Place Field Plasticity and Directionality in a Spatial Memory Task

Patrick D. Martin

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Department of Anatomy and Developmental Biology,
University College London.

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Abstract

In order to determine whether hippocampal units display firing which is modulated by the demands of a spatial memory task rats were trained in an enclosed "cue controlled environment" (CCE) consisting of four platforms and six spatial cues that identified one of the four platforms as the goal. Rats learned to select the goal platform at the end of the trial even when the cues defined the goal platform were removed mid-way through the trial.

In a previous study (O'Keefe and Speakman, 1987a) place fields were shown to be controlled by the spatial cues such that rotation of the cues caused concomitant rotation of the fields. They also found that the place fields continued to fire after removal of the cues. Thus although the place fields were controlled by the six spatial cues they were not dependent on them.

In the present study we confirm the above findings and report two new responses. Some hippocampal place units were observed to increase or initiate place specific firing after cue removal. Others decreased or ceased place specific firing after cue removal. Thus place field location and place field intensity appears to be governed by the presence or absence of the six spatial cues.

Previous work has shown that place fields show directionality when a rat traverses an open field in a restricted and stereotypical manner (McNaughton et al, 1983a; Marcus et al, 1995). Rats trained on the CCE spatial memory task were shaped to run in a raster pattern to insure uniform platform coverage. The place fields recorded in this situation were found to display directionality oriented with respect to the six spatial cues. Moreover the directionality of place fields was found to persist after the six spatial cues were removed. Thus place field directionality appears to be initially configured by the six spatial cues but is subsequently independent of them.

O'Keefe and Speakman (1987a) examined error trials in which the rat failed to choose the goal platform. They found that the place field firing displayed by the hippocampus before the rat chose a platform could be used to predict the
rat's choice. In the present study the hippocampal error trial firing could be
use to predict the choice the rat made in some instances as per O'Keefe and
Speakman (1987a). However in the majority of instances the hippocampal
error trial firing was completely unrelated to that seen in previously recorded
correct trials and in one instance the hippocampal error trial firing predicted
that the rat would choose correctly.
Abbreviations

CCE cue controlled environment
CC controlled cue
SBC static background cue
PP perceptual-perceptual trial
PP1 first two minute control phase of the PP trial, cues are present
PP2 second two minute test phase of the PP trial, cues are present
PM perceptual-memory or cue removal trial
PM1 first two minute control phase of the PM trial, cues are present
PM2 second two minute test phase of the PM trial, cues are absent
Chapter 1: Background
1.1 Why the Hippocampus?

In 1953 the patient HM underwent bilateral removal of mesial temporal lobe structures in an effort to treat epilepsy (Corkin, 1984). He subsequently displayed a profound anterograde amnesia. This finding led to a surge of interest in the mesial temporal lobe structures. The most prominent of these structures is the hippocampus. The conclusion initially drawn was that the locus of the mnemonic deficit was the hippocampus. However Mishkin (1978) found that both the amygdala and hippocampus had to be removed in order to produce a memory deficit in monkeys. Furthermore later MRI studies have shown that HM sustained extensive damage to mesial temporal lobe structures other than the hippocampus and retains five eighths of his hippocampus (O'Keefe, personal communication). This has cast some doubt as to the identity of the structures participating in the anterograde amnesia of HM.

With the spotlight now on the mesial temporal lobe structures more patients were identified with damage to these structures (Squire and Zola-Morgan, 1988; Squire, 1992; Bechara et al, 1995; Kartsounis et al, 1995). They were found to display various memory deficits. McLardy (1970) reviewed the patient pool available at the time and concluded that they invariably sustained damage to structures adjacent to the hippocampus as a result of their diverse diseases. He concluded the mnemonic functions often ascribed to the hippocampus proper were located in structures adjacent to the hippocampus.

Despite the uncertainty regarding the locus of the mnemonic deficit numerous neuropsychologists were inspired to perform a large number of studies in which the hippocampi of standard laboratory animals were lesioned, stimulated, recorded from, and otherwise manipulated while the animal performed a variety of mnemonic tasks. Numerous hypotheses of hippocampal function were advanced. The evidence presented for many of these hypotheses has been heavily biased towards lesion data and in some cases no electrophysiological evidence is presented in their favor.
During this period in which research into the possible mnemonic role of the hippocampus was at a peak O'Keefe and Dostrovsky published a paper in which they observed a correlation between the rat's location and single unit hippocampal discharge (O'Keefe and Dostrovsky, 1971). They found that units in the CA1 layer of Ammon's Horn fired when the rat was in specific location on a box and failed to fire in all other locations. They termed the location in which the unit fired a "place field" and the unit itself was called a "place unit". Numerous other workers soon observed place units and began exploring their properties. This growing body of data prompted O'Keefe and Nadel (1978) to propose that the hippocampus was not involved in generalized mnemonic functions but rather was exclusively concerned with spatial processing.

1.2 Outline of the Thesis

The aim of this thesis is to examine whether hippocampal units display firing which is modulated by the demands of a spatial memory task.

This thesis is organized into four main parts. The first part consists of Chapters 2, 3, and 4. Together these form an overview of the anatomy, physiology, and hypothesized functions of the hippocampus respectively, the background to the research described herein.

The second part consists of Chapter 5, in which the methods used to collect the data in this thesis are detailed. Only one experimental paradigm was employed to gather this data, namely recording from rat hippocampal place cells while animal performed a spatial memory task.

The third part consists of Chapters 6, 7, 8, and 9, in which the data is analyzed in four different ways. In each of these four chapters the rational for the analysis procedure is introduced, followed by a description of the analysis paradigm, the results of the analysis, and a discussion of these results.

The fourth part consists of Chapter 10, in which the results of this thesis and other work in the field are examined. This is followed by an attempt to draw
conclusions as to the function of the hippocampus, drawing on three currently influential hypotheses of hippocampal function. Lastly possible fruitful directions for further research are proposed.
Chapter 2: Anatomy
2.1 Gross Anatomy of the Hippocampal Formation

Ramón y Cajal (1911) and Lorente de Nó (1933, 1934) first provided detailed descriptions of the hippocampal formation, including the hippocampus proper and the surrounding structures. In rodents the hippocampal formation lies just beneath the cortex, occupying a large fraction of the total brain area. In primates the hippocampal formation is located in the medial temporal cortex, occupying a considerably smaller fraction of the total brain area. (Figure 1.)

This thesis will be predominantly concerned with the rat unless stated otherwise. Opinions vary as to which structures constitute the hippocampal formation. In this thesis the convention of Amaral and Witter (1989) will be followed. Thus the structures that will be considered part of the hippocampal formation are Ammon's Horn, dentate gyrus, subiculum, and entorhinal cortex.

In the rat the two interlocking C shapes of Ammon's Horn and dentate gyrus start at the midline of the hippocampus, just posterior to the septum. They curve both ventrally and posteriorly around the dorsal surface of the thalamus to their deepest and most lateral extent adjacent to the amygdala. This complex three dimensional morphology has lead to the adoption of the septal-temporal axis nomenclature. This axis follows the curves of the hippocampus. The septal pole or direction is located at the midline or septum. The temporal pole or direction is located laterally and posteriorly, just below the medial temporal lobe, and adjacent to the amygdala. The subiculum lies posterior and medial to the mass formed by the interlocking Ammon's Horn and dentate gyrus. The entorhinal cortex lies posterior and lateral to the mass formed by the interlocking Ammon's Horn and dentate gyrus. (Figure 2.)

Ammon's Horn is a C shaped layer of cells that is subdivided into subfields, known as CA1, CA2, and CA3 where CA is an abbreviation for Cornu Ammonis. The CA2 layer is physically small and functionally obscure. Consequently in most studies it is lumped into the CA3 field for convenience although Ishizuka et al (1990) has suggested this is an oversimplification. In the original studies by Lorente de Nó (1934) the CA3 region was said to give rise to the "CA4" layer. Later work has classified the "CA4" layer neurons as
being more closely associated with the hilus of the dentate gyrus than Ammon's Horn (Blackstad, 1956). The latter classification will be followed in this thesis. (Figure 3.)

The dentate gyrus is composed of a C shaped layer of cells that curves around the lower blade of Ammon's Horn such that the two C shapes interlock. The dentate gyrus is subdivided into the upper or buried blade and the lower or exposed blade. Enclosed by the C shaped layer of dentate gyrus cells is the dentate hilus. (Figure 3.)

The subicular complex lies beside the CA1 layer. The subiculum is divided into either four or five subdivisions by different authors (Swanson, 1979; Amaral et al, 1991). The prosubiculum is the area between the end of the CA1 layer and the subiculum. However some authors consider the prosubiculum to be the area in which the CA1 and subiculum overlap and consequently do not recognize the prosubiculum as a separate area (Witter and Groenewegen, 1990). Posterior to the prosubiculum is the subiculum proper, the presubiculum, and the parasubiculum. The postsubiculum is an invagination of the subiculum present only at the septal end. (Figure 3.)

The entorhinal cortex lies beside the parasubiculum. Based on cell morphology the entorhinal cortex can be divided into lateral and medial halves (Blackstad 1956). The medial entorhinal cortex lies between the subiculum and the lateral entorhinal cortex. The lateral entorhinal cortex is lies between the medial entorhinal cortex and the perirhinal fissure. (Figure 3.)

2.2 Layers of the Hippocampal Formation

Ammon's Horn is divided into five layers. Descending from the ventricular surface these are the alveus, striatum oriens, striatum pyramidale, striatum radiatum, and striatum lacunosum-moleculare. In the CA3 cell layer there is one further layer, the striatum lucidum, located just below the striatum pyramidale. These cell layers curve as Ammon's Horn curves. (Figure 4.)
The dentate gyrus is subdivided into three layers, the molecular layer, granule layer, and polymorph layer or hilus. The hilus or polymorph layer is the innermost zone, curving around with the curve of the dentate and enclosed by the granule layer.

There is little detailed histological information on the various components of the subiculum. However most authors agree the prosubiculum, subiculum, and the septally located postsubiculum consist of a single diffuse layer of loosely packed cells (Blackstad, 1956; Swanson, 1979; Amaral et al, 1991). Blackstad (1956) states the presubiculum and parasubiculum consist of six layers. However this is not readily apparent in photomicrographs. (Figure 3.)

The medial and lateral entorhinal cortex consists of six cell layers (Blackstad, 1956; Stewart, 1976; Alonso and García-Austt, 1987; Amaral et al, 1987). Layers I, II, and III are termed superficial and layers IV, V, and VI are termed deep. Between the superficial and deep layers is a cell free zone called the lamina dissecans.

2.3 Cells of the Hippocampal Formation

Ammon's Horn contains two main classes of cells, pyramidal neurons and non-pyramidal cells. In Ammon's Horn the soma of pyramidal neurons are organized into the C shaped striatum pyramidale. Ammon's Horn pyramidal neurons have both basilar and apical dendrites and an axon emerging from the soma base. (Figure 5.)

In the CA3 layer the pyramidal cell soma is relatively larger than the CA1 pyramidal cell soma. CA3 pyramidal neurons also have large spines close to the soma. These spines are found in the striatum lucidum, the layer peculiar to CA3.

The most important of the non-pyramidal neurons of Ammon's Horn are the basket cells first identified by Cajal. The term "basket" was used to describe the basket like plexus of axons that enveloped the soma of adjacent pyramidal cells. Sik et al (1995) using intracellular labeling determined single basket cell
axons ramify widely and contact approximately 1500 pyramidal neurons as well as approximately 60 other interneurons.

The dentate gyrus consists predominantly of granule cells. Granule cells are physically smaller than pyramidal cells and have only apical dendrites extending up into the molecular layer and an axon extending downwards into the polymorph layer or hilus. The axons of the dentate granule cells are known as mossy fibers due to the numerous varicosities along their length giving them a fuzzy mossy appearance.

There are basket cells in the dentate gyrus with soma located on the border between the granule and polymorph layers (Amaral, 1978). In this case the term "basket" is a misnomer as the axons do not form basket like plexuses that envelope the soma of the granule cells but rather display beaded axons that are interspersed with the granule cells.

In the hilus of the dentate there are numerous cell types including pyramidal, mossy, pyramidal basket, pyramidal-like stellate, giant aspiny stellate, large spiny stellate, wavy multipolar, and peanut cells among others (Amaral, 1978; Ribak et al, 1985). However the two main cell types are pyramidal cells and the mossy cells. Both apical and basal dendrites of the hilar pyramidal neurons display thorny excrescences at their proximal end. The hilar mossy cells also have numerous thorny excrescences on their soma and dendrites that give them the appearance of being covered by moss.

The prosubiculum, subiculum, postsubiculum, presubiculum, and parasubiculum are chiefly composed of pyramidal neurons (Blackstad, 1956; Amaral et al, 1991).

The medial and lateral entorhinal cortexes do not differ greatly in terms of their cell composition (Blackstad, 1956; Germroth et al, 1989; Schwerdtfeger et al, 1990; Germroth et al, 1991; Belichenko, 1993). Layer I consists of horizontal cells and sparsely spinous multipolar cells. Layer II contains pyramidal cells, spiny stellate cells, and multipolar cells. Layer III contains pyramidal neurons and large spinous multipolar neurons. Layers V and VI collectively contain
pyramidal neurons, spinous multipolar neurons, spinous bipolar cells, and spinous tripolar cells.

