firing must have been extensive, reflecting

the high rates that were found in the SWS states that followed. It should also be noted that the firing rates in the present study reflect rates that have been averaged over a long, continuous period of time as opposed to the short interval, representative data reported in previous studies (O'Keefe, 1976; Suzuki and Smith, 1985). The rates of unit firing reported by other researchers may represent a pure behavioral state but may not be representative of the overall behavioral state since there is great variability in the activity of these cells. On the other hand, the rates reported in the present study may not be of a pure behavioral state but are perhaps more representative of that state because of the longer recording session.

The present results also indicate that rewarding an animal while exposing a cell to its place field had no noticeable effect either on the in-place-field firing or the firing rates in the sleeping states that followed. It was originally expected that reward or novelty in the place field would greatly increase the firing rates of these cells. The insensitivity of these units to reward, however, has been alluded to previously (O'Keefe, 1976).

The possible interpretation of the significance of these results and their relevance to memory consolidation will appear in the final discussion.

CHAPTER 4

Experiment 2: Descriptive analysis of hippocampal 'place cells'.

Since the original discovery of hippocampal place cells many experiments have been conducted to study various characteristics of these cells. Most of these studies have focused on providing a descriptive analysis of the sensory inputs that make these cells fire in a spatial manner. There have been very few studies that have looked at the firing characteristics of these cells in different behavioral states. Furthermore, in all of these studies the primary measure used has been the rate of firing. The use of the firing rate as the only measure occurred despite the fact that place cells fire both in complex bursts as well as in single spike action potentials. The objective of the present study was to record from complex spike cells during a number of waking and sleeping behavioral states and to provide a detailed analysis of the firing patterns of these cells. Besides analyzing the rate of firing, analysis was also made for a number of other parameters. These included the following: (i) the rate of firing (all spikes including single-, double-, triple-spikes, etc.); (ii) the number of bursts; (iii) the ratio of bursts to spikes; (iv) the inter-burst-interval, as well as finer subdivisions of these; (v) the average number of spikes per burst; (vi) the number of single spikes as well as the number of bursts with two, three spikes, etc.;

(vii) the average interspike interval as well as finer subdivisions of the ISI (0-2, 2-4, 4-6, 6-8 and 8-10 msec.).

Methods

A number of criteria, similar to those used in the first study, had to be met before units would be selected for further recording and analysis. To recapitulate, these criteria were as follows: (i) the units had to display complex spikes during the initial course of testing; (ii) units had to have a signal to noise ratio of at least 3-to-1; (iii) the cells had to display clear and discrete place fields; (iv) at least two units with the above mentioned characteristics had to be present simultaneously and to display diverse, non overlapping place fields. It was also preferable that the two units be recorded from different electrodes (see methods section for details). It should be noted that although the primary objective in recording these cells was to include them in the first (counterbalanced) study, it was observed at the time of the off-line data analysis that exposure of the first cell to its place field inadvertently made the second cell fire in a high rate as well. The reason for this is that these pairs of cells had overlapping place fields which were not evident at the time of the original recording. These cells were, therefore, excluded from the first study and were included in the present study. In looking at the data, however, it should be noted that these cells were exposed to their place fields

extensively at least two or three times during the course of recording and this may have in fact influenced their rates of firing.

Recording of the 24 units included in the second study took place over an extended period of time (usually 4 to 5 hours) and covered a number of different behavioral states. These included: exploration, still alert, in-place-field, quiet awake, slow wave sleep, pre-rapid-eye-movement sleep and rapid eye movement sleep. The firing characteristics of all the complex spike cells reported here represent averages of unit firing in many episodes, within a particular behavioral state, recorded over the entire period of observation.

Determination of the animal's behavioral state, unit analysis and statistical analysis of the data were identical to those described in the first study (see methods chapter as well as methods section of second study). Briefly, data reduction was done by observing both the animal's behavior and also the electroencephalogram (EEG). These two criteria were used to subdivide the animal's behavior into the seven different categories. Almost all data was analyzed by the primary investigator. In some cases when a third person performed the data reduction, complete supervision by the primary investigator was employed. At times, value judgements had to be made as to whether the neuronal firing observed was actually generated by the unit under investigation or the result of other units with similar spike characteristics traversing the discriminator settings. In such cases the discriminator settings were readjusted to exclude the other units.

In the event that the readjustment did not produce proper results, the unit was precluded from the final analysis.

Results

B. Overall Review of the Findings

1- Two types of cells have been encountered- theta cells and complex spike cells.

2- Complex spike cells in the CA3 field fired at higher rates than cells in the CA1 hippocampal field.

3- Differential sleep firing between CA1 and CA3 subfields was detected, with the majority of complex spike cells in the former firing at higher rates in SWS than in REM while in the latter higher rates were seen in REM than in SWS.

4- Higher bursting was observed for cells in the CA3 field than for cells in the CA1 field.

5- Significantly longer bursts (i.e. more spikes within a burst) were seen for cells found in the CA3 hippocampal field compared to the CA1 field and also for the QA and PREM states compared to the other behaviors.

6- Lower ISI's were detected for the CA1 cells than cells found in the CA3 field. Shorter ISI's were also obtained in the REM and XPL states than all the other behavioral states tested.

7- Shorter interburst intervals were observed during the in-place-field intervals than the other behavioral states. No differences in the IBI were detected for the two hippocampal fields tested.

8- Possible plasticity of place fields following sleep was observed.

C. Histology

Twenty four units were included in the second study. Of these, eight were located in the CA1a field, five were located in the CA1c subfield, eight were located in the CA3a subfield and the remaining three units were located in the CA3c subfield (see Figures 24a,b and 25a,b). All of the units were located in the pyramidal cell layer and are assumed to be pyramidal cells.

D- Cell types

Two basic types of units were encountered in the dorsal hippocampus- theta cells and complex spike cells. This finding is in agreement with the two types of units that have been originally reported by Ranck (1973) and later confirmed by others (O'Keefe, 1976;