2.4 Connections Within the Hippocampal Formation

Trisynaptic Pathway

Within the hippocampus there exists a series of three synaptic connections. These run from entorhinal cortex to dentate gyrus, from dentate gyrus to CA3, and then from CA3 to CA1. This trisynaptic pathway was first proposed by Andersen et al (1971b). It has since been shown to be an oversimplification. However it is still useful as a means of characterizing the major excitatory pathways within the hippocampal formation as well as organizing the exceptions and extensions to the basic trisynaptic idea.

First Synapse of the Trisynaptic Pathway

The first synapse of the trisynaptic pathway is that between entorhinal cortex and dentate gyrus. The entorhinal cortex gives rise to the perforant path, so named because it perforates the hippocampal fissure. When the entorhinal cortex is lesioned extensive degeneration of terminals is seen in the dentate gyrus and regio inferior of Ammon's Horn (Blackstad, 1958). Andersen et al (1971b) found that the perforant pathway forms predominantly excitatory synapses on the dendrites of the dentate granule cells. Amaral and Witter (1989) showed the entorhinal cortex projects to a large septo temporal extent of the dentate gyrus.

The perforant path has since been shown to excite Ammon's Horn pyramidal directly (Steward, 1976; Yeckel and Berger, 1990). Yeckel and Berger claim the entorhinal to Ammon's Horn projection is at least as strong as the entorhinal to dentate connection. However using neuroanatomical tracers Amaral and Witter (1989) showed the entorhinal to Ammon's Hörn projection is not as wide spread as that between entorhinal cortex and dentate gyrus.
The perforant path also synapses on inhibitory basket cells. The entorhinal projection to the basket cells of Ammon's Horn was demonstrated anatomically by Seress and Ribak (1985) and electrophysiologically by Schwartzkroin and Mathers (1978). By stimulating the perforant path Mizumori et al (1989a) and Jung and McNaughton (1993) exploited the lower threshold of basket cell discharge to distinguish basket cells from granule cells during acute single unit recording.

Stewart (1976) has also shown a projection from the entorhinal cortex to the molecular layer of the subiculum.

**Second Synapse of the Trisynaptic Pathway**

The second synapse of the trisynaptic pathway is that between dentate gyrus and CA3. Dentate gyrus granule cells give rise to axons know as mossy fibers due to the large number of varicosities along their length that give them a mossy appearance under the microscope (Andersen et al, 1966). The mossy fibers synapse on the proximal apical dendrites of CA3 pyramidal. These synapses have been shown to be excitatory. The mossy fiber system is topographically organized in a lamellar fashion in which a given septo temporal extent of dentate gyrus will synapse with the corresponding point in Ammon's Horn, with the exception of the septal hippocampus. Near the septum the mossy fibers from the dentate turn abruptly upon reaching the border between CA3 and CA1 where upon they course in a caudal direction for several millimeters before proceeding into Ammon's Horn and forming synapses (Gaarskjaer, 1978; Gaarskjaer, 1986; Amaral and Witter, 1989).

As well as projections from the dentate gyrus to CA3 the area dentata also has extensive connections within itself. Amaral and Witter (1989) and Swanson and Cowan (1978) found the cells in the polymorphic layer of the dentate project to the inner third of the dentate molecular layer. The projection is very extensive medially up to the septal pole but it is not as extensive laterally. This pathway distributes activity along the long axis of the hippocampus. Swanson et al (1981) determined that cells tentatively identified as mossy cells project to the ipsilateral and contralateral dentate hilus. Ribak
et al (1985) claimed that cells unambiguously identified as mossy cells project to the contralateral hilus.

**Third Synapse of the Trisynaptic Pathway**

The third synapse of the trisynaptic pathway is that between the CA3 and CA1 layers. In Ammon's Horn the CA3 layer projects to the ipsilateral CA1 cell layer via Schaffer collateral associational fibers. Andersen et al (1971b) stimulated Schaffer collateral associational fibers and recorded population spikes along the septo-temporal extent of the ipsilateral CA1. They found the population response was maximal at the same septo temporal beam as the stimulation site and dropped dramatically as the recording electrode was moved in either the septo or temporal direction. They concluded that the connection was divided into lamella organized perpendicular to the septo-temporal axis.

This conclusion was contested by Amaral and Witter (1989) who used neuroanatomical tracers to show that the CA3 to CA1 Schaffer collateral associational fibers reached across the postulated lamina of Andersen et al. According to Amaral and Witter the ipsilateral projection from CA3 to CA1 changes in a regular fashion as a function of the particular transverse level observed. Moving septally the CA3 associational fibers terminate preferentially in the CA1 areas closest to the CA1/CA3 border, and deeply in the striatum radiatum and encompassing the striatum oriens. Moving temporally the CA3 associational fibers terminate preferentially in the CA1 areas closest to the CA1/subiculum border, and in the superficial levels of the striatum radiatum. The septal temporal extent of the CA3/CA1 associational system is approximately 90% of the long axis of the hippocampus.

The CA3 layer also projects to the contralateral CA3 and CA1 cell layers. The contralateral projection is mediated by commissural fibers that travel in the fibria. Andersen et al (1966) found commissural afferents to CA3 were monosynaptic and excitatory.
Buzsáki and Eidelberg (1982) stimulated the contralateral commissural and ipsilateral associational pathways arising in the CA3 layer and recorded individual units in the CA1 layer. They found both pathways effectively produced negative field potentials at one location in CA1. Moreover both pathways could be tetanized by high frequency stimulation. They concluded that both the ipsilateral associational and contralateral commissural projections converged onto individual CA1 neurons and formed excitatory synapses.

Andersen et al (1963) described a synaptic loop between pyramidal cells and basket cells in Ammon's Horn. (Figure 6.) Pyramidal axon collaterals make excitatory synapses on basket cells. Andersen et al (1963) determined that the majority of basket cells gave rise to inhibitory afferents that synapse on the soma of pyramidal cells. Thus pyramidal cell firing excites basket cells that then recurrently inhibit the originally active pyramidal neurons. Using intracellular labelling Sik et al (1995) found the lateral spread of the basket cell axon was comparatively large, extending to approximately 1500 pyramidal neurons in the local area. Thus one basket cell could modulate the firing of numerous pyramidal units.

**Extensions to the Trisynaptic Pathway**

The trisynaptic pathway as first postulated by Andersen et al (1971b) does not include three of the major projection pathways in the hippocampal formation, namely the projection from Ammon's Horn to the subicular formation, the projection from Ammon's Horn to the entorhinal cortex, and the projection from the subicular formation to the entorhinal cortex.

Swanson and Cowan (1977) found that all of Ammon's Horn projects to the subiculum, presubiculum, and parasubiculum. Tamamaki and Nojyo (1990) and Amaral et al (1991) found that the projection from CA1 to the subiculum is organized into slabs approximately 2 mm wide. CA1 pyramidal near the CA3 border projected to the distal third of the subiculum near the presubiculum whereas CA1 pyramidal near the CA1 subicular border projected just across the border to neighboring subicular neurons. Van Groen and Wyss (1990) found a strong projection from the CA1 layer to the postsubiculum.
The second pathway in the hippocampal formation not included in the trisynaptic pathway is the projection from Ammon's Horn to the entorhinal cortex (Swanson and Cowan, 1977; Beckstead, 1978; Tamamaki and Nojyo, 1995). As the entorhinal cortex is the first structure in the trisynaptic pathway it can be seen that the connectivity loops around onto itself. The functional implications of this looping are discussed below.

The last major pathway in the hippocampal formation not included in the trisynaptic pathway is the projection from the subicular formation to the entorhinal cortex (Swanson and Cowan, 1977; Sørensen and Shipley, 1979; Van Groen and Wyss, 1990). This projection is weaker than the projection between the CA1 layer and the entorhinal cortex (Tamamaki and Nojyo, 1995).

While the trisynaptic pathway captures the essence of the feedforward hippocampal formation connectivity, there exists evidence for feedback connections as well. Scharfman (1994) found CA3 pyramidal neurons innervated hilar mossy cells in the slice preparation. Sik et al (1994) found inhibitory feedback projections from CA1 to CA3 and hilus. They speculate that this pathway allows for the synchronization of activity in Ammon’s Horn.

2.5 Afferents to the Hippocampal Formation

Septal Formation Afferents

The septum gives rise to the most widespread afferents to the hippocampal formation. There is a projection from the medial septum to both the medial and lateral entorhinal cortex, the CA3 field of Ammon's Horn, the dentate gyrus hilus, the subiculum, the presubiculum, and the parasubiculum (Swanson and Cowan, 1979). Freund and Antal (1988) demonstrated a projection from the septum to the inhibitory interneurons of Ammon's Horn. McNaughton and Miller (1985) recorded complex field potentials in the dentate granule layer and hilus upon stimulation of the medial septum. However it is possible that this projection is indirect and polysynaptic. The septal afferents
to the hippocampal formation are postulated to provide a pacemaker input to
the hippocampal neurons. (See below.)

Other Subcortical Afferents

Room and Groenewegen (1986b) examined subcortical afferents to the
entorhinal cortex. They found the magnocellular basal forebrain, basolateral
amygdaloid complex, claustrum, nucleus reuniens of the thalamus, supra-
mammillary region of the hypothalamus, ventral tegmental area of the
mesencephalon, dorsal raphe nucleus, nucleus centralis superior, locus
coeeruleus, and medial septum gave rise to projections to the entorhinal cortex
in the cat.

Maglóczky et al (1994) found the supra-mammillary bodies of the
hypothalamus project to CA3 pyramidal and dentate granule cells in a widely
diffuse fashion. Moore and Halaris (1975) demonstrate a projection from the
raphe nuclei to Ammon's Horn and the dentate gyrus. Van Groen and Wyss
(1990) found various thalamic nuclei project to the presubiculum,
parasubiculum, and postsubiculum.

Thus it appears that while a minority of subcortical structures synapse on
various components of the hippocampal formation the majority of the
subcortical inputs to the hippocampal formation are channelled through the
entorhinal cortex.

Cortical Afferents

The entorhinal cortex receives projections from the anterior piriform cortex,
diagonal band nucleus of Broca, retrosplenial cortex, infralimbic cortex,
prepiriform cortex, olfactory bulb, olfactory cortex, prelimbic cortex, infralimbic
cortex, cingulate cortex, and insular cortex (Beckstead, 1978; Sørensen,
1985; Room et al, 1985; Room and Groenewegen, 1986a).

Vogt and Miller (1983) found projections from visual cortex areas 17 and 18b
and the retrosplenial cortex to the parasubiculum and postsubiculum. Room
et al (1985) found the infralimbic cortex projects to the subiculum in the cat. Witter and Groenewegen (1984a) demonstrated a weak projection from the perirhinal cortex to Ammon’s Horn and the dentate gyrus.

Thus in common with subcortical inputs it appears that the majority of the cortical inputs to the hippocampal formation are channelled through the entorhinal cortex. A minority pass through the subicular complex or travel directly to the hippocampal formation.

2.6 Efferents from the Hippocampal Formation

Subicular Formation Efferents

The most intriguing efferent pathway originating in the subicular formation (subiculum and prosubiculum) is the one to the nucleus accumbens (Kelley and Domesick, 1982) since this pathway has been implicated in the control of locomotion (Mogenson and Nielsen, 1984; Annett et al, 1989; Schacter et al, 1989). Moreover the nucleus accumbens has been shown to display increases in activity following feeding and drinking (Mark et al, 1992).

Swanson and Cowan (1977) found the subiculum projected to the mammillary bodies, perirhinal cortex, ventral hypothalamus, and ipsilateral lateral septal complex. Finch et al (1984a) found the subiculum projected to the posterior cingulate cortex. Sørensen (1980) and Vogt and Miller (1983) found a connection from the subiculum to the retrosplenial cortex. Canteras and Swanson (1992) found the subiculum also projected to the amygdala, bed nuclei of the stria terminalis, and the rostral hypothalamus.

Swanson and Cowan (1977) found the presubiculum and/or parasubiculum projected to the mammillary complex and the thalamus. Finch et al (1984a) found a projection from the presubiculum to the posterior cingulate cortex. Finch et al (1984b) stimulated the presubiculum and recorded excitatory field potentials in cingulate cortex thus revealing these projections to be excitatory. Vogt and Miller (1983) found a connection from the parasubiculum to the retrosplenial cortex and visual cortex areas 17 and 18b. Van Groen and Wyss
Swanson and Cowan (1977) found the postsubiculum has a bilateral projection to both the cingulate and retrosplenial cortices. Vogt and Miller (1983) found a connection from the postsubiculum to the retrosplenial cortex and visual cortex areas 17 and 18b. Van Groen and Wyss (1990) examined the postsubiculum further and found an additional projection to adjacent perirhinal cortex.

Maren and Fanselow (1995) implanted electrodes in the ventral angular bundle and recorded monosynaptic responses in the basolateral amygdala. They note that ventral angular bundle fibers arise from CA1, ventral subiculum, and lateral entorhinal cortex. However they concluded that the majority of the hippocampal efferents to the basolateral amygdala originate in the subiculum.

**Entorhinal Cortex Efferents**

Kosel et al (1982) found projections from the entorhinal cortex to the inferior temporal cortex, perirhinal cortex, and auditory cortex. Sørensen (1985) found a projection from the entorhinal cortex to retrosplenial cortex, cingulate cortex, prelimbic cortex, infralimbic cortex, perirhinal cortex, prepiriform cortex, and insular cortex as well as the subcortical lateral thalamic nucleus, accumbens nucleus, and striatum.

Swanson and Köhler (1986) studied connections between entorhinal cortex and cortical and subcortical areas in the rat. They found the entorhinal cortex projects to the olfactory cortex, cingulate gyrus, medial prefrontal cortex, perirhinal cortex, and insular cortex. They also found connections between the entorhinal cortex and the caudoputamen, nucleus accumbens, and the basolateral complex of the amygdala.

In common with the subicular formation the entorhinal cortex is the origin of numerous efferent pathways. However these appear to be predominantly to higher order cortical areas. Thus while the subicular formation is a relatively
unrestricted way station the entorhinal cortex is more specialized for efferents to other cortical areas.

Other Efferents

Raisman et al (1966) found Ammon's Horn projects to the anterior thalamic nuclei, mammillary nucleus, medial and lateral septum, diagonal band nucleus, and the nucleus accumbens. Meibach and Siegel (1977) and Swanson and Cowan (1977) confirmed the projection from Ammon's Horn to the septum. Swanson and Cowan (1977) also found a weak projection from the CA1 layer to the perirhinal cortex and another weak projection from the CA3 layer to the cingulate cortex. In general Ammon's Horn efferents are weaker than subicular formation and entorhinal cortex efferents.

The dentate gyrus is not thought to project outside the hippocampus (Raisman et al, 1966; Swanson and Cowan, 1977).

2.7 Anatomical Models of Hippocampal Function

A number of models of hippocampal function have been put forward based predominantly on the postulated function of its efferent and afferent structures. The first of these is the Papez circuit postulated to mediate emotion (Papez, 1937). The Papez circuit consists of the reciprocal connections between medial temporal structures and the hypothalamus. Papez considered the hippocampal formation and cingulate gyrus to be the necessary medial temporal structures and the mammillary bodies to be the requisite hypothalamic ones. The hippocampal formation is directly connected to the mammillary bodies whereas the connection between the cingulate gyrus and mammillary bodies was thought to be channeled through the anterior thalamic nuclei. According to Papez the hypothalamus was the collection point for somatic and visceral components of emotion which were then passed to the cortex by way of the above circuit. A number of deficiencies have been noted with respect to the Papez circuit, particularly the omission of the amygdala. However in the same manner as the trisynaptic circuit the Papez circuit is still useful as a benchmark for the construction of subsequent anatomical models.
Based on the topology of the connections between the hippocampal formation and cortical structures Teyler and DiScenna (1984, 1986) proposed that it serves to construct an index of neocortical areas active during a learning episode. Upon subsequent activation of a subset of these neocortical areas the index stored in the hippocampal formation is reactivated and leads to the retrieval of the entire neocortical representation.

The trisynaptic pathway loops back onto itself via a direct projection from CA1 to the entorhinal cortex. There is also an indirect loop from CA1 to the entorhinal cortex via the subiculum. It has been proposed that this physical loop of connectivity is designed to facilitate the maintenance of electrical loops of activity in the hippocampus and related structures, thus creating a pattern completion or memory device (McNaughton and Nadel, 1990; Mizumori et al, 1989b; Buzsáki et al, 1990).

Risold and Swanson (1996) proposed that information processing in different regions of the hippocampus mediates different behaviors. This postulate was based on the observation that different hippocampal regions were connected via the lateral septal nucleus to hypothalamic systems which mediated different behaviors. The CA3 region was found to project to the lateral supramammillary nucleus. The bulk of CA1 and subiculum was found to project to the medial zone nuclei of the hypothalamus thought to be involved in social behavior. The ventral tip of CA1 and subiculum was found to project to the medial preoptic nucleus and hypothalamic periventricular region. According to Risold and Swanson (1996) these regions may be involved in ingestive behaviors.
Chapter 3: Physiology
3.1 Theta Rhythm

When a rat runs, rears, or jumps a regular sinusoidal EEG wave termed "rhythmic slow activity" (RSA) or the "theta rhythm" can be recorded in Ammon's Horn and dentate gyrus (Vanderwolf, 1969). This rhythm consisted of a 6 to 12 Hz sinusoid with an amplitude of 300 µV or greater. Later work showed that there was two types of theta rhythm, atropine sensitive and atropine resistant (Vanderwolf, 1975). Atropine sensitive theta rhythm was found to occur during motionless awake alert behaviors and tonic REM sleep and could be abolished by the injection of atropine or other muscarinic cholinergic antagonists. Atropine resistant theta rhythm was found to occur during voluntary movement and phasic REM sleep and was not affected by muscarinic cholinergic antagonists but could be abolished by the application of anesthetics (ether, urethane) and morphine (Vanderwolf et al, 1978).

In the rat the hippocampal theta varies with depth (Winson, 1976). Starting at the striatum oriens it increases in amplitude until it reaches a local maximum at the level of the stratum pyramidale. As the recording electrode moves further the theta wave shifts phase and drops in amplitude until at the level of the hippocampal fissure it is greatly attenuated. It then picks up in amplitude until it peaks at the level of the granule cell layer. At this level the theta is substantially greater in amplitude than at the level of the stratum pyramidale and 180° phase reversed.

The hippocampal theta pacemaker is thought by some to be located in the medial septum (Stewart and Fox, 1990). Cholinergic and GABAergic fibers originate in the septum and project to the hippocampal pyramidal and interneurons (Freund and Antal, 1988). It is this synchronized septally driven variation in the membrane potentials of numerous hippocampal cells that is thought to give rise to the extracellular voltage changes characteristic of theta.

Others believe the hippocampus generates the theta rhythm independent of external pacemakers (Konopacki et al, 1987; Konopacki et al, 1992). In the experiments of Konopacki et al hippocampal slices were bathed in the
cholinergic agonist carbachol. It was possible to record a rhythmic slow wave in the dentate gyrus of the slice with a frequency that was comparable to that of theta found in intact hippocampus, despite the absence of septal afferents.

3.2 Large Irregular Activity

When a rat is sleeping or resting quietly the hippocampus displays irregular bursts of cell firing that summate to form a large low frequency electrical events (Vanderwolf, 1969; O'Keefe and Nadel, 1978; Ylinen et al, 1995a). This EEG activity has been termed large-amplitude irregular activity (LIA) (Vanderwolf, 1969) or the sharp wave (Ylinen et al, 1995a). A sharp wave consists of a relatively high amplitude and long duration EEG wave. Successive sharp waves occur at irregular intervals, they do not display rhythmicity.

Positive going monophasic sharp waves are observed in the stratum oriens above the pyramidal cell layer. The amplitude increases as the electrode approaches the cell layer. Sharp waves reverse polarity at the level of the stratum pyramidale, displaying biphasic waveforms in the pyramidal layer itself. They then became relatively smaller in amplitude and negative going in the stratum radiatum. Sharp waves are postulated to arise as a result of spontaneous and synchronous discharge of numerous pyramidal neurons. (O'Keefe and Nadel, 1978). Sharp waves form a useful marker for positioning electrodes in the CA1 cell layer as the sharp wave polarity is determined by the location of the recording electrode with respect to the stratum pyramidale.

3.3 Hippocampal Ripple

Found at the level of the CA1 cell layer is a characteristic EEG wave referred to as the "hippocampal ripple" (O'Keefe, 1976; O'Keefe and Nadel, 1978). Ripples are observed during LIA riding the sharp wave. They consist of a burst of 4 to 10 low amplitude waves of 4 to 8 ms duration.

The hippocampal ripple may be due to abortive pyramidal action potentials that accompany the simultaneous firing of many cells observed during LIA
(O'Keefe and Nadel, 1978). An alternate hypothesis is that the ripple arises as a result of a barrage of inhibitory inputs from basket cells resulting in a train of inhibitory post-synaptic potentials in the pyramidals (Buzsáki et al, 1992; Ylinen et al, 1995a).

The presence of the ripple on the sharp wave is another phenomenon that can be exploited to locate electrodes in the CA1 cell layer. When the amplitude of the ripple is at its maximum the recording electrode is located close to the stratum pyramidale.

3.4 Long Term Potentiation

Long term potentiation is the term given to the increase in the extracellular field potential following stimulation of afferent fibers either by a paired pulse or by a burst of pulses (Bliss and Collingridge, 1993). LTP was first described in the projection from the entorhinal cortex to the dentate gyrus (Bliss and Lømo, 1973) but has since been found in many limbic and neocortical pathways (Racine et al, 1983; Bear and Kirkwood, 1993; Fox and Daw, 1993). The stimulation parameters originally used to induce LTP were not very physiologically plausible. However subsequent studies found that stimulation at the theta frequency (Larson et al, 1986; Greenstein et al, 1988) or artificial sharp waves (Buzsáki et al, 1987) produced LTP. Furthermore the ease with which LTP can be induced varies at different phases of the theta rhythm (Pavlides et al, 1988), providing further evidence for a physiological role. The phenomenon of LTP can last for several weeks in chronic recording setups (Bliss and Gardner-Medwin, 1973).

In order to induce LTP at a synapse the postsynaptic cell must be depolarized when the action potential reaches the presynaptic terminal and causes neurotransmitter release (Gustafsson and Wigström, 1986; Wigström and Gustafsson, 1986; Wigström et al, 1986). This potentiation of transmission as a result of co-incident activity is similar to a learning rule first suggested by Donald Hebb (Hebb, 1949). Hebb postulated that if one cell repeatedly caused a second to fire the connection between the two cells would be strengthened. This Hebbian learning rule is realized in the conditions
necessary for the induction of LTP (Kelso et al, 1986) and is postulated to underlie some forms of learning and memory.

3.5 Ammon's Horn Complex Spike Units

Ranck (1973) first described extracellular single unit recording in Ammon's Horn of the hippocampus in the freely moving rat. Based on this study he divided the units of Ammon's Horn into two broad groups, complex spike units and theta units.

Complex spike units would fire single spikes or multiple "complex spikes" of decreasing amplitude. (Figure 7.) The duration of their extracellularly recorded waveform was greater than that of theta units and the magnitude of the first phase was greater than the second. Their firing frequency, counting a complex spike burst as one spike, was lower than theta units and they were never observed to fire continuously for seconds at a time. The longest burst observed was typically less than two seconds. Occasionally a complex spike unit would fail to fire for minutes at a time. Relative to theta units complex spike units displayed low frequency, irregular firing.

Complex spike units are found in association with other large amplitude spikes in the stratum pyramidale of Ammon's Horn. Thus the complex spike unit identified electrophysiologically is postulated to be one and the same as the pyramidal cell identified histologically (Ranck, 1973; Fox and Ranck, 1975; Fox and Ranck, 1981). Complex spikes have been recorded in areas other than Ammon's Horn such as the stratum granulosum of the dentate gyrus. However in this thesis the term "complex spike" will be used to refer to units recorded in Ammon's Horn unless stated otherwise.

Observation of Place Fields

A strong correlation between hippocampal unit discharge and location was first described by O'Keefe and Dostrovsky (1971). They found units in the CA1 layer of Ammon's Horn that fired when the rat was in a specific location on a box and that failed to fire at other locations on the box. The location in which
the unit fired came to be known as a "place field" and the unit was referred to as a "place unit" (O'Keefe, 1976).

By taking into account the work by Ranck (1973) in classifying Ammon's Horn cell types into pyramidal cells and basket cells O'Keefe (1979) determined that place fields were identified primarily with pyramidal cells. This conclusion was based on the observation that units displaying place fields also displayed spike bursts of decreasing amplitude and that the histological location of the units displaying place fields was the same location of the complex spike pyramidal cells of Ranck, namely the stratum pyramidale. Thus pyramidal cells display location specific firing.

CA1 and CA3 Place Fields

Initially due to methodological constraints units displaying place fields were only described in the CA1 field. Subsequent work revealed units displaying place fields in CA3 (Olton et al, 1978). According to Olton et al (1978) place fields in CA3 were essentially identical to those seen in CA1. However with the advent of more precise techniques to quantify the identity of individual units and the spatial location of the rat this view was modified. McNaughton et al (1983a) found that place units in CA3 were less spatially exact than those in CA1. They put forward the hypothesis that CA1 place unit responses were constructed from the intersection of a number of CA3 place units. This would account for their higher spatial selectivity. Muller et al (1987) stated that the spatial specificity of CA1 and CA3 place fields was about the same. Barnes et al (1990) claimed that CA3 place fields are more spatially specific relative to CA1 place fields. The resolution of this issue must await further experimentation.

Dorsal and Ventral Place Fields

Units displaying place fields were initially only recorded in the dorsal hippocampus. Poucet et al (1994) recorded place units in the dorsal and ventral Ammon's Horn. They found the size, shape, and cue responsiveness of ventral hippocampal fields to be the same as dorsal hippocampal fields but
their overall firing rate was lower. Jung et al (1994) also compared Ammon's Horn dorsal and ventral place units. They concluded that a significantly smaller fraction of the ventral units displayed fields and the ventral units had fields that were significantly less spatially specific. Contradictions between these two studies must await further experimentation to be resolved.