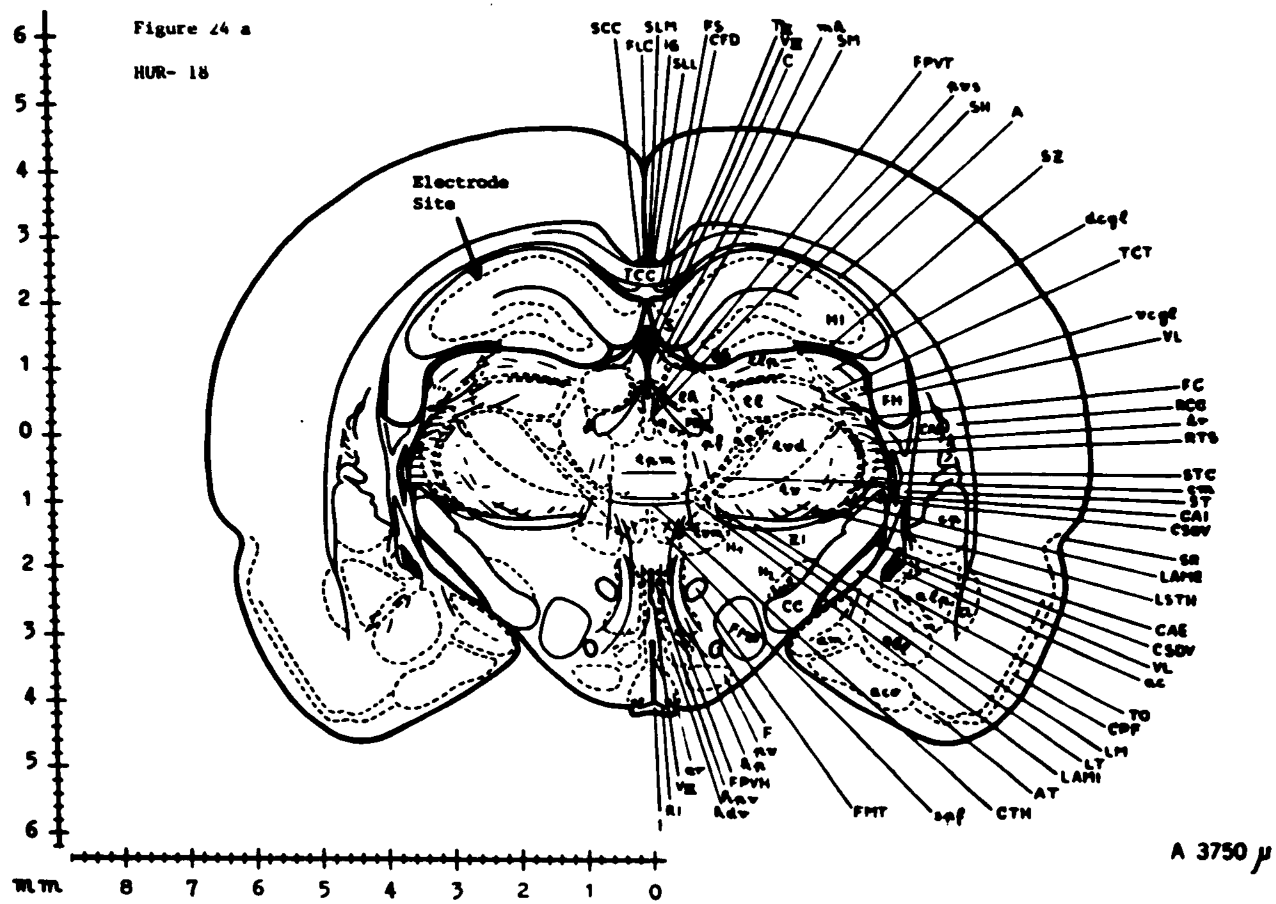
Figure 24.

a. Coronal schematic of the rat brain at the approximate position of the recording electrodes, in animal HUR-18 (Koenig and Klippel, 1974 atlas). Abbreviations: HI- hippocampus; GD- dentate gyrus; tl- lateral thalamus.

b. Coronal photomicrographs of the histological sections containing the recording electrodes in animal HUR-18. Note that the final position of the electrode passed through the hippocampal formation and entered the lateral thalamic nucleus (tl). For this animal, the electrodes traversed through the CA1b field, the dentate gyrus and the CA3c field. The units reported in the second study came from both the CA1 and the CA3 pyramidal cell fields. There is some indentation of the hippocampal formation caused by the electrode assembly. This is due to the fact that the animal was sacrificed approximately 3 months from the time of the original implantation. The recording of units, however, took place within the first 2 months following implantation. (Abbrev. DG- Dentate gyrus; Pyr- Pyramidal cell layer).

Figure 24 a

HUR- 18



A 3750 μ

Figure 24b

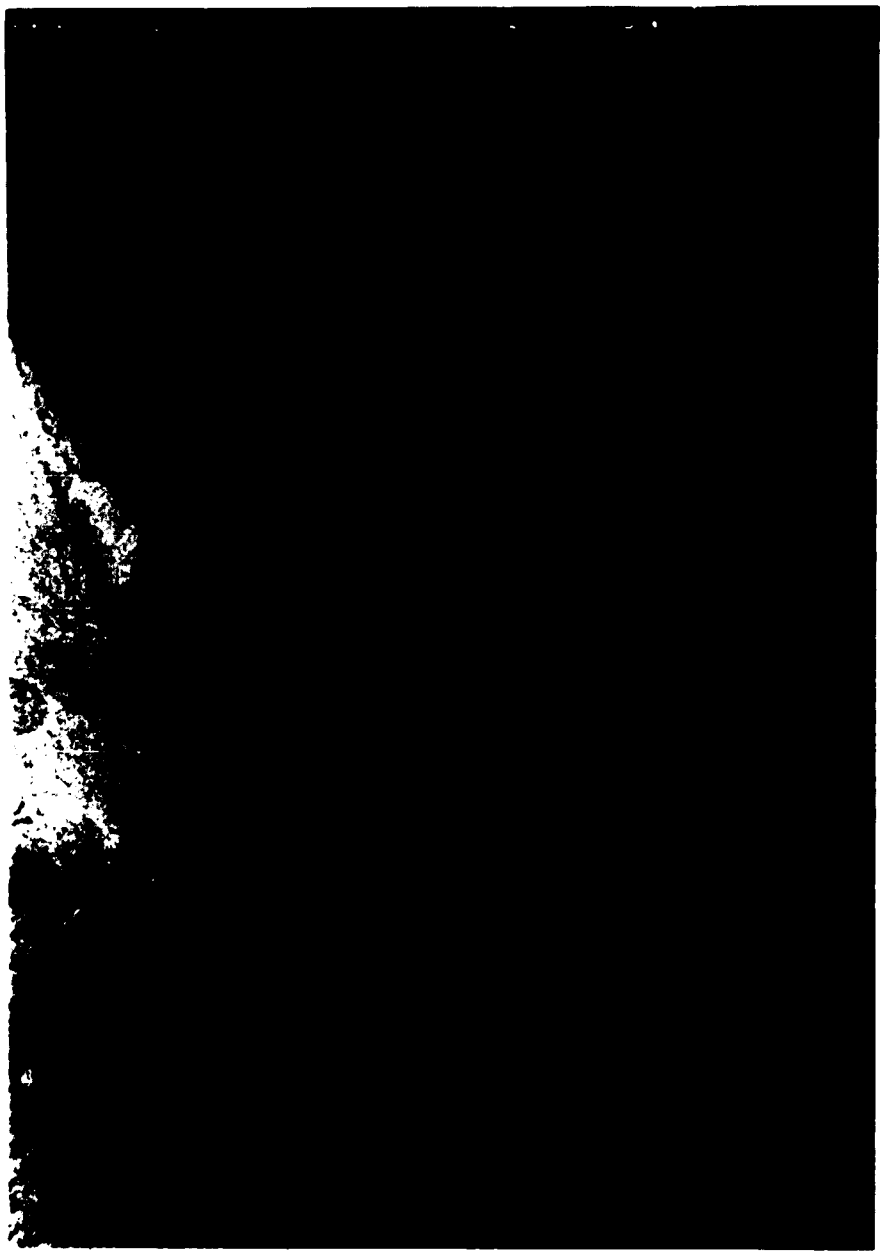


Figure 25.

a. Coronal schematic of the rat brain at the approximate position of the recording electrodes, in animal HUR-20 (Koenig and Klippel, 1974 atlas). Abbreviations: HI- hippocampus; GD- dentate gyrus.

b. Coronal photomicrographs of the histological sections containing the recording electrodes in animal HUR-20. On the right upper corner, the electrode tract and the prussian blue stain (marking the final position of the electrodes) could be detected. The position of these electrodes were in the CA3 pyramidal cell layer. (Abbrev. Cx- Cortex; DG- Dentate gyrus; Pyr- Pyramidal cell layer).