**Place Fields are Controlled by Multiple Distal Cues**

In the first description of place fields O'Keefe and Dostrovsky (1971) examined their dependence on environmental cues. The location of the place field was insensitive to the removal of single cues. However it was altered when large changes were made to the environment. O'Keefe and Conway (1978) also found that place field firing could be maintained by a subset of the environmental cues. Thus it appeared the place specificity of place units is determined by a constellation of cues.

O'Keefe and Conway (1978) found that rotations of controlled cues within a circular curtained enclosure reliably rotated CA1 place fields. If a subset of the cues were removed before placing the rat in the environment the place fields were unaffected. However if all of the controlled cues were removed before placing the animal in the enclosure the place specific firing of the units was altered or abolished.

Muller and Kubie (1987) recorded place units in a standard cylindrical apparatus with a single polarizing cue (a white card). When the polarizing cue was rotated the place fields would rotate in register. Breese et al (1989) also found that a polarizing cue card could rotate place fields.

**Place Fields Appear Insensitive to Cue Removal**

O'Keefe and Conway (1980) extended the findings on cue responsiveness of place cells by removing all environment cues after the animal was exposed to them but before it could chose a goal. They found that the animals reliably choose the goal despite not having immediate access to the cues defining the
goal. However if the cues were not present from the start of the trial the rat would chose an arm at random, and performed at chance levels.

O'Keefe and Speakman (1987a) succeeded in recording units in rats while they performed the above task. They found that place field firing was set by the environment cues and rotated in synchrony when the environment cues were rotated. Moreover when the controlled cues were removed the place fields persisted. They concluded that Ammon's Horn place specific firing was initiated but not maintained by the environmental cues.

Quirk et al (1990) recorded the response of Ammon's Horn place units when rats were placed in an environment that was initially lit but then darkened half way through the recording session. For the majority of place units darkening the environment did not significantly affect the spatial specificity of their firing. However for a small minority of place units darkness abolished the spatial specificity of their firing. They contrasted these results with a second recording paradigm in which the rat was placed in the same environment, but it was darkened throughout recording. They found about half of the units had vastly changed place fields in the darkened environment. They concluded that the spatial specificity of hippocampal units depended on recently viewed visual information but could be maintained in the absence of visual information by either non-visual information or by the rat constantly tracking the previous and current locations ("dead reckoning").

Markus et al (1994) recorded place units while the rat performed an eight arm radial maze task in an environment that was alternately lit and darkened between trials. They found a higher proportion of Ammon's Horn complex spike units displayed place fields in the lit trials than in the darkened trials. Moreover the place units that were observed in darkness were less specific and reliable than their lit counterparts. However some place fields were found to appear only when the trials were run in darkness.

The results of Quirk et al (1990) and Markus et al (1994) have implications for cue removal studies. The changes observed in place unit firing after cue removal may be due to the misapprehension on the rat's part that the old
environment has been exited and a new one entered. The place units specific to the dark situation may be neurons coding for the new environment. Alternately changes observed in place unit firing after cue removal may simply be due to the degradation of place unit firing due to the paucity of visual information.

Place Fields Differ in Different Environments

O'Keefe and Conway (1978) recorded place fields in two environments. They found that single place units had place fields in either the first, the second, or both environments. For those units with fields in two environments there was no clear relationship between the shape or location of the two place fields. They concluded a single place unit can participate in the representation of different environments.

Kubie and Ranck (1983) recorded place cells in three different environments, an eight arm maze, an operant conditioning cage, and the rat's home cage. All environments were centered in the same room and thus occupied the same location in real space. They found a given unit would have completely dissimilar place firing in the three environments. Moreover upon rotation of the eight arm maze about the center Kubie and Ranck found the fields failed to rotate whereas upon rotation of the operant chamber and the home cage about the center the fields also rotated. This differential response to the rotation of the environment clearly shows that the same place units were responding differently to different environments.

Muller and Kubie (1987) recorded place units in two environments, a circular enclosure and a square enclosure of comparable size and color. They also found that for a given unit the location and shape of its place fields in one environment did not resemble that seen in the other.

Place Fields Depend on Both Visual and Vestibular Inputs

Sharp et al (1995) addresses the question of whether visual or vestibular information is used to maintain stability of place field firing. They designed an
apparatus in which the floor and walls could be rotated independently or in concordance. If the floor was rotated and the walls were not rotated 100% of Ammon's Horn fields would rotate in register with the floor. Sharp et al claim this is due to the rat being able to view the walls going round while the floor rotated, therefore the vestibular and visual inputs are in agreement as to the magnitude and direction of the rotation. If the lights were turned off and the floor rotated only 40% of the fields would rotate in register with the floor. They maintain this is due to the rat not being able to view the walls going round while the floor rotated. Thus the vestibular input indicates rotation but the visual input was unavailable. If both the floor and walls were rotated less than 20% of the fields would rotate in register with the floor and walls. They assert this is due to the rat not being able to view the walls going round while the floor rotated, therefore the vestibular input indicates rotation but the visual input does not. Sharp et al concluded that rats use both vestibular and rotational information to set up place fields. If one form of information is unavailable the stability of place fields is moderately impaired. However if one form of information contradicts the other the stability of place fields is greatly impaired.

Knierim et al (1995) compared place fields recorded in a cylinder with a single polarizing cue. They pre-trained two groups of rats to forage for pellets in the cylinder. One group was disoriented before foraging and the second was not. They found the rats that were pre-trained with repeated disorientation had unstable place fields in the cylinder that did not reliably rotate when the polarizing cue was rotated. The rats in which pre-training did not involve repeated disorientation had stable place fields in the cylinder that reliably rotated when the polarizing cue was rotated. They concluded that by disorienting the rat before training it was prevented from learning that the polarizing cue is a stable directional reference. Consequently the effect it later had over place unit firing was minimal.

Place Fields and Environment Scaling

Muller and Kubie (1987) recorded place units in a standard cylindrical apparatus. When the cylinder was scaled up in size the place fields of some
units (36%) were also scaled up in size while remaining in the same angular location. The place fields of other units (52%) were completely altered by the scaling of the cylinder. When barriers were placed within place fields they were found to attenuate or abolish place field firing. Breese et al (1989) also found that a barrier placed within a place field attenuated the firing of the corresponding place unit.

Wilson and McNaughton (1993) further explored the dynamics of place unit response upon environment scaling. They placed rats in an environment with a partition in the middle and recorded from place units. Half way through the recording session the partition was raised. They found that place units that fired in the original environment continued to do so in the new expanded environment. However they also found that units that did not display place fields in the original environment began to fire in the new half of the environment. Initially the units fired in a manner that was not spatially robust but they soon settled down and began to display clear place fields. It appears as though when the environment was increased in size the hippocampal place units not already involved in the spatial representation were recruited in order to "fill in" the newly opened space.

O'Keefe and Burgess (1996) studied the dependence of place fields on the environment shape. They recorded place fields in four differently shaped boxes in the same room, namely a small square, horizontal rectangle, vertical rectangle, and large square. They found some place fields that remained in a constant location relative to two orthogonal walls, for example tucked up in a corner. Thus as the rat was moved from one box to another differently shaped box the field would remain in the same corner. However the location of other place fields was dependent on two opposite walls. If the two walls were pulled further apart the place field would be elongated and in some cases pulled apart into two separate components. The striking observation of the dependence of place fields on one or more of the edges of the environment has implications for all experiments in which place fields are recorded.
The environment scaling manipulation of Muller and Kubie (1987), the partition removal manipulation of Wilson and McNaughton (1993), and wall manipulation of O'Keefe and Burgess (1996) are comparable in design. The experiments of Muller and Kubie (1987) can be characterized as ones in which all of the walls of the environment were moved simultaneously, the experiments of Wilson and McNaughton (1993) can be characterized as ones in which only one wall of the environment was moved, whereas in the experiments of O'Keefe and Burgess (1996) all of the environment walls were moved. Thus the scaling of place fields seen by Muller and Kubie (1987) and the elongation and separation of fields seen by O'Keefe and Burgess (1996) may have taken place via the same mechanism.

However in the partition removal experiment of Wilson and McNaughton (1993) the fields were found to be unchanged. This may have been due to how the experiments were run. In the studies of Muller and Kubie (1987) and O'Keefe and Burgess (1996) the rat was removed from one environment and placed in another, differently shaped environment. In the study of Wilson and McNaughton (1993) the rat was in the environment when the partition was removed. The fact that the rat was in place when the environment was manipulated may have caused it to view the new environment as essentially the same as the old environment. Thus the fields were relatively unchanged. In the studies of Muller and Kubie (1987) and O'Keefe and Burgess (1996) the rat was physically removed from the old environment. Thus the spatial map may have been partially or completely dissociated from the old environment such that when the rat was replaced in the new environment the hippocampal fields could display novel firing more readily.

Place Fields and Directionality

O'Keefe (1976) and McNaughton et al (1983a) observed that the firing rate of some place units was greater when the rat ran through the unit's place field in one of two directions on a radial arm maze. However it is possible the directional specificity in place unit firing seen in these studies was due to constraints on the rat's movement that were imposed by the narrow maze arms. Muller et al (1987) found little to no directional specificity of place unit
firing in rats run trained to run around randomly in a high walled cylindrical enclosure.

Muller et al (1994) performed a more extensive analysis to test the hypothesis that place unit directional specificity is due solely to geometric constraints of an environment. They assumed the firing rate of a place unit smoothly decreased from the maximum in the center and postulated that the illusion of direction specific firing arose as a result of the inhomogeneous distribution of rat traversals within this region. They found this assumption was valid when place unit recording was performed in a high walled cylinder but invalid when recording was performed on an eight arm radial maze. They concluded that there was an intrinsic directionally component to place unit firing for fields recorded on environments consisting of narrow arms.

The directional specificity of place field firing was further examined by Markus et al (1995). They also found place fields displayed more directional selectivity on radial arm mazes than on an open circular platform. However they also observed that shaping a rat to traverse a circular open field between reward sites in a stereotypical and invariant manner caused place field to show directionality. They conclude it is the rat's behavior in an environment rather than the geometry of the environment that determines whether place fields recorded in the environment display directionality.

**Topography of Place Fields**

Other areas of the brain such as visual and somatosensory cortex (Kandel et al, 1991) and auditory cortex (Takahashi, 1989) demonstrate clear topographic correspondence between the external world and the neural representation. Researchers have examined hippocampal place fields in an effort to see whether there is a similar topographic organization. Using single unit electrodes it is normal to record a number of place units on one electrode due to both the electrode's small size and the close packing of Ammon's Horn neurons. Units recorded simultaneously on one electrode are necessarily physically close or else they would not be able to produce measurable potentials on the electrode. Muller et al (1987) and O'Keefe and Speakman
(1987a) observed that the fields produced by physically close units recorded on one electrode are spread throughout the environment. There was no relationship between the locations of place fields in the environment and the physical locations of their corresponding place units in the hippocampus. This finding was disputed by Eichenbaum et al (1989). They found a slight bias towards the grouping of place fields recorded simultaneously from one electrode.

**Goal Location and Place Fields**

It has been claimed that the locations of place fields are independent of the goal location (O'Keefe and Speakman, 1987b; Speakman and O'Keefe, 1990). Rats were trained on a spatial memory task with a given goal and the place fields were recorded. The rats were then trained to select a new goal. The locations of the place fields that were previously recorded were unchanged.

However Eichenbaum et al (1987), Deadwyler et al (1989), Breese et al (1989), and Wiener et al (1989) found the strength of hippocampal firing could be modulated by goal location. Eichenbaum et al (1987) and Wiener et al (1989) described hippocampal "goal-approach" units which fired preferentially when the rat approached a reward cup. However it is possible that the increased hippocampal firing near the reward cup may simply be due to their being a place field in that location. Breese et al (1989) and Deadwyler et al (1989) described place fields which shifted such that they were superimposed onto water ports which had recently delivered water.

**Multiple Place Fields**

O'Keefe and Speakman (1987a) showed numerous units with double and triple fields. The potentials were recorded on two closely spaced wires ("stereotrode") and characterized using hardware that recorded the maximum voltage, minimum voltage, and time between maximum and minimum voltage (McNaughton et al, 1983b). The relative voltage values on each wire were used to identify individual spikes. However it was possible to erroneously assign two or more units to one cluster. In subsequent experiments the
spikes were recorded on four closely spaced wires ("tetrode") and interrupt driven analogue to digital recording was used to capture the entire spike waveform (O'Keefe and Recce, 1993). Recce (1994) demonstrated that the use of tetrodes made it theoretically possible to separate spikes unambiguously. He concluded that using stereotrodes to record place fields leads to the illusion of multiple place fields due to erroneously classifying two or more place units as one. Since each unit gives rise to its own place field the result is a pseudo double field. Nevertheless studies using tetrodes still uncovered what appear to be single units that give rise to double or multiple fields. Muller et al (1987) found a double place field that remained in a constant location even as the waveform of the unit was altered by moving the electrode. Recording in a symmetrical environment with two cards Sharp et al (1990) found some units would express two fields. In some instances one field would become attenuated after repeated recording sessions. Jung and McNaughton (1993) found that in rare instances CA3 units would display two fields.

Quantitative Properties of Place Fields

O'Keefe (1976) observed that place units had tonic firing rates of 1 Hz or less outside their place fields. Within the fields the frequency of place unit firing could rise to as high as 30 Hz. Muller et al (1987) found Ammon's Horn place fields occupied an average of 22% of the total environment area. The CA1 place units had a maximal firing rate of 15 Hz whereas the CA3 place units had a maximal firing rate of 21 Hz. The field centers were uniformly distributed throughout the environment. O'Keefe and Recce (1993) later found CA1 place units with rates as high as 28 Hz. Thompson and Best (1990) recorded place fields over a period of weeks. They found the position of a place field would remain stable for up to 153 days.
Place Fields Are Abolished by Restricting Movement

Foster et al (1989) found that when a rat was restrained within a place field the within field firing was abolished. They hypothesized that place unit firing represents the association between movement and spatial translation.

Complex Spike Firing is Correlated with Motor Behaviors

Ranck (1973) found complex spike units would fire when the rat groomed, ate, moved its body, and drank. The strongest motor correlated firing occurred during sniffing behavior when there was no object present. This work was done prior to the discovery of place fields. Thus it is possible that what were thought to be complex spike motor responses may actually have been place specific firing. However there is evidence that the unit firing within a place field can be modulated by various motor behaviors such as grooming, eating, and drinking (O'Keefe, personal communication). Furthermore Wiener et al (1989) examined hippocampal neuronal firing and found correlations between hippocampal neuronal firing and the speed, direction, and turning angle of the rat within place fields.

McNaughton et al (1983a) and Recce (1994) found the firing rate of complex spike units increased in linear proportion to running speed when the rat traversed the unit's place field.

Complex Spike Firing May be Correlated with Sensory Stimuli

Workers endeavoring to test the working memory hypothesis have reported correlations between visual, auditory, and olfactory stimuli and Ammon's Horn pyramidal discharge in various mnemonic tasks (Berger et al, 1976; Wible et al, 1986; Eichenbaum et al, 1987; Otto and Eichenbaum, 1992; Hampson et al, 1993; Sakurai, 1994). However the reliability of these responses appears low, firing approximately 20% to 40% of the time.
3.6 Ammon's Horn Theta Units

The second type of unit characterized by Ranck (1973) was the theta unit. Theta units were described as never displaying decreasing amplitude "complex spikes" but rather always had single action potentials. Extracellularly recorded theta spikes were shorter in duration than complex spike waveforms and unlike complex spikes the first and second phases were approximately the same amplitude. (Figure 7.) Theta units could show higher firing rates relative to the firing rates of complex spike units, firing continuously for many seconds. The firing rate of theta units is maximal in presence of the theta rhythm.

Theta units were found both with other large amplitude spikes in Ammon's Horn and on their own in otherwise unit free areas. Theta units have since been shown to be basket cells identified histologically (Ranck, 1973; Fox and Ranck, 1975; Fox and Ranck, 1981; Barnes et al, 1990). Theta units are found in Ammon's Horn, dentate gyrus, subiculum, presubiculum, parasubiculum, and medial entorhinal cortex.

Ranck (1973) isolated approximately one theta unit for every three complex spike units in Ammon's Horn. However he notes this measure may be affected by sampling bias. Since the theta units fire with higher frequency they are more likely to be detected as compared to the quieter complex spike units.

**Theta Unit Firing is Strongly Correlated with Motor Behaviors**

No report of theta unit firing in response to pure sensory stimulation or head direction has been made. However theta unit firing is highly correlated with movement and other motor behaviors (Ranck, 1973; Feder and Ranck, 1973; O'Keefe, 1976). O'Keefe (1976) observed that theta units displayed irregular low frequency when the rat was stationary but shifted to a regular high frequency bursting pattern when the rat displaced itself. Other vigorous movements such as grooming did not elicit regular theta unit bursting. Combined extracellular and intracellular recording (Ranck, 1973; Ylinen et al
1995b) has showed that theta unit firing occurs in phase with the theta rhythm.

**Theta Unit Firing is Weakly Correlated with Spatial Location**

McNaughton et al (1983a) recorded from CA1 theta units and found their firing was weakly correlated with the spatial location of the rat. However they note theta unit firing is strongly correlated with movement. Thus the apparent spatial firing may have been due to the rat displaying different behavior in different locations. Christian and Deadwyler (1986) also recorded from theta units and concluded that theta unit firing is not correlated with location.

Kubie et al (1990) performed a more extensive study. They recorded theta units in both CA1 and CA3 while the rat chased pellets in a circular apparatus with a single white cue card taped to the wall. They found theta units display statistical significant spatial firing. This spatial firing was widespread and diffuse but nevertheless constant and reproducible. Moreover the spatial firing pattern of theta units could be rotated by moving the cue card to other locations on the cylinder wall.

Both McNaughton et al (1983a) and Kubie et al (1990) note that theta units receive excitatory connections from numerous complex spike units. Therefore the diffuse spatial firing of theta units could be the result of their being driven by complex spike units each mediating its own place field. Thus theta unit place fields are the intersection of a number of complex spike unit place fields.

### 3.7 Subicular Formation Units

Ranck (1973) recorded from theta units and complex spike units in the dorsal subiculum. He found the firing of subicular theta units was essentially identical to that of Ammon's Horn theta units. However the subicular complex spike units fired more rapidly than Ammon's Horn complex spike units.

Barnes et al (1990) recorded from units in the subiculum. Unlike Ranck (1973) they claimed it was not possible to classify subicular units into theta and complex spike types. The units in the subiculum did not display clear
complex spike-like bursting nor theta-like rhythmic firing but behaved in an intermediate fashion, displaying some burst spiking and some rhythmic spiking.

Taube et al (1990a) recorded two clear classes of units in the postsubiculum. The first class displayed triphasic or biphasic waveforms and never fired in a complex spike burst ("head direction" units, see below.) The second class resembled the theta units of other hippocampal areas both in terms of the spike morphology, spike timing, and relationship to the hippocampal EEG.

No report of a clear sensory correlate of subicular formation unit firing has been made. Ranck (1973) recording units in the dorsal subiculum found that most of them increased their firing rate when the rat moved.

Postsubicular Unit Firing is Strongly Correlated with Head Direction

In a short abstract Ranck (1984) reported units in the postsubiculum that fired preferentially when the head of a rat was pointing in a given direction. Taube et al (1990a) followed up this result. Approximately one quarter of subicular units were sensitive to head direction. The direction of peak firing did not vary when the rat moved. The head direction vectors were parallel throughout the environment. As such their firing behavior is as highly spatial as the firing of place units while the nature of their spatial selectivity is completely different. Place units are specific to the locations of a rat on a maze surface and in general the direction the rat is pointing is irrelevant. Head direction units are specific to the direction the rat is pointing and the location of the rat on a maze surface is irrelevant.
Head Direction Firing Can be Entrained by a Single Cue

Taube et al (1990b) found the head direction units could be governed by a single cue in a cue controlled environment. Head direction units were recorded in a cylindrical environment containing only one polarizing cue, a white card. When the card was rotated by a given angle the head direction units shifted their preferred direction by the same angle. If the cue card was removed while the rat was not in the environment and then the rat replaced within the environment they found the preferred direction of the head direction units was altered. However the unit continued to fire in a direction specific manner. When two head direction units were recorded simultaneously the angle between the two preferred directions was found to be invariant despite rotations of the cue card or transferal to differently shaped environments. Therefore it appears that the postsubicular head direction units differ from hippocampal place fields in the manner in which the unit population represents different environments. Different environments elicit place unit firing from different but overlapping pools of hippocampal pyramidal neurons. In contrast the same pool of postsubicular units appear to display head direction firing when the rat is in different environments and moreover the head direction units are coupled such that the angular relationships between their preferred directions is invariant.

Head Direction Firing is Controlled by External and Internal Cues

Goodridge and Taube (1995) examined the effects of external environmental cues and internal idiothetic sensory systems on the firing of postsubicular head direction units. They recorded one session in a cylinder with one salient cue and established the preferred direction of the head direction units. They then removed the cue and recorded a second session. They found that about half of the units significantly shifted their preferred direction after removal of the salient cue. If the salient cue was replaced in the cylinder in its original position almost all of the head direction units shifted their preferred firing back to the original direction. They concluded that both environment cues and idiothetic sensory systems were able to maintain head direction firing. However if the environment cues and the idiothetic sensory systems were in
conflict the environmental cues override the idiothetic sensory systems and set the preferred direction of head direction units.

Taube and Burton (1995) also examined the interrelationship between environmental cues and idiothetic sensory systems on postsubicular head direction firing. In their task a familiar cylindrical environment containing a cue card was connected to an unfamiliar rectangular environment with a single prominent cue via a U-shaped passageway. When a rat with extensive experience in the cylinder was allowed to travel to the rectangle the directional firing of the postsubicular units was relatively unchanged. It appeared as though the rat used its idiothetic sensory systems to remain aware of its orientation as it traversed from one environment to the other. On the second trial Taube and Burton (1995) set up a conflict situation in which the cylinder cue card was rotated by 90° but the rectangle was left unchanged. When the rat was re-introduced to the cylinder the preferred direction of the head direction units was found to have rotated in register with the rotated cue card. However when the passageway was opened and the rat entered it the preferred direction of the head direction units shifted back to their original orientation and remained in this orientation during the time the rat spent in the passageway and the rectangle. Upon returning to the cylinder the preferred direction of the head direction units shifted such that they were in register with the cylinder cue card, or remained in the original passageway/rectangle orientation, or shifted to an intermediate value that lay between the cylinder cue card and rectangle/passageway values. Taube and Burton (1995) concluded that postsubicular head direction units could maintain directionally specific firing using a combination of external environmental cues and internal idiothetic sensory systems. However in situations where the two systems were in conflict the preferred direction of the postsubicular head direction units would be locked to the external environmental cues.

The possible candidates for the idiothetic sensory systems include proprioceptive, vestibular, and kinesthetic systems. The involvement of idiothetic sensory systems in spatial navigation has already been demonstrated (Mittelstaedt and Mittelstaedt, 1980; Etienne, 1992; Sharp et al, 1995; Etienne et al, 1996). Which of the possible candidates or combination
of candidates actually participates in navigation in various situations has not been established.

**Subicular Unit Firing is Weakly Correlated with Spatial Location**

Barnes et al (1990) reported "spatially consistent firing" in the subiculum when the rat performed a radial arm maze task. They note that subicular unit spatial firing could result from the convergence of CA1 axons onto subicular neurons. The spatially specific firing of CA1 place fields would thus be summated to create the spatially consistent firing of the subicular units. Taube (1995) reported that a small percentage of the units in the parasubiculum displayed place fields. These fields were larger and had a higher level of background firing than those in the hippocampus.

Sharp and Green (1994) reported subicular place fields whose strength was modulated by head direction. It appears as though the subicular units may be an integration point in which the place field information from CA1 afferents is modulated by head direction information originating from the postsubiculum. Similarly the parasubiculum place units reported by Taube (1995) showed some directional correlates in when the rat chased scattered pellets in a cylindrical apparatus, a situation in which Ammon's Horn place units do not show directional correlates.

**3.8 Dentate Gyrus Granule Units**

Ranck (1973) and Olton et al (1978) recorded what they claimed to be complex spike units in the dentate gyrus. They based their identification on the unit's firing characteristics, chiefly a low mean firing rate (between 0.1 and 11 Hz), and the occasional display of the characteristic "complex spike" composed of individual spikes of decreasing amplitude. They claimed the units were located in the dentate gyrus on the basis of the recording depth and the histological tract. However there exists the possibility that the units classified as dentate gyrus complex spike units were actually located in the hilus or CA3 layer.
Mizumori et al (1989a) recorded single units in the dentate gyrus of acutely implanted rats. They used paired pulse stimulation applied to the perforant path to distinguish between granule units and basket units. The first perforant path stimulus produced a field potential in the dentate gyrus and the second, timed to arrive during the field potential refractory period, did not. Assuming the field potential is chiefly composed of granule unit activation the second stimulus does not excite granule units. Therefore single units which responded only to the first stimulus were classified as granule units and those which responded to both the first and second stimulus were classified as basket units. The putative granule units had mean spontaneous firing rates of about 1 Hz. The putative dentate gyrus basket units had mean spontaneous firing rates of about 8 Hz, compared with 11 Hz for CA1 theta units. The similarity in rates for putative dentate gyrus basket units and CA1 theta units further strengthens the idea that theta units recorded extracellularly are identical to histologically identified basket cells.

Jung and McNaughton (1993) attempted to use the paradigm of Mizumori et al (1989a) in chronically implanted animals to distinguish between granule units and basket units. However the results were inconclusive and the units classified as granule units may have not been correctly identified. They found the postulated granule units would fire in bursts of either equal amplitude spikes or decreasing amplitude spikes. The spike width of the postulated granule units was intermediate between that of Ammon's Horn pyramidals and theta interneurons. It is possible that the units observed by Ranck (1973) and Olton et al (1978) to give rise to complex spikes were actually dentate granule units.

No report of has been made of granule unit showing firing correlated to purely sensory stimulation or motor behavior. Moreover granule units appear insensitive to head direction.