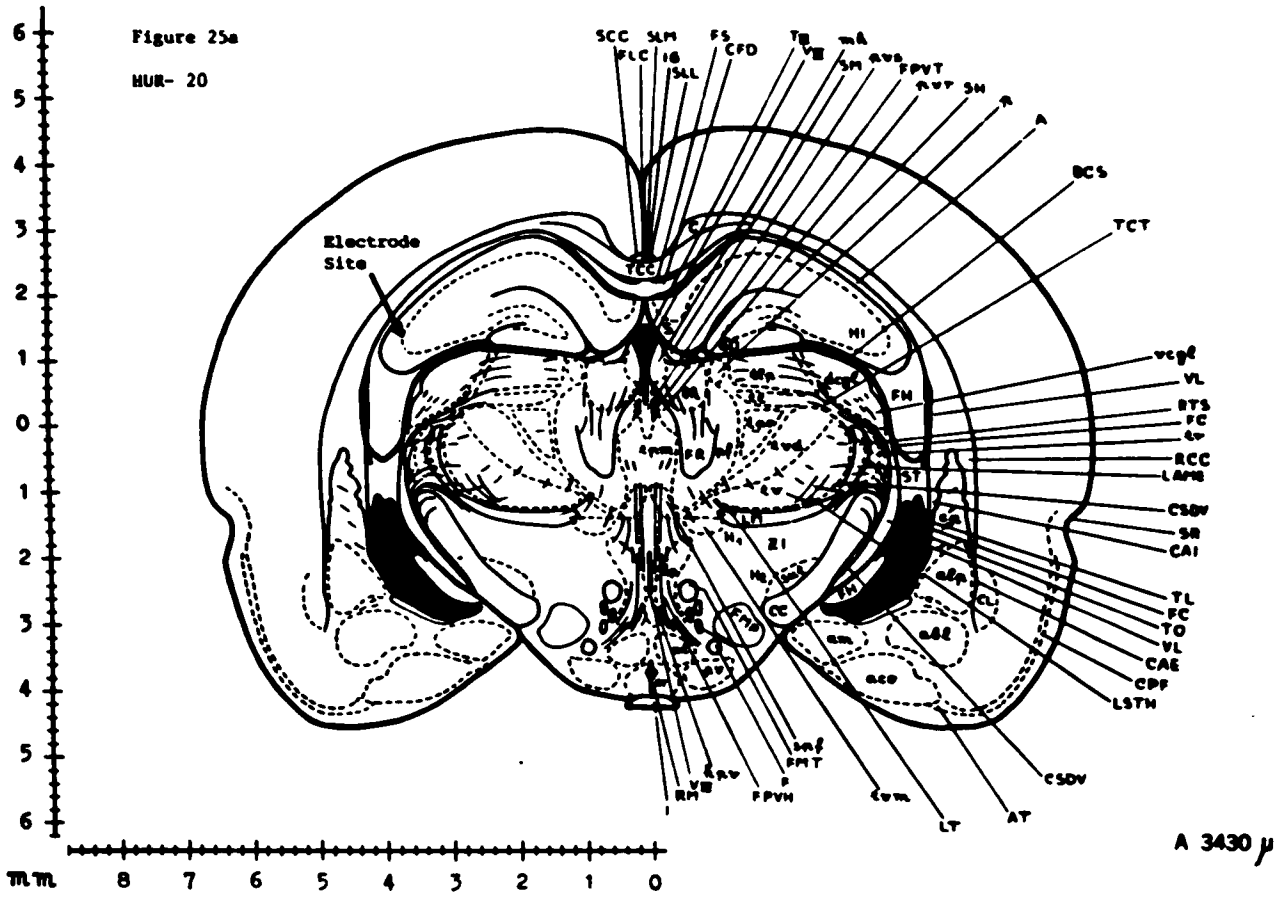


Figure 25a
HUR- 20
Electrode Site
SCC
SLM
FS
CFD
FPVT
SH
A
BCS
TCT
VCpl
VL
RTS
FC
RCC
CSDV
SR
CAI
TL
FC
TO
VL
CAE
CPF
LSTH
CSDV
AT
LT
FPVH
A 3430 μ
mm
8
7
6
5
4
3
2
1
0

Figure 25b

Olton, et al., 1978). Although a number of theta cells were encountered, only on-line observations were made on these cells and no attempt was made to record them on tape. A number of characteristics, similar to those reported by Ranck (1973) were noted. These included the observation that these cells always fire in single spike, short duration action potentials. Their rate of firing was relatively high most of the time but an increase in the rate was observed in states that were accompanied by extracellularly recorded theta rhythm (in the rat, locomotion and REM sleep; Winson, 1972). Their firing was also seen to be phase locked with the theta wave. No further attempts at analyzing the behavioral and firing characteristics of these units were made. In contrast, both the spike and firing characteristics of complex spike cells were analyzed extensively.

E. Spike Characteristics of Complex Spike Cells.

a- Duration of Action Potentials.

All of the units recorded displayed spikes with an initial negativity followed by positivity. The duration of the action potentials was based on the falling phase of the filtered signal (primary filters on Grass amplifier (Model 7P511): 3 Hz - 3 KHz; secondary active highpass filters: 3 - 5 KHz, 24 dB/octive) as measured near the baseline. The mean time interval of the action potentials was .25 msec. with a range of .15 to .36 msec.. The mean time interval of CA1 complex spike cells (.24 msec.) was lower than

the CA3 cells (.26 msec.), however, this did not represent a statistically significant difference.

b- Amplitude of Action Potentials.

The mean negative to positive peak amplitude of the 37 complex spike cells recorded (for both studies) was 105 uV with a range of 60 to 170 uV. It should be noted that although the electrodes were often moved so as to obtain a higher signal to noise ratio, no attempt was made to maximize the response. The reason for this is that recording was done on two or more units simultaneously. Since the ten microelectrodes advanced as a bundle it was feared that in trying to maximize the amplitude of one cell the amplitude of the second cell might be reduced. Due to the fact that all of the recordings made were extracellular, the displacement of the electrode from the cell being recorded would greatly influence the observed amplitude of the action potential.

F-Firing Characteristics of Complex Spike Cells.

Table 2 presents the average firing rates, in all the behavioral states tested, of the 24 units included in the analysis. Twenty two of these units showed place cell characteristics in that there was a statistically significant increase in their firing rates in specific parts of the testing apparatus (over all other places on the maze) or over their spontaneous still-alert state (Schaffer-Welsch test; $P <$

Table 2.

This table presents the rate of firing (spikes/sec., including all spikes within a burst measured separately) of the 24 hippocampal complex spike (place cells) included in the second study. The units coming from the CA1 hippocampal pyramidal cell layer are grouped on the top portion of the graph while the units that came from the CA3 pyramidal field are grouped on the bottom. Separate means are given for the two groups as well as a grand mean for the two groups combined (this organization will be true for all of the tables that follow). With the exception of cells 15 and 22, which show lower rates in the in-place-field state, all the other cells show well formed place fields (increase in firing rate in this state over all others). Surprisingly, similar rates of firing are seen for all the other behavioral states (see discussion). One other point to be made is that while the units from the CA1 hippocampal field show decreased firing in REM sleep, as compared to SWS, the units found in the CA3 field show the opposite results.

Table No. 2

Rate

		XPL	SAL	IPF	QA	SWS	PREM	REM
CA1	Mean	.51	.60	3.14	.66	.78	.79	.67
	SD	.47	1.20	2.62	.54	.70	.66	.69
CA3	Mean	.97	1.03	2.87	.96	.96	.94	1.02
	SD	.58	.68	1.67	.67	.66	.58	.83
Grand Mean		.72	.83	3.0	.81	.86	.86	.83
SD		.55	.99	2.2	.59	.66	.60	.74
Min.		.04	.08	.8	.17	.03	.13	.07
Max.		2.28	4.60	10.6	2.40	2.50	2.30	2.40

.05). Of the 24 cells presented in the table, two of them (cells number 15 and 22) appeared to decrease their firing rate in the IPF state over their XPL and SAL states. This may have been due to a shift in their place fields.

With the exception of a significant increase of the firing rate in the IPF state, none of the other behavioral states appeared to be significantly different from each other (Schaffer-Welsch test; $P > .05$). Thus the cells seemed to fire at relatively similar rates in all of the tonic behavioral states. A possible explanation for the lack of significance may be due to the very large variability in the rate of unit firing.

The 24 units in this group have been separated into two groups on the basis of the histological findings- those found in the CA1 field and those that were located in the CA3 field. An ANOVA performed on the firing rates of the two categories did not reveal significant differences in the firing rates of the two groups in any of the behavioral conditions [$F(1,22) = .53$; $P > .05$]. Close observation of Table 2, however, reveals that although not significant, the rate of firing for units in the two subfields varied somewhat with CA3 units showing higher firing rates in all of the behavioral states. Note also that while the average spikes per second of the CA1 units decreased in REM sleep in comparison to SWS, the average spikes per second of the CA3 units increased in REM sleep in comparison to SWS. In the CA1 field 11 of the 13 cells recorded fired at a higher rate in SWS than in the REM state. The other two of these units fired at higher rates

in REM than in SWS. In contrast, approximately half of the cells in the CA3 field fired with higher rates in REM than in SWS (see Figure 26).

Table 3 presents the average events per second in all the different behavioral states. An ANOVA of behavioral state and hippocampal field revealed a significant main effect of behavior. Schaffer-Welsch post-hoc analysis ($a = .05$) showed significantly higher bursting in IPF in comparison to all other behaviors. None of the other behaviors reached significance. Nonetheless, in the CA1 subfield somewhat higher bursting was also seen in the SWS and PREM states. Lower bursting rates were seen in REM sleep and the two awake states (SAL and XPL). A significant main effect of hippocampal field, was not seen. Close examination of table 3, however, reveals that in comparison to the CA1 subfield, the average bursts per second in the CA3 field was higher, although not significant. Within the CA3 field, besides a higher rate of bursting in the IPF state, the bursts per second within the remaining behaviors were rather uniform (see Table 3).

Table 4 presents the average percent bursts (versus single spikes) of the 24 cells in the seven different behavioral states. The mean percent bursts of all the cells is rather low (less than 20%) with slightly increased bursting seen in the majority of cells during the IPF (18 %) and also in the QA-SWS (17.5 %) and PREM (17.8 %) states. The lowest percentages are seen in the out-of-place-field exploration and also during REM sleep (approximately 12 %). Perhaps

Figure 26.

Line graph of the firing rate (spikes/sec.) of the twenty four units included in the second study, for each of the behavioral states tested. The left side of the graph presents the units recorded in the CA1 hippocampal field, while the right side of the graph displays the units recorded in the CA3 hippocampal field. Note that the rate of the highest unit from the CA1 field is much higher (10.6 spikes/sec.) than is actually depicted in the graph. The range of firing rates in the CA3 field appear to be larger than that of the CA1 field. Notice also that while only two units from the CA1 field show higher rates in REM than in SWS, for the CA3 field more than half of all cells show such a trend.

* actual rate of firing is 10.6 spikes/sec.

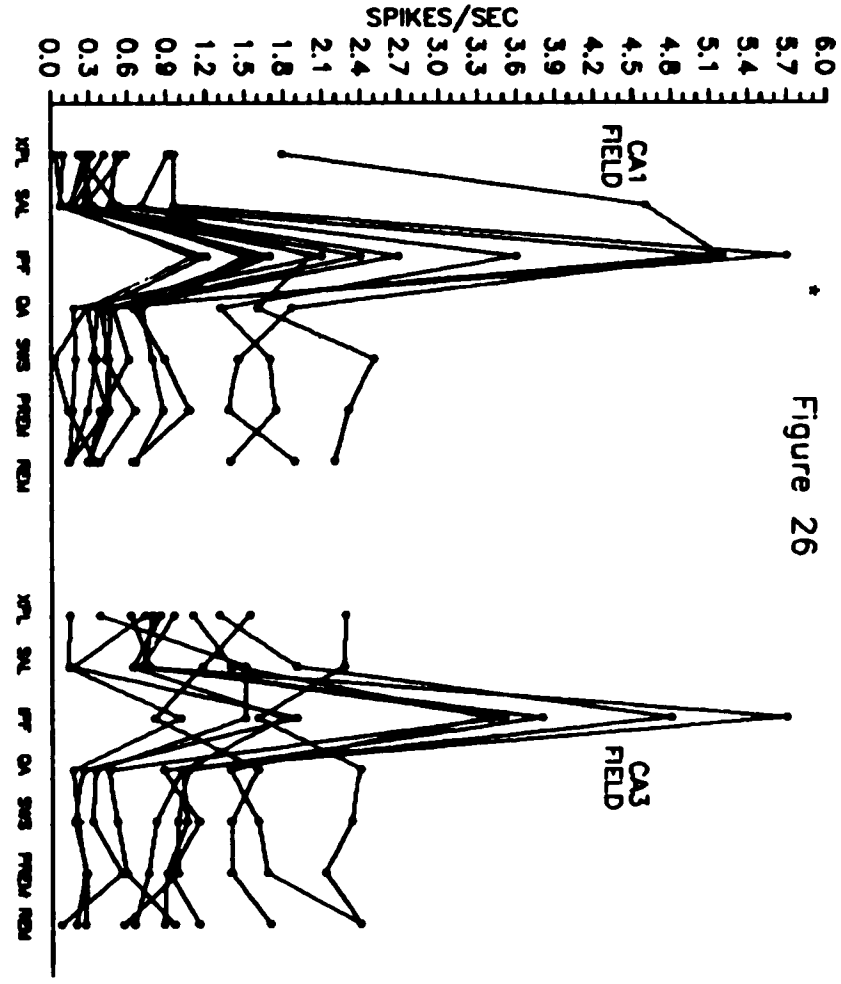

SPIKES/SEC
0.0
0.3
0.6
0.9
1.2
1.5
1.8
2.1
2.4
2.7
3.0
3.3
3.6
3.9
4.2
4.5
4.8
5.1
5.4
5.7
6.0
CA1
FIELD
CA3
FIELD

Figure 26

Table 3.