**Granule Unit Firing is Weakly Correlated with Spatial Location**

Jung and McNaughton (1993) reported place fields mediated by what they postulated to be dentate granule units. These fields were more likely to be
multiple in character relative to CA3 units, with some displaying 6 subfields. As with Ammon's Horn place units, no topographic relationship between the postulated granule unit fields was found.

However McNaughton et al (1989) found that when the dentate gyrus was selectively destroyed the spatial firing of the CA3 layers was unaffected. Thus even though dentate granule units may display spatial firing in light of the experiments of McNaughton et al it is difficult to see what role they play in giving rise to Ammon's Horn place fields.

3.9 Entorhinal Cortex Units

Barnes et al (1990) recorded from units in the entorhinal cortex. They found their mean firing rate was higher than that of Ammon's Horn pyramidal units but lower than that of theta units and subicular units. The units in the entorhinal cortex did not display clear complex spike-like bursting nor theta-like rhythmic firing but behaved in an intermediate fashion, displaying some burst spiking and some rhythmic spiking.

Quirk et al (1992) reported results of single unit recording in the superficial zone of the medial entorhinal cortex. Superficial medial entorhinal cortex units did not display decreasing amplitude complex bursts but did display short constant amplitude bursts. The width of the individual medial entorhinal unit spikes was comparable to that of the pyramidal units recorded by Ranck (1973). Quirk et al (1992) reported difficulties in obtaining recordings from units in the deep zone of the entorhinal cortex, perhaps due to the recording electrode damaging afferents.

No report of has been made of entorhinal cortex units showing firing correlated to purely sensory stimulation. However Dickson et al (1995) and Jeffery et al (1995) reported entorhinal cortex unit firing that was correlated with theta and movement. Entorhinal cortex units appear insensitive to head direction.
Entorhinal Unit Firing is Weakly Correlated with Spatial Location

Barnes et al (1990) reported what they described as spatially consistent firing in the entorhinal cortex when rats performed a radial arm maze task. Spatially consistent firing differed from classical place field firing on the eight arm maze in that it was not restricted to a single arm or two adjacent arms but was spread throughout all maze arms. However it was consistently higher on some arms relative to others, unlike spatially insensitive units that displayed the same firing rate on all arms.

Quirk et al (1992) recorded from units in the superficial zone of medial entorhinal cortex place fields. When the environment was a circular enclosure with a polarizing white card they found some units displayed diffuse place fields with locations controlled by the cue card such that rotations of the card resulted in comparable rotations in the place fields. However when environment was changed from a circular to a square enclosure of comparable size they found the entorhinal units stretched their fields such that they occupied the same position in both environments.

Entorhinal place fields appear to respond to environment geometry in a manner different to that of Ammon’s Horn place fields. Upon alterations in environment geometry Ammon’s Horn place fields can completely change location in some cases (O’Keefe and Conway, 1978; Muller and Kubie, 1987; Wilson and McNaughton, 1993) and stretch in others (Muller and Kubie, 1987; O’Keefe and Burgess, 1996). However to date entorhinal cortex place units have only been observed to stretch in response to alterations in environment geometry.

Chapter 4: Function
4.1 Spatial Map Hypothesis

Inspired chiefly by the observation of hippocampal place fields O'Keefe and Nadel (1978) proposed a hypothesis in which the hippocampus is the locus of a cognitive map of space. They began by considering two competing representations of space, space existing independent of any objects within it or absolute space and space defined relative to objects within it or relative space. A special category of relative space is egocentric space in which the key object is one's self. The world is defined relative to one's own position in it. In contrast to egocentric space, allocentric space is defined relative to a fixed grid in the environment. According to the cognitive map hypothesis the hippocampus represents spatial environments using an allocentric coordinate system. This allocentric representation is derived from egocentric information about the distance and direction of all the cues within the environment the animal attends. The animal can update this representation by monitoring its own movements and feeding this information back into the mapping system.

Since the first publication of the spatial map hypothesis more detailed descriptions of its operation have been presented (O'Keefe, 1990; O'Keefe, 1991; O'Keefe, 1993). The spatial map is thought to be composed of two components, a centroid defined by the geometric center of the cues within an environment and the eccentricity defined by direction of the vector average of all pairs of cues. From the forgoing definitions it can be seen that in an environment with cues distributed at random the location of the centroid would be approximately in the center of the environment and the eccentricity would point in an arbitrary direction. In an environment with linearly distributed cues the location of the centroid would again be approximately in the center of the environment but the direction of the eccentricity would be along the long axis of the distributed cues. In both case the centroid and eccentricity are fixed and unvarying as long as the cues remain stationary. The rat is postulated to calculate the environment centroid and eccentricity and to use this information to construct an allocentric spatial map that it can use to navigate in the environment.
The place field firing of Ammon's Horn, entorhinal cortex, and subicular formation units, the head direction firing of postsubiculum units, and the movement specific firing of Ammon's Horn theta units are postulated to be electrophysiological manifestations of the spatial map located within the hippocampal formation (O'Keefe, 1990; O'Keefe, 1991; O'Keefe, 1993).

Ammon's Horn place field firing is postulated to be the electrophysiological manifestation of the calculations used to determine the rat's relationship to the environment centroid and eccentricity (O'Keefe, 1990; O'Keefe, 1991). If a line is drawn in space that originates at the rat's current location and ends at the centroid the length of this line and the angle between it and the environment eccentricity can be used as an allocentric location parameter. If the rat moves within the environment both the length of the line and the angle it makes with the environment eccentricity is altered. Concomitant with the altered angle is a change in the population of units displaying place specific firing. If the rat rotates in one place the length of this line and the angle it makes with the environment eccentricity remains unchanged and therefore Ammon's Horn place field firing is unchanged.

The postsubicular head direction unit firing is postulated to be the electrophysiological manifestation of the calculations used to determine the angle between the rat's head and the environment eccentricity (O'Keefe, 1990; O'Keefe, 1991). As the rat moved within the environment in straight lines this angle remains unchanged. The directional firing of the postsubicular head direction units similarly remains unchanged. If the rat rotates the angle between its head and the environment eccentricity is altered. This is reflected by the alteration in the firing rate of postsubicular head direction units.

Lastly the movement related theta unit discharge is postulated to be the electrophysiological manifestation of a clocking mechanism used to update the current position within the cognitive map (O'Keefe and Nadel, 1978; O'Keefe and Recce, 1993; O'Keefe, 1993). Theta units fire when the rat performs voluntary movement that cause translation in an environment, such as running or jumping (Ranck, 1973; Feder and Ranck, 1973; O'Keefe, 1976). The firing of theta units provides the hippocampal cognitive mapping system with
information on translations being performed by the rat so that the system may update the current position. The theta discharge may shift the focus of excitation within the hippocampus on the basis of the animal's movement through the environment.

O'Keefe and Recce (1993) found there was a precise relationship between place unit firing and the theta cycle such that place unit spikes occurred at a definite point on the theta cycle and shifted to earlier points on subsequent cycles as the rat traversed the place field. There appears to be an intimate relationship between the postulated "place" signal contained in the place unit spikes and the "displacement" signal contained in the theta cycle such that the two signals considered together contain more information than each considered separately.

O'Keefe and Conway (1978, 1980) developed the cue controlled environment (CCE) in order to examine the electrophysiological manifestation of the cognitive map in more detail. The essential elements of a CCE are a small set of controlled cues distributed around the perimeter of the environment. Dim lighting and a floor length curtain were used to exclude visual cues outside of the environment and the orientation of the controlled cues was randomly varied by multiples of 90° between trials in order to prevent the rat from using auditory, olfactory, and visual cues outside of the environment. The result of these manipulations is a situation in which the rat must only attend to those cues under the control of the experimenter in order to navigate within the environment successfully.

According to the spatial map hypothesis the centroid is located in the center of the CCE and the eccentricity is in a random but fixed direction. When the controlled cues are rotated the eccentricity is rotated by the same amount but the location of the centroid remains unchanged. Since the controlled cues are the only ones relevant to the performance of the task it is reasonable to assume that after removing these cues the rat no longer has sensory access to the information necessary to perform the task. Consequently if the rat is able to successfully navigate within the CCE after cue removal this must be due to either its remembering the location of the cues or continuously updating
an internal map of the environment. Any sustained and consistent unit firing that is related to the former locations of these cues is likely to be the electrophysiological manifestation of this process.

O'Keefe and Conway (1978) placed a T maze within the CCE and recorded place fields in the CA1 field of Ammon's Horn. They found that the place units would fire reliably in locations defined by the controlled cues. When the controlled cues were rotated the place unit fields would rotate in register. This demonstrated the ability of the place units in Ammon's Horn to attend to the subset of controlled cues and disregard the external cues, thus verifying the assumptions inherent in the design of the CCE. This experiment provided evidence in favor of the hypothesis that Ammon's Horn is either the locus or downstream of a spatial mapping system that can compute spatial location information from egocentric sensory information.

O'Keefe and Speakman (1987a) extended the results of O'Keefe and Conway (1978). They placed a plus maze in the CCE and defined one arm with respect to the controlled cues as the goal arm. The actual physical goal arm varied as the controlled cues were pseudo-randomly rotated but it always had a fixed relationship to the controlled cues. They trained rats to go to this goal arm while randomly varying the orientation of the controlled cues with respect to the external world. They then implanted the rats with micro-electrodes to record Ammon's Horn place units. When the rat was placed in the CCE with all cues present the place units would fire reliably in locations as defined by the controlled cues. If all the cues were removed while the rat was in the CCE the place units would almost always continue to fire reliably in the same locations defined by the controlled cues. In the cue-absent phase of the trials it appeared that the spatial firing of Ammon's Horn place units was not due to sensory or perceptual inputs but rather due to a maintained map or model of the environment. This environment map could be created using either memory for the previous location of the cues or some sort of idiothetic based dead reckoning system. When the rat was permitted to choose an arm by raising the movable central platform they found the rat almost always chose the goal arm despite the absence of the controlled cues defining the goal arm location. This experiment provided evidence in favor of the hypothesis that
Ammon's Horn is either the locus, or downstream of the locus, of a spatial mapping system that can store spatial location in the absence of sensory inputs.

In control trials the controlled cues were removed before the rat was placed within the CCE. In these instances the rat performed at chance levels. However in these error trials the eventual choice the rat made could be predicted by comparing the constellations of place field firing with that seen on correct trials. This suggests that the spatial representation in the hippocampus is made available to other parts of the rat brain in order to enable it to make decisions as to which arm is the goal arm. If the spatial representation is flawed then the choice is flawed in a manner consistent with the erroneous spatial representation. These results provide even stronger evidence that the hippocampus is involved in spatial navigation since the eventual choice the rat was to make, whether correct or erroneous, could be predicted from Ammon's Horn place unit firing upwards of three minutes in advance. By observing the place unit firing one could read out the contents of the rat's spatial memory.

4.2 Working Memory Hypothesis

Olton et al (1979) proposed a hypothesis in which memory is divided into two broad categories, working memory and reference memory. Working memory is defined as memory used for one trial and not for subsequent trials. Reference memory is defined as memory used in many trials, often all the trials in a given experiment. The working memory hypothesis states that damage to the hippocampus impairs performance in working memory procedures and spares performance in reference memory procedures. The locus of the working memory system is the hippocampus.

The working memory hypothesis of Olton et al (1979) was originally framed in terms of lesion damage to the hippocampus. Since it was assumed that the working memory system resides in the hippocampus numerous experiments have attempted to detect electrophysiological manifestations of hippocampal working memory. Three major studies will be considered.
Wible et al (1986) recorded from CA1 units during a brightness discrimination delayed match to sample task. The apparatus consisted of a T maze with a black and a white goal box at the end. On every trial one box was randomly chosen to be the goal box. In the sample phase the entrance to the non-goal box was blocked and the rat was rewarded for entering the goal box. In the match phase the rat was rewarded for entering the goal box. The position of the goal and non-goal boxes were randomly varied between phases and across trials. Wible et al (1986) found CA1 unit firing that depended on the goal box color, the goal box position, and the phase of the trial. They also found unit firing that was affected by the combination of the goal box color and the phase of the trial, the goal box color and the goal box position, and the goal box position and the phase of the trial. Thus the CA1 units appeared to be coding for the demands of the individual trials, the working memory information, although not in any clearly defined manner.

Otto and Eichenbaum (1992) recorded from CA1 units during an olfactory delayed non-match to sample task. Trials were given to the rat in a series. In each trial an odor was presented to the rat. If the odor differed from that presented on the preceding trial the rat was to make a nose poke to receive a reward. They found units that increased their firing in response to trial initiation, cue sampling, reward approach, and during the delay interval. Certain cue sampling units were notable in that they displayed firing changes in response to the cue history, either firing maximally if the previous and current odors differed or firing maximally if the previous and current odors were similar regardless of whether the rat made a nose poke. These units appeared to be coding for the demands of the individual trials, the working memory information. Other cue sampling units were notable in that they displayed firing changes that predicted the behavioral response by 400 ms, either firing maximally if the response was "go" (a nose poke) or if the response was "no-go" (no nose poke). These units most clearly appear to be mediating the working memory demands of the task since by observing their firing it is possible to predict the eventual response of the rat.