Seen here are the average events (either single spikes or bursts) per second of the hippocampal complex spike (place cells) included in the second study. These rates include all bursts (2, 3, 4, 5, 6+ spikes) but exclude the single spikes.

Table No. 3

Average Events/Sec.

	XPL	SAL	IPF	QA	SWS	PREM	REM
CA1 Mean	.52	.54	2.44	.59	.67	.65	.52
SD	.31	.82	1.76	.40	.55	.51	.47
CA3 Mean	.87	.87	2.17	.76	.82	.86	.83
	.52	.53	1.27	.50	.55	.64	.64
Grand Mean	.69	.70	2.30	.67	.73	.71	.66
SD	.40	.69	1.50	.44	.54	.46	.55
Min.	.13	.98	.55	.17	.06	.14	.08
Max.	2.00	3.09	6.20	1.84	2.12	1.90	1.90

Table 4.

This table presents the percentage of spikes that occur in bursts over those occurring as singles. Higher percentages are seen for the CA3 hippocampal cells than for the CA1 cells. Relatively higher percentages are also seen during the QA, SWS and PREM behavioral states.

Table No. 4

Average Percent Bursts vs. singles

	XPL	SAL	IPF	QA	SWS	PREM	REM
CA1 Mean	11.69	12.75	16.32	16.10	15.09	16.43	10.64
SD	7.6	8.85	10.57	7.88	9.42	6.51	5.05
CA3 Mean	14.43	19.37	20.30	19.74	19.88	19.36	13.88
SD	4.51	11.69	6.60	9.55	11.21	8.61	7.12
Grand Mean	12.94	15.90	18.00	17.80	17.30	17.80	12.12
SD	6.30	10.40	8.90	8.50	10.12	7.40	6.04
Min.	3.10	3.20	5.60	6.90	.00	6.15	.80
Max.	25.90	47.90	35.70	42.60	50.70	38.60	29.80

the homogeneity of the average percent bursts can be attributed to the large variability among the cells. By inspecting each of the cells individually it was apparent that while some cells fire at a low percentage of bursts, other cells fire in bursts more frequently.

The average number of spikes within a burst were also analyzed (see Table 5). An ANOVA of hippocampal subfield and behavioral state revealed significant main effects for both measures. The interaction of subfield by behavioral state was not significant. Post-hoc analysis for behavioral state (Schaffer-Welsch; $P < .05$) revealed significant differences between the QA and PREM and the REM and XPL states. None of the other behavioral states reached significance. Cells in the CA3 field had significantly higher spikes per burst than the CA1 field.

Finer analysis of the number of spikes within a burst was also performed separately for single-, double-, triple-, and quadruple-spikes. Although originally, subcategories for bursts with five and six (+) spikes were included in the analysis, the occurrence of such long bursts was very rare that it was decided to exclude them from the final analysis. Tables 6 and 7 present the proportion of bursts with one, two three and four spikes, for each of the behavioral states. As can be observed from these tables the occurrence of bursts with four spikes is rare. The largest number of action potentials (approximately 80%) are single spikes. The number of bursts with double spikes was approximately 13%. The number of triple spikes was approximately 13% and the number of quadruple spikes was approximately 2%. The number of quintuple spikes was less than 1%.

Table 5.

Presented here is the average number of spikes per event (event is defined as either a single spike or a burst) for the 24 units included in the second study. Slightly higher numbers are seen for the CA3 than the CA1 field.

Table 6.

This table presents the proportion of events with single (S), Double (D), Triple (T), and Quadruple (Q) spikes for the three awake behavioral states included in the second study. Note that approximately 80% of all events are single spikes. Slightly higher bursting can be seen for the CA3 than the CA1 hippocampal field.

Table 7.

The same analysis as presented in table 5 was done for the sleeping behavioral states.

Table No. 5

Average Spikes/Event

Unit No.	Hipp. Field	XPL	SAL	IPF	QA	SWS	PREM	REM
CA1	Mean	1.62	1.85	2.16	2.30	2.00	2.10	1.77
	SD	.62	.63	.39	.45	.71	.42	.53
CA3	Mean	2.27	2.51	2.32	2.59	2.42	2.59	2.01
	SD	.61	.54	.48	.55	.87	.68	.94
Grand Mean		1.92	2.16	2.20	2.40	2.20	2.30	1.88
SD		.67	.65	.42	.49	.78	.60	.73
Min.		.85	.85	1.60	1.50	.00	1.25	.40
Max.		3.30	3.60	3.40	4.20	3.90	4.30	3.55

Table No. 6

Proprotion of Spikes/Event

	XPL				SAL				IPF			
	S	D	T	Q	S	D	T	Q	S	D	T	Q
CA1 Mean	.84	.11	.03	.01	.83	.12	.04	.01	.82	.13	.03	.02
SD	.11	.08	.03	.01	.07	.04	.04	.01	.09	.05	.03	.03
CA3 Mean	.68	.15	.04	.01	.78	.14	.05	.01	.80	.14	.04	.01
SD	.07	.05	.02	.01	.11	.07	.04	.02	.06	.03	.03	.02
Grand Mean	.82	.13	.03	.01	.81	.13	.05	.01	.81	.14	.04	.02
SD	.09	.06	.03	.01	.09	.05	.04	.02	.07	.04	.03	.03
Min.	.66	.00	.00	.00	.58	.05	.00	.00	.68	.06	.01	.00
Max.	.99	.24	.09	.05	.92	.25	.14	.08	.93	.24	.09	.12

Table No. 7

Proportion of Spikes/Event

	QA				SWS				PREM				REM			
	S	D	T	Q	S	D	T	Q	S	D	T	Q	S	D	T	Q
CA1 Mean	.79	.13	.06	.01	.84	.11	.04	.01	.81	.11	.04	.02	.81	.14	.03	.01
SD	.11	.05	.09	.01	.09	.06	.03	.02	.08	.05	.02	.01	.08	.06	.02	.01
CA3 Mean	.79	.13	.05	.02	.79	.14	.05	.04	.77	.17	.05	.02	.81	.13	.04	.03
SD	.11	.05	.09	.01	.09	.06	.03	.02	.08	.05	.02	.01	.09	.07	.03	.04
Grand Mean	.79	.13	.06	.02	.82	.13	.04	.22	.79	.15	.04	.02	.81	.14	.03	.02
SD	.09	.04	.06	.03	.09	.06	.03	.45	.13	.13	.03	.02	.08	.06	.02	.03
Min.	.54	.07	.01	.00	.61	.00	.00	.00	.24	.06	.01	.00	.65	.02	.00	.00
Max.	.91	.22	.34	.13	.99	.23	.11	.23	.93	.72	.11	.08	.98	.28	.10	.13

Table 8 presents the average inter-burst intervals in all the behavioral states tested. An analysis of variance of behavioral state and hippocampal field revealed significant differences for behavioral state [$F(6, 132) = 6.176$; $P < .001$] but not for hippocampal field. Post-hoc comparisons of behaviors (Schaffer-Welsch test; $P < .05$) showed that the IPF state displayed lower IBI's than any of the other behavioral states tested. None of the other states, however, were significantly different from each other. Somewhat lower IBI's were observed during the awake behaviors and also in the PREM and REM states for the cells in the CA3 subfield. During QA and SWS, lower IBI's were observed in the CA1 subfield.