Hampson et al (1993) recorded from CA1 and CA3 units during a delayed match to sample task. During the sample phase the rat was presented with
one of two retractable levers that it had to press. After pressing the lever the rat had to run to the opposite end of the environment and poke its nose in a port until a light was extinguished. The match phase followed in which both levers were extended and the rat had to press one. If it pressed the lever previously pressed in the sample phase it was rewarded (the reinforcement phase). They found units that increased their firing during the sample, match, and reinforcement phases, during the match and reinforcement phases, during the sample and match phases, and during the match phase only. On the basis of this study it appeared that hippocampal units were active in various phases of a working memory task, although as in the Wible et al (1986) the relationship between unit activity and task demands was not clear cut.

4.3 Relational Memory Hypothesis

Cohen, Eichenbaum, and Otto (Cohen and Eichenbaum, 1991; Eichenbaum et al, 1992; Cohen and Eichenbaum, 1993) have presented a hypothesis of hippocampal function in which they attempt to integrate the findings of their own work and the work of others. According to their relational memory hypothesis the hippocampus is required in order to form flexible associations between multiple cues. An intact and functioning hippocampus is required to perform in tasks where the correct response is determined on the basis of multiple cues presented either simultaneously or sequentially. Moreover an intact and functioning hippocampus is required to perform tasks in which the correct response during one phase of the task differs from that in another phase, such as the delayed non-match to sample task.

According to the relational memory hypothesis the function of the hippocampus is to store processed experiences in a highly interconnected network. The interconnections between the various processed experiences are possible relations between them. External stimuli impinging on the animal cause the stored processed experiences to be flexibly activated in order to produce behavior appropriate to the current situation. According to the relational memory hypothesis, spatial cues are external stimuli that are processed by the hippocampal relational system. Spatial cues presented
simultaneously activate hippocampal representations that can flexibly guide behavior in various situations. However the relational memory hypothesis states that the hippocampus is required to process the relations between other olfactory, visual, and auditory stimuli as well.

The electrophysiological recording studies of O'Keefe and Conway (1978) and O'Keefe and Speakman (1987a) that have been advanced in favor of the spatial map hypothesis have also been taken to be in support of the relational memory hypothesis. Whereas in the spatial map hypothesis the hippocampal place fields are postulated to be the electrophysiological manifestation of the calculation of spatial variables, in the relational memory hypothesis the unit firing is thought to be due to the units coding for the relationships between the multiple environmental cues.

Similarly the studies of Wible et al (1986), Otto and Eichenbaum (1992), and Hampson et al (1993) have been advanced in support of the relational memory hypothesis as well as the working memory hypothesis. The difference is in what the working memory hypothesis and the relational memory hypothesis postulate to be occurring in the hippocampus during the trials. In all of these studies hippocampal units were described that displayed variations in firing rate in response to the different demands of the tasks. According to the working memory hypothesis the variations in firing rate are the electrophysiological manifestation of the storage of information that is to be used on the given trial and is not applicable to subsequent trials. According to the relational memory hypothesis the variations in firing rate are the electrophysiological manifestation of the activation of a highly interconnected hippocampal representation that is then used to guide behavior.

However the studies of Eichenbaum et al (1987) and Wiener et al (1989) that claim to show hippocampal unit activity correlated with location, olfaction, as well as more abstract mnemonic processing appear to have been advanced in support of the relational memory hypothesis alone.

Eichenbaum et al (1987) recorded from CA1 and hilus during an olfactory discrimination task. During a sample phase the rat would poke its nose into
an odor port. If the odor was a reward odor it was required to keep its nose in
the port for two seconds whereupon it would run to the opposite end of the
arena to obtain a water reward. If the odor was a non-reward order the rat
was not required to do anything. They found units that increased their firing
rate when the rat sniffed at the odor port, approached the odor port, and
approached the reward cup.

Wiener et al (1989) recorded from CA1 units during performance of a place
task and an olfactory discrimination task. In the place task they describe units
that showed firing rate modulation correlated with place, speed, direction, and
turning angle. In the olfactory discrimination task two odors were presented
simultaneously in two adjacent odor ports. If the rat poked its nose into the
"correct" port as defined by its odor a water reward would be sent to the port
located between the two odor ports. They found hippocampal unit activity that
was time locked to the approach to the odor ports, trial initiation, nose poke,
and approach to the water port.

Cohen and Eichenbaum (1993) claim these studies show that the
hippocampus is involved in non-spatial information processing. More
importantly, the observation that hippocampal units are active in tasks
designed to involve relational memory has lead Cohen and Eichenbaum
(1993) to conclude that the hippocampus appears to participate in relational
memory.

4.4 Comparison of the Hypotheses

The spatial map hypothesis of O'Keefe and Nadel (1978) explicitly excludes a
non-spatial function for the rat hippocampus. Conversely, the working memory
hypothesis of Olton et al (1979) and the relational memory hypothesis of
Cohen, Eichenbaum, and Otto (Cohen and Eichenbaum, 1991; Eichenbaum et
al, 1992; Cohen and Eichenbaum, 1993) does not exclude a spatial function
for the hippocampus. According to the proponents of both the working
memory hypothesis and the relational memory hypothesis, when a memory
task includes a spatial component the firing pattern of the hippocampal units
reflect this in the form of place field firing and when a memory task does not
includes a spatial component the firing pattern of the hippocampal units reflects the non-spatial memory demands of the task (Eichenbaum and Cohen, 1988; Eichenbaum et al, 1989; Eichenbaum and Wiener, 1989; Olton et al, 1989; Wiener et al, 1989; Squire and Cave, 1991; Eichenbaum et al, 1992; Eichenbaum et al, 1994).

Olton et al (1979) originally framed the working memory hypothesis in terms of damage to hippocampal structures. Damage to the hippocampus was postulated to result in impaired performance in working memory procedures. However Jarrard (1993) has since demonstrated that many hippocampal lesion studies are flawed since the lesions encompassed structures adjacent to the hippocampus. Numerous lesion studies conclude the hippocampus is required for spatial memory and not working memory (e.g. O'Keefe et al, 1975; Jarrard, 1978; Morris et al, 1982; Jarrard, 1983; Sutherland et al, 1983; Aggleton et al, 1986; Rasmussen et al, 1989; Gallagher and Holland, 1992). Numerous others conclude the hippocampus is required for working memory and not spatial memory (e.g. Olton and Papas, 1979; Olton and Feustle, 1981; Raffaele and Olton, 1988; Eichenbaum et al, 1988; Wan et al, 1994). Thus it does not appear to be possible to make any strong claims based solely on lesion studies.

Cohen, Eichenbaum, and Otto (Cohen and Eichenbaum, 1991; Eichenbaum et al, 1992; Cohen and Eichenbaum, 1993) claim the relational memory hypothesis can subsume all the other hypotheses by virtue of its generality. According to Eichenbaum et al (1992) the functions attributed to the hippocampus by the spatial map hypothesis of O'Keefe and Nadel are only a subset of the broader functions the hippocampus mediates. Cohen, Eichenbaum, and Otto also claim that the hippocampal relational system can associate stimuli across time. This enables it to subsume the working memory hypothesis of Olton et al.

The most powerful evidence in favor of any memory hypothesis would be the ability to predict both correct and erroneous responses in a memory task on the basis of single unit electrophysiological activity. If this condition is fulfilled then the recorded electrophysiological activity is clearly correlated with the
mnemonic demands of the task and nothing else. In the non-spatial memory tasks of Wible et al (1986) Hampson et al (1993), Eichenbaum et al (1987), and Wiener et al (1989) the future response of the rat could not be predicted based on the observed hippocampal unit firing. In the non-spatial memory task of Otto and Eichenbaum (1992) some hippocampal units predict the future response of the rat, but by only 400 ms. In the spatial memory task of O'Keefe and Speakman (1987a) the response of the rat can be predicted by observing the electrophysiological activity of the hippocampal pyramidal units. Moreover this prediction can be made upward of three minutes prior to the response.

The spatial map hypothesis explicitly excludes non-spatial functions for the hippocampus. Thus apparently n-onspatial tasks which appear to be accompanied by hippocampal firing are frequently cited as evidence against spatial map hypothesis. However the studies of Wible et al (1986), Otto and Eichenbaum (1992), and Hampson et al (1993) that support both the working memory and relational memory hypotheses and the studies of Eichenbaum et al (1987), and Wiener et al (1989) that support the relational memory hypothesis the majority of alterations in hippocampal unit firing occur concurrently with either sensory presentations or motor responses. Thus the changes in hippocampal unit firing seen in these non-spatial memory tasks may be due to the changes in sensory inputs or motor outputs or changes in the animal's arousal level as a result of its past reward history and be entirely unrelated to mnemonic processing. Ranck (1973) recorded from single Ammon's Horn units while the rat performed various behaviors in simple foraging tasks with no discernable mnemonic component and noted that they displayed striking increases in firing rate when the rat ate, drank, and sniffed as well as when it displayed other more non-specific behaviors.

The changes in hippocampal unit firing seen in some of the non-spatial memory tasks may also be due to the different motor response required. In the non-spatial memory tasks of Eichenbaum et al (1987) and Otto and Eichenbaum (1992) a different response is required depending on the cue presented. The tasks are go or no-go. The differences in hippocampal unit firing could be due to the framing of the imminent motor responses or the
suppression of a motor response and not have any mnemonic correlates at all. In the spatial memory task of O'Keefe and Speakman (1987a) the response of the rat is always go, to one of three platforms. Therefore the differences in unit firing are unlikely to be due to the imminent framing of a motor response or the suppression of a motor response. Vanderwolf (1969) and Ranck (1973) both note that the hippocampus displays alterations in both unit and slow wave activity in response to spontaneous motor behavior.

The experimental evidence considered appears to favor the spatial map hypothesis. This thesis will be predominantly concerned with the spatial map hypothesis and its predictions. However the data will also be considered in light of the predictions made by the working memory and relational memory hypotheses in the Conclusions.
Chapter 5: General Methods
5.1 Introduction

The experiment of O'Keefe and Speakman (1987a) was designed to test the effects of cue removal on place field responses. To this end controlled cues were placed inside a circular enclosure and rotated between trials in order to eliminate the information content of background cues that are invariably present. Rats were trained to find a goal arm that was defined solely in relation to the controlled cues. This was the essence of the spatial task. Once the rats had learned the spatial task they were trained to find the goal without the presence of the controlled cues. Upon being placed in this cue controlled environment (CCE) and being allowed to view the controlled cues the cues were then removed before the rats were permitted to make a choice. This was the essence of the spatial memory task.

The experiment described herein is an extension of the study of O'Keefe and Speakman (1987a). The arms used in the earlier study were replaced with platforms. By using square platforms rather than narrow arms it was postulated that place field responses would more closely approximate those seen in a more natural open field situation.

In the present study the overall hypothesis to be tested was: "Do hippocampal Ammon's Horn units display electrophysiologically observable phenomena that is correlated to the demands of a spatial memory task". Thus a CCE was employed in order to produce a spatial navigation task with an explicit memory component.

5.2 Subjects

The subjects used in the present study were male Lister hooded rats between 350 and 450 g in weight. The rats were housed in a dedicated animal house separate to the experiment room and maintained on a twelve hour light twelve hour dark cycle with lights out at three PM. The rats were housed two or three to a cage. Each cage had an unlimited water supply.
Training took place on weekdays at 1 PM. Rats were transported to and from the experiment room in an opaque box. After the training session the rats were returned to the animal house and weighed. Twenty grams of rat chow were placed in the cage for each rat. On weekends each cage had 20 g for each rat placed within it at approximately one PM. No training took place on weekends.

Rats chosen for implantation were separated from their companions before surgery and housed separately for the remainder of the study. During recording implanted rats were fed between 15 and 20 g of food a day in order to control motivation in the task. Feeding was modulated such that at no time did weight loss in excess of 80% of the free feeding weight occur.

5.3 Environment

Structure of the Environment

The CCE was located in the north end (as defined by the door location) of a rectangular room 591 cm by 316. (Figure 8.) It consisted of a black curtained circular enclosure 244 cm in diameter. The curtains were hung from a matte black false ceiling 205 cm above the floor. There was no gap between the top of the curtains and the false ceiling or the bottom of the curtains and the floor. At four equally spaced points along the margin of the CCE false ceiling there was one dim 12 V light bulb. In front of each of these lights there was a black card to prevent direct lighting of the environment. These diffuse and oblique "house lights" were on at all times during training and recording.

Within the enclosure there were four platforms, each 81 cm above the floor. The platforms supported removable square covers measuring 54.5 cm on a side with a lip 1.5 cm wide and 1.5 cm high running along all four edges. The distance between the inner edges of the covers was 18.5 cm. Platform covers were employed in order to protect the actual platforms from damage and to provide control over olfactory or textural cues on the platform surface. The lip along the cover's edges was added in order to prevent the rats from distracted
walking off the edges of the platform, which they would do when the edges were not mechanically delimited.

In the center of the CCE was a central platform that could be moved up or down by means of a pneumatic piston (Kay Pneumatics). The movable center platform was covered by a removable cover measuring 31 cm on a side with notches at each corner to allow it to mesh with the center corners of the four stationary platform covers. The center platform cover did not have a lip.

The four stationary and central movable platforms and all platform covers were painted matte grey.