The interspike intervals (of spikes within a burst) were also analyzed. Table 9 presents the average interspike intervals (in msec.) for the 24 units in each of the behavioral states. An ANOVA revealed significant main effects both for behavioral state [$F(6,132) = 6.36$; $P < .001$] as well as for hippocampal field [$F(1,22) = 16.79$; $P < .001$]. The average ISI's, in decreasing order of magnitude were: IPF (6.5), QA (6.0), SWS (5.87), PREM (5.7), SAL (5.6), XPL (5.2) and REM (4.78). Schaffer-Welsh post-hoc comparisons for behavioral state revealed significantly lower ISI's for REM and XPL over all of the other states tested. REM and XPL were not significantly different from each other. Significantly lower ISI's were obtained for the CA1 than the CA3 hippocampal subfields.

The ISI's were further subdivided into smaller intervals for closer analysis. Tables 10 and 11 present the proportions of cells

Table 8.

Presented here are the time intervals between bursts.

Table No. 8

Average IBI (Msec.)

	XPL	SAL	IPF	QA	SWS	PREM	REM
CA1 Mean	2760	2601	1221	2350	2154	2250	1991
SD	813	1095	509	812	931	922	801
CA3 Mean	1955	2191	1335	2428	2208	2171	1830
SD	763	969	774	1423	1465	1101	882
Grand Mean	2391.2	2005.2	2277.9	2386.5	2220.5	2214.4	1917.6
SD	856.9	1011.5	595.6	999.6	1161.1	965.4	805.9
Min.	952.3	432.5	241.4	783.5	555.9	799.9	667.9
Max.	4586.9	4948.5	2586.8	5077.4	4613.1	3942.4	3587.7

Table 9.

Presented in this table are the average interspike intervals for spikes within a burst. Lower interspike intervals can be seen for the CA1 than the CA3 hippocampal place cells.

Table No. 9

Average I-S-I (Msec.)

	XPL	SAL	IPF	QA	SWS	PREM	REM
CA1 Mean	4.11	4.54	5.90	5.41	5.11	4.91	4.35
SD	1.44	1.73	1.18	.91	1.74	1.03	1.28
CA3 Mean	6.30	6.54	7.19	6.55	7.02	6.48	6.96
SD	1.76	.67	1.26	.87	1.16	1.05	2.31
Grand Mean	5.11	5.50	6.46	5.90	5.76	5.60	4.75
SD	1.88	1.61	1.30	1.03	1.84	1.26	1.80
Min.	2.25	2.00	3.96	4.20	.00	3.50	1.07
Max.	8.30	7.98	8.70	8.00	8.50	7.90	7.77

Table 10.

The ISI's were subdivided and analyzed in shorter intervals (0-2, 2-4, 4-6, 6-8 and 8-10) and the proportions of spikes falling within these subcategories are presented here for the three waking states.

Table 11.

This table is similar to table 9 except that it presents the proportions of ISI's for the sleeping behaviors. It is apparent from this analysis that the interspike intervals are not constant but could change between bursts.

Table No. 10

Proportion of Interspike Intervals (msec.)

	XPL					SAL					IPF				
	<2	<4	<6	<8	<10	<2	<4	<6	<8	<10	<2	<4	<6	<8	<10
CA1 Mean	.06	.19	.30	.29	.16	.02	.17	.40	.33	.07	.01	.16	.35	.30	.19
SD	.10	.12	.09	.08	.11	.03	.14	.19	.25	.07	.01	.12	.10	.07	.13
CA3 Mean	.14	.22	.30	.17	.18	.02	.27	.32	.19	.16	.03	.12	.35	.26	.22
SD	.26	.11	.15	.09	.14	.03	.15	.17	.09	.07	.04	.07	.08	.09	.07
Grand Mean	.09	.21	.30	.24	.17	.02	.22	.36	.26	.11	.02	.14	.35	.28	.20
SD	.19	.11	.11	.10	.12	.02	.15	.18	.20	.09	.03	.09	.09	.08	.10
Min.	.00	.00	.07	.04	.00	.00	.00	.00	.00	.00	.00	.03	.23	.14	.00
Max.	.78	.50	.57	.42	.52	.07	.68	.80	1.00	.31	.10	.38	.65	.47	.39

Table No. 11

Proportion of Interspike Intervals (msec.)

		QA					SWS					PREM					REM				
		<2	<4	<6	<8	<10	<2	<4	<6	<8	<10	<2	<4	<6	<8	<10	<2	<4	<6	<8	<10
CA1	$\bar{X}$	.05	.18	.39	.26	.10	.08	.21	.41	.14	.09	.02	.24	.38	.24	.11	.02	.25	.41	.20	.12
	SD	.06	.10	.08	.13	.08	.15	.12	.12	.09	.06	.02	.10	.06	.09	.06	.03	.14	.12	.07	.10
CA3	X	.08	.27	.33	.18	.15	.01	.28	.32	.22	.17	.01	.27	.35	.20	.17	.01	.22	.33	.23	.21
	SD	.13	.13	.08	.07	.07	.02	.13	.09	.09	.07	.01	.12	.09	.06	.06	.03	.15	.14	.13	.27
Grand	$\bar{X}$	.06	.22	.36	.22	.12	.05	.24	.37	.10	.13	.02	.25	.36	.22	.13	.02	.23	.37	.21	.16
SD		.09	.12	.08	.11	.07	.11	.12	.11	.44	.08	.02	.11	.07	.08	.06	.03	.14	.13	.10	.19
Min.		.00	.01	.18	.02	.00	.00	.05	.10	.22	.00	.00	.10	.19	.10	.00	.00	.00	.00	.00	.00
Max.		.44	.54	.52	.49	.28	.56	.50	.11	.09	.27	.08	.47	.49	.47	.25	.09	.45	.69	.50	1.00

with ISI's falling into one of the subcategories, for each of the behavioral states tested. As can be observed from these tables, the ISI's fall within an approximately normal distribution. The majority of spikes within a burst fire with an interspike interval of 4-6 msec. (approximately 35%). Approximately 20% of the spikes fire with 2-4 msec. ISI's. Approximately 20% fire with 6-8 msec. ISI's. Bursts with ISI's at the higher and lower ends of this range are rare.

A number of other observations concerning the firing of complex spike cells, although not quantified, were made at the time of the recording. Firstly, place field firing appears to be more intense when an animal enters its place field, and habituates to a small extent as the animal remains there for a period of time. Secondly, upon awakening from sleep (either SWS or REM sleep) for a short period of time (approximately 10-15 seconds) there is a complete silence in unit activity. Similar observations have been made by other investigators (O'Keefe, 1979, Suzuki and Smith, 1985; Ranck, personal communication). A further observation has been made in REM sleep firing. It has been noticed that although a good portion of the REM sleep episode is free of unit firing, at times there appears to be intense bursts of firing that lasts for short periods of time. These short bursts of firing perhaps may account for the fact that although, on the average, REM sleep unit activity appears to be very low, when analyzed over long periods of time this stage of sleep turns out to be as active as all other behavioral states.

As previously stated, place field firing has been shown, by a number of investigators (O'Keefe, 1979; Olton, et al., 1978; and others) as well as in the present studies, to be stable over long periods of time (which in most cases could extend over a number of days) and even after the removal of an animal from the particular environment. Place field firing, however, may be more plastic than originally believed. It was noticed in a number of cases that increased firing of a place cell occurred in places other than the place field when the animal first awakened from sleep and engaged in exploration. While in most cases this new increased firing did not last for a long time and new place fields did not develop, in a few cases the increased firing continued for the remainder of the experimental session.

Discussion

The results of the present experiment confirm many of the observations made previously on hippocampal complex spike cells. In particular, the observation that the majority of hippocampal complex spike cells of freely behaving rats increase their firing in correlation with a specific spatial location (O'Keefe and Dostrovsky, 1971; Olton, et al., 1978; Hill, 1978) has been confirmed. Approximately two-thirds of the complex cells encountered in a single environment displayed place field firing characteristics. This number is somewhat lower than the 95% estimate given by O'Keefe (1979) but is

comparable to more recent estimates (Best and Thompson, 1986). The in-place-field firing rates of the cells in the present studies are also similar although slightly lower than those reported earlier (Hill, 1978; O'Keefe 1976; Olton, et al., 1978). One reason for the lower rates is that the IPF rates reported here reflect firing rates averaged over an extended period of time. In fact the rates reported for all behaviors may be lower than those reported previously for the same reason.

There are no previous studies available that have analyzed the detailed firing characteristics of place cells during tonic waking and sleeping behaviors with the exception of a few studies that have analyzed the overall firing rates. Many of the observations originally made by Ranck (1973) were confirmed in the present study. For example, Ranck reported that the firing rates of complex spike cells were highest in SWS than in other tonic behavioral states. The great majority of the complex cells that he had observed fired in lower rates during REM than SWS. Ranck (1973), however, also noticed a few neurons whose firing rates were higher in REM than SWS. It should be noted that classification of cells in accordance to hippocampal field were not made at that point. Ranck (1973) also reported that in REM sleep, the majority of units were mostly silent with the exception of 'groups of action potentials for a few seconds'. This last observation was precisely the mode of firing for the majority of units observed in the present study. Although the firing rate during REM sleep was very low to practically absent, the unit would suddenly burst into firing both in single as well as complex spikes. This sudden burst of firing

was unpredictable and was not correlated with the phasic mode of firing which is characterized with increased amplitude EEG, panting, whisker and eye movement, heavy breathing and a decrease in muscle tone. Neither did the increased firing appear to be phase locked to theta rhythm.

Olmstead, et al. (1973) also recorded complex spike cells from all hippocampal subfields. They subdivided their cells in accordance with the hippocampal region in which they were found. The majority of complex spike cells found in the CA1 and CA2 fields fired at higher rates in SWS than in REM sleep. Conversely, in the CA3 and CA4 hippocampal fields, the majority of complex spike cells fired at higher rates in REM than in SWS. The results of the present studies confirm the observations made by Ranck (1973) and are also in agreement with the findings of Olmstead et al., (1973).

Suzuki and Smith (1985) subdivided their CA1, CA3 and DG complex spike cells into fast and slow firing cells. Their results indicate that while the slow firing cells fire at higher rates in SWS than in REM sleep, the two high firing complex spike cells they encountered showed higher rates in REM than SWS. Seven complex spike cells in the present study could be classified as fast firing. One out of four in the CA1 field fired at higher rates in REM than SWS while in the CA3 field all three fast complex spike cells fired at a higher rate in REM than in SWS. It is possible that Suzuki and Smith's (1985) fast firing cells, which fired at higher rates in REM sleep, came from the CA3 field.

Rather surprising is the apparent uniformity of the firing rates of these cells in many of the behavioral states (see Table 2). A number of possible explanations for this variability come to mind. For one, the uniformity may be due to a lack of differentiation of behavioral state produced by the length of unit analysis. This is an unlikely explanation, however, since many of the behavioral states are so different that misinterpretation of the animal's behavior and EEG characteristics is almost impossible. This not withstanding, behavioral mixup may be a legitimate explanation. A second explanation may be due to the large variability between the individual units. It is also possible, although unlikely in light of the findings of the fist study, that the firing rates of these cells is equal in all behavioral states when observed over long periods of time. The uniformity may be a product of the particular group of cells included in this study. These cells were originally believed to be discrete place cells but at the off-line data analysis they were shown to posses multiple, non-discrete place fields. Another possible, although unlikely, explanation is that the uniformity in the firing rate in all of the sleep states is due to the substantial degree of place field firing that these cells underwent during the awake states.

Besides the higher firing rates that were observed in the CA3 hippocampal place field, there was also a higher number of bursts per second as well as a higher number of spikes within a burst seen for the cells coming from this field as opposed to the CA1 hippocampal field. In contrast, shorter interspike intervals for spikes within a burst were observed in the CA1 as opposed to the CA3 field.

For a number of units for which correlational analysis was performed, the hippocampal complex cells observed in this study appeared to maintain their characteristic rate of firing across the various behavioral states (Pearson r = .73 to .87). That is, fast firing and slow firing cells maintained their mode of firing throughout all behavioral states.

A discussion of the significance of these results will appear in the final chapter.

CHAPTER 5

General Discussion

Evidence for the involvement of the hippocampus in learning and memory is derived from studies employing a number of diverse techniques. The lesion studies indicate that destruction of the hippocampal formation produces working memory deficits in rats. Similar memory deficits for episodic memory are also reported in humans. Recent studies have indicated that the memory deficit does not require destruction of the entire hippocampus but that severe memory deficits can also be elicited with lesions restricted to the CA1 hippocampal field. Furthermore, these memory deficits are of a compound nature in that they involve both an anterograde (inability to form new memories) as well as a retrograde component (inability to remember events for a short period of time prior to the lesion). These findings indicate that the hippocampus is essential for the consolidation and/or retrieval of some kinds of newly formed memories.

Hippocampal complex spike cells present a most remarkable correlation between unit activity and environmental stimuli. A number of investigators have reported that in the freely behaving rat, the majority of these cells increase their firing rates in correlation with the animal's position in space (Olton, 1980; O'Keefe, 1976; O'Keefe and Dostrovsky, 1971). More remarkable is the fact that once a

place field has been established (in most cases soon after an animal visits a particular place; Hill, 1978) it maintains its increased firing to that particular place field over extended periods of time (days to weeks, O'keefe, 1976; Best, 1986, personal communication; observations on present studies) and even after removal and replacement of an animal from the environment containing the place field. The ability of rats to perform spatial and working memory tasks is diminished following hippocampectomy (Olton, 1983). It would appear, therefore, that a main function of the hippocampal formation, at least in the freely behaving rat, is the formation and maintenance of information related to space and to working memory tasks (O'Keefe and Nadel, 1979; Olton, 1984). Furthermore, consolidation of such memories must be an active process that takes time to be completed. A critical question that arises then is the time course of such a process and the nature of the underlying neural events accompanying it.

As described in the introduction, neuronal transmission through the hippocampal formation is dependent on the behavioral state of the animal (i.e. neuronal gating; Winson and Abzug, 1978). To recapitulate, through the use of field potential analysis, it was demonstrated that transmission of a stimulus through all three stages of the hippocampal trisynaptic chain was restricted during the still alert state. Conversely, such transmission was totally unrestricted during the SWS state (gate open). Variable gating was seen in the two behavioral states accompanied by theta rhythm (in the rat) XPL and REM sleep. The findings in neuronal gating suggest that information,

perhaps related to memory consolidation, may be occurring in the rat hippocampal formation in a behaviorally dependent manner.

The results of the descriptive study to a large extent confirm the findings reported using field potential analysis. In the tonic behavioral states, complex spike cells fire at a slow rate during the still alert state and also during random exploration. Complex spike cells, however, also fire in a phasic mode, namely in place fields, which could not be detected using field potential analysis. During the sleep states, unit firing was significantly higher during SWS and also during QA (it should be noted that in terms of behavioral and EEG characteristics, these two behaviors are rather similar). In contrast, the firing rates during the REM state were significantly lower, comparable to some extent with the field potential studies which show that the CA1 field is completely closed to neuronal transmission during REM and that the DG and CA3 fields are only relatively restricted during this state.

The findings of the counterbalanced study, further compliments and extends on the findings reported with field potential analysis. It is suggested that processing of information acquired in the awake state may occur during the subsequent sleep states. Thus, when a rat was placed in a unit's place field and the unit increased its firing there, there was also enhanced firing in the sleep states that followed. The enhanced unit activity was observed in all of the sleep states, the highest increases, however, occurred in SWS and REM sleep.

The role of sleep, and more specifically REM sleep, in memory consolidation has also been supported by REM deprivation experiments (Fishbein, et al., 1971; Fishbein and Gutwein, 1977; Horne and McGrath, 1984). What came to be known as the REM memory-retention hypothesis suggests that memory remains labile until the occurrence of REM sleep. Keeping in mind that there are a number of methodological problems in the REM sleep deprivation experiments (see Ellman, et al. 1978 for review), the majority of these studies suggest that REM sleep is a necessary prerequisite for memory consolidation to occur. A basic paradigm used in these studies is one in which, a rat or mouse is given a one trial passive avoidance task following which the animal is selectively deprived of REM sleep. At various time intervals following REM sleep deprivation the animal is given a session of electroconvulsive shock (ECS) and is then tested for retention of the passive avoidance task learned previously. Control animals are allowed normal REM sleep or are given the ECS at longer time intervals after the REM deprivation, having been allowed a chance to engage in REM sleep (for review see Ellman, et al., 1978). The results in these studies indicate that ECS produces the biggest memory deficits if applied between one and three hours following 48 hours REM sleep deprivation. Longer intervals for the application of the ECS were not effective (Fishbein and Gutwein, 1977). The conclusion drawn from these studies is that REM sleep is necessary for memory consolidation to take place.

The results obtained in the present studies to some extent complement and add another dimension to the findings made in the REM

sleep deprivation experiments. The present results also suggest that sleep (both SWS and REM) may play a role in the memory consolidation process. Thus, spatial information, as revealed by the increased firing in particular places during the awake state, may also be reprocessed, as revealed by the enhanced firing of these cells in the sleep states that follow exposure. Since hippocampal place fields have been shown to maintain their place field specificity over extended periods of time it is reasonable to assume that the increased firing of these units in the subsequent sleep states is related to the awake, place field firing. The increased firing activity was evidenced for a number of the parameters tested (i.e. spike rate, burst rate, number of spikes within a burst, and interspike interval) and occurred both in SWS and REM sleep. Furthermore, the increased firing appeared to be limited to an approximately three hour period during which time a number of SWS-REM episodes had transpired. In contrast to sleeping enhanced firing activity, the firing mode of these cells remained relatively unchanged in the awake states following exposure to place field. It would appear, therefore, that if memory processes are dependent on the increased firing of these cells then such a process could take place during sleep.

A second question concerning memory consolidation relates to the underlying mechanism of such a process. Long-term potentiation, the enhancement of synaptic facilitation by application of brief electrical stimulation to the perforant pathway has been proposed as a mechanism for mnemonic processes (Bliss and Lomo, 1973). If indeed hippocampal long term potentiation is a naturally occurring phenomenon

it must be induced by entorhinal cortex and hippocampal cell firing. The hippocampal formation and its connectivity with cortical and limbic system structures presents a potential feedback loop that may underlie the neural networks necessary for particular forms of memory (Halgren, 1984). Furthermore, the nature of the hippocampal innerconnectivity (the trisynaptic chain) is such that incoming stimulation produces an enhancement in these neural networks that could last for long periods of time and that may underlie the memory consolidation process.

In light of this, the findings of the present studies are of great relevance for two reasons. First, the descriptive analysis of hippocampal complex spike cells during the various waking and sleeping behavioral states has provided, for the first time, a detailed description of the spiking characteristics of these cells. Besides measuring the firing rate, which had been reported previously, an analysis of the number of bursts, the interburst intervals, the number of spikes within a burst as well as the interspike intervals were analyzed in all the behavioral states. All of these parameters may play a crucial role in the natural induction of long-term potentiation. Our findings, for the first time, provide the opportunity to utilize normally occurring patterns of CA1 and CA3 cell firing to investigate LTP in anesthetized animals or in the hippocampal slice. This provides a further link between LTP and naturally occurring memory processes.

Findings reported in LTP research revealed the existence of three sensitive parameters for its induction. One way of effectively inducing LTP is by increasing the number of stimuli that constitute the LTP stimulus train. The findings of the present studies suggest that such a process may be happening physiologically in that an increase in the number of spikes within a burst occurred, in the sleeping behaviors, following increased activity of the hippocampal place cells. Second, LTP is induced more effectively by increasing the number of bursts that constitute the LTP stimulus. Increased bursts were also seen in the present study after exposure of the cells to their place fields. Following exposure, increased bursting took place both in the waking as well as in subsequent sleeping behaviors. Finally, there appears to be a frequency sensitive effect in that the most effective frequency for inducing LTP is 400 Hz (2.5 msec. ISI). Following exposure of the place cells to their place fields there was an increase in the number of bursts with ISI's in the 2-4 msec. range-approximately 400 Hz.. Our present experiments reveal changes in cell firing characteristics which are more likely to produce LTP. Furthermore, the present findings indicate that assuming that LTP is a naturally occurring phenomenon, it may occur in a behaviorally dependent manner.

In summary, an underlying reason for undertaking these experiments was an attempt to further understand mnemonic processes at the neuronal level. Previous research dealing with memory suggests that a necessary prerequisite for memory consolidation is the processing of information through the hippocampal trisynaptic chain,

the last two stages of which are the CA3 and CA1 neurons. One possible interpretation of our results is that memory processing is evidenced, at least for the hippocampal place cells, by the enhancement of the firing characteristics that have been noted here to take place during sleep, following exposure in the waking state.

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