Cues in the Environment

Four matte black vertical posts were present in the CCE, in each of the four corners just inside the curtain and below the house lights. The posts ran the height of the CCE from the floor to the false ceiling. Each of the posts had an identical 12 V electric cooling fan (Radio Spares) mounted 107 cm above the floor. On each of the posts above the fan there was a low power 12 V light mounted 125 cm above the floor.

A circular railing with a diameter of 129 cm was attached to the bottom of the false ceiling. Four small metal plates were attached to the railing. Each plate was used to hang a removable hanging cue via a small lip on which the cues were hooked. These were a white card 51 cm square hung 51 cm from the ceiling, a scented white plastic ball nine cm high by 7 cm diameter hung 83 cm from the ceiling, an 83 cm white towel hung 19 cm from the ceiling, and a 15 cm square cage containing a toy plastic rat hung 78 cm from the ceiling. The angular relationship between these cues was constant. (See Figure 9.)

If the card is defined as 0° then the scent ball was at 71° clockwise, the light was at 135°, the towel was at 194° clockwise, the fan was at 225°, and the cage was at 267° clockwise. These angles remained unchanged for the duration of the study.
The combination of SBC's and CC's enabled the CCE platforms to be viewed in two separate reference frames. In the SBC reference frame the four platforms were designated NW, NE, SW, and SE, taking the experiment room door to be the north. In the CC reference frame the four platforms were named according to the nearest CC. Thus there was the fan platform, the light platform, the platform between the ball and the card (subsequently referred to as the ball platform for brevity), and the platform between the card and the cage (subsequently referred to as the cage platform).

If the card is viewed as the apex of a triangle then the left vertex is formed by a powered fan and the right vertex is formed by a lit light. All other hanging cues were positioned with respect to the card by virtue of being fixed to the same rotatable rail. All other electrical cues were off. The goal platform was defined solely within the CC frame of reference. It was set to the platform between the card and the cage and remained unchanged for the duration of the study.

The stationary platform covers were numbered from one to four. The central platform cover was oriented with respect to stationary platform cover one. During the initial training the platform covers were rotated in register with the rotations of the CC's. At later stages of training the platform covers were fixed in the NE goal orientation for all trials. (See below.)

5.4 Training

The rats were brought into the experiment room daily at approximately one PM and placed on a wooden holding platform south of the CCE. There were four groups of six animals, one group of three animals, and two groups of four animals for a total of thirty five animals that were trained on the CCE task.

Familiarization

The rats were initially familiarized with the CCE. It was set up with the goal in the NE with respect to the SBC's and the center platform in the up position. The goal platform was baited with 8 pellets of rat chow (Campden
Instruments) in the far corner. The rats were placed on one of the three non-goal platforms and allowed to explore at random. When they entered the goal arm and consumed the pellets the platform was re-baited. After approximately three minutes the rats were returned to the holding platform. Three familiarization trials were given each day for four days, always with the goal in the NE with respect to the SBC’s.

Initial familiarization was carried out separately for each rat or by placing the entire group of rats on the platform simultaneously. This was found to have no effect on subsequent training.

Static Goal

Once familiarized the rats were then trained on the static goal task. The goal platform was held constant during the day’s trials. The far corner of the goal platform was baited and the movable center platform was in the up position. The rat was allowed to select platforms until it found the goal platform and consumed the reward.

Three static goal trials were given each day for four days. Across the three trials given in one day all possible start platforms with respect to the CC’s were tested in pseudo-random order. These were the fan start platform, light start platform, and ball start platform. (See Figure 9.)

Across the four days all possible goal platforms with respect to the SBC’s were tested. These were the NW, NE, SW, and SE platforms. Across the four blocks of trials given in four days all possible goal platforms with respect to the SBC’s were tested in pseudo-random order.

To illustrate the resulting pattern consider the SBC reference frame alone. On day one the goal may have been set to be the NE. Thus the rat would be started from the SW, SE and NW platforms, or some other combination. On day two the goal could be set to the SW platform. Thus the rat would be started from the SE, NE and NW platforms, and so forth.
All the rats in the training group were given the three trials in rotation, thus there was an interval of at least fifteen minutes between trials for each rat. Three static goal trials were given each day for four days. Each rat was started from each of the three start platform locations with respect to the CC’s four times and from each of the four platform locations with respect to the SBC’s three times. Thus all twelve possible combinations of CC and SBC defined start platforms were tested once.

**Shifting Goal**

The rats were then trained on the shifting goal task. In this task the goal platform location was varied between trials as opposed to between days, subject to the stipulations that the same goal platform location could not occur twice in succession and across four days all possible goal platforms with respect to the SBC’s must occur three times each.

Again to illustrate consider the SBC reference frame alone. On day one and trial one the start platform may have been set to be the NE and the goal platform to the SW. On trial two the start and goal could be SW and SE, and on trial three the start and goal could be NW and NE. On day two the start and goal could then be SE NE, SE NW, and NE SE for the three trials respectively, and so forth. It can be seen that within each day all possible CC defined start platforms occur once (Day one: light platform, ball platform, and fan platform. Day two: ball platform, light platform, and fan platform.) Moreover in four days all twelve possible combinations of CC and SBC defined start platforms occur once.

Rats were trained on the shifting goal task until they selected the goal platform on their first choice on nine out of ten trials.

**Movable Center**

The task was then modified to include the movable center platform. Instead of being up throughout the trial, the platform was initially in the down position when the rat was placed in the start platform. The center was then raised
immediately and the animal was allowed to make a choice. Once the rat had chosen a non-start platform the center platform was lowered, stranding the animal on its chosen platform. If the rat chose correctly it was allowed to consume the food reward and then removed to the holding platform. If the rat chose incorrectly it was left on the incorrect platform for one minute and then removed to the holding platform.

The movable center was necessary in order to delay the point in time in which the rat could make a choice. When the center was up well trained rats would chose the goal platform immediately, preventing the recording of hippocampal activity in the start platform.

Rats were trained on the movable center / shifting goal task until they achieved a criterion performance of three out of three correct.

**Delay of Reward**

The task was then modified to include delay of reward. Instead of baiting the goal platform before the rat was placed in the start platform, the far corner was baited if and when the rat reached it. Delay of reward was introduced in order to insure the rat was not choosing the goal platform based on the sight or smell of the food reward.

Rats were trained on the reward delay / movable center / shifting goal task until they achieved a criterion performance of three out of three correct.

**Delay of Choice**

The time before the raising of the center platform was then increased in increments of fifteen seconds until it was between two and three minutes. This proceeded at different rates for different animals. Some were distraught at very slight increases in the delay and would evidence their distress by not choosing a platform or choosing incorrectly. When this occurred the rat would be given another trial at the lesser delay until it appeared confident, whereupon the delay would be increased again.
The long delay before the rat was permitted to choose a goal platform was necessary in order to accurately record place fields. An adequate amount of time was needed in order to enable the rat to traverse the environment numerous times such that location and magnitude of place fields could be unambiguously characterized.

Rats were trained on the choice delay / reward delay / movable center / shifting goal task until they achieved a criterion performance of nine out of ten correct.

Segregation Cylinder

Rats that had learned the choice delay / reward delay / movable center / shifting goal task were separated and trained separately. The animal was brought into the experiment room and placed in a steel unmarked circular enclosure 23 cm in diameter and 35 cm in height that rested on the holding platform. The cylinder was carried into the CCE. The sides of the cylinder were sufficiently high to prevent the animal from viewing the cues. At the beginning of each trial the rat was taken from the cylinder and placed on the start platform. The cylinder was removed from the CCE. At the end of the trial the cylinder was brought back into the CCE and rat was replaced within it. The cues were then rotated and preparations were made for the next trial. The cues were never moved when the rat was able to observe them.

The use of the cylinder was necessitated by the recording arrangement. When recording in the CCE the head cap of the rat was connected to wires that transmitted the neural signals to the recording equipment. In order to remove the rat from the environment it was necessary to unplug these wires from the head cap. However it was not feasible to continually connect and disconnect the rat between trials in order to remove it from the environment. Therefore the cylinder was used to conceal the changing of the environment cues. The rats slept or groomed while in the cylinder.
There was no need for a criterion performance level for the addition of the segregation cylinder to the running paradigm as it was found to have no effect on performance.

Cue Removal

At this point the memory component of the task was introduced. The animal was placed in the start platform and allowed to explore. The cues were then removed half way through the trial. Cue removal was accomplished by cutting the power to the fan and light cues and physically removing the card, towel, ball, and cage. (See Figure 9.) The hanging cues were hung outside the environment. The center platform was then raised and the rat was allowed to choose a platform. Upon choosing a platform the center platform was lowered. If the goal platform was chosen food was deposited in the far corner. If a non goal platform was chosen the rat was left there for a minute and then returned to the holding platform. The delay between removing the cues and allowing the animal to make a choice was gradually increased until it was between two and three minutes.

Rats were given cue removal / choice delay / reward delay / movable center / shifting goal trials in alternation with choice delay / reward delay / movable center / shifting goal trials until they achieved a criterion performance of ten out of ten correct.

Perceptual-Perceptual and Perceptual-Memory Tasks

At this point in training the rat was able to perform the two trial types used in subsequent recording. The choice delay / reward delay / movable center / shifting goal trials will henceforth be referred to as perceptual-perceptual (PP) trials. The cue removal / choice delay / reward delay / movable center / shifting goal trials will henceforth be referred to as perceptual-memory (PM) trials.

In the first (control) phase of the perceptual-perceptual trials (PP1) and the perceptual-memory trials (PM1) the CC's were present. If the trial was a PM
trial the cues were removed half way through the trial. In the second (test) phase of perceptual-perceptual trials (PP2) the cues were present, whereas they were absent in the second phase of the perceptual-memory trials (PM2). Any differences in behavior or unit firing due to spatial memory for the cues would be manifested as differences between the PP and PM trials during the test phase.

In an effort to control for the disruption caused by the removal of the cues in PM trials, at the half way point in PP trials the electrical cues were pulsed on and off and the hanging cues were lifted out of their holders and immediately replaced.

Rats were trained on alternating PP and PM with shifting goal locations. They were given three trials a day encompassing all three CC defined start platforms until they achieved a criterion performance of ten out of ten correct.

Fixed Platform Covers

During all preceding phases of training the platform covers were rotated in register with CC's. It is possible the rats may have been able to differentiate the platforms by accumulated odors or textures on the platform covers. In order to prevent the rat using the platforms as goal defining cues the covers were placed in the NE goal configuration and left there for all subsequent trials, regardless of the location of the goal platform. Thus the platform covers were reduced to being a SBC. Rats were given trained on PP and PM trials with fixed platform covers until they achieved a criterion performance of ten out of ten correct.

Coverage Paradigm

The last stage of training consisted of shaping the animals to chase wooden insulated forceps manipulated by the experimenter in a stereotypical and invariant pattern. (Figure 10.) This was done in order to ensure complete and uniform coverage of the start platform area. The forceps were baited with one food pellet and moved in alternating sets of horizontal and vertical rows just
out of reach of the rat. The rat quickly learned to follow the forceps closely, typically within one or two trials. The rat was permitted to catch up and eat the pellet on a sufficient number of occasions to continue to follow the forceps but not often enough to unduly disrupt coverage. This number ranged from four to ten. When the rat ate a pellet the forceps were immediately re-baited.

**Priming Trial**

During all phases of training and subsequent recording the first trial of the day was a simple familiarization trial in which the center remained up at all times, the goal was baited before the rat was placed in the environment, and exploration was allowed. The start and goal platforms were determined by the training schedule. The behavior of the rat on the priming trial was not used in determining whether the rat had achieved any given criterion performance level and place units were not recorded.

**5.5 Implantation**

After training there remained nine animals that could perform the PP and PM tasks reliably. These were implanted bilaterally with two movable electrode microdrives.

**Electrodes and Microdrives**

Four 25 μm platinum 10% iridium wires insulated with H-ML ceramic (California Fine Wire) were twisted together to produce a tetrode (Recce and O'Keefe, 1989; Recce et al, 1991). The loose ends of the tetrode were stripped by passing them through an alcohol flame. The electrodes were placed in a microdrive modified from the original design of Ainsworth et al (1969). (Figure 11.) The stripped free ends of the tetrodes were wire wrapped under a microscope to the terminal posts. The wrapped wires were then coated with silver print paint to ensure electrical continuity and then with nail varnish to provide mechanical stability. The tips of the electrodes were plated with platinum using the method of Merrill and Ainsworth (1972) so as to reduce the impedance to less than 500 kΩ.
Surgery

Anesthesia was induced in a sealed chamber with a mix of 3% isoflurane (Abbott) in oxygen at three L/min. Anesthesia was maintained with a mixture of isoflurane (1% to 3%) in oxygen (1.5 L/min) and nitrous oxide (3 L/min) delivered via a scavenging rodent mask. The percentage of isoflurane was varied in order to maintain the heart rate between 250 and 350 beats/min.

The head was securely locked in a stereotaxic frame via ear bars and a bite bar. The skull was exposed and leveled and four skull screw holes of two mm were drilled in the four corners of the skull. Skull screws of five mm length were machined to a range of diameters and stored in alcohol. A skull screw providing a snug fit for a given hole was selected and tightly mounted in the hole. The right anterior skull screw was soldered to an Amphenol pin before being mounted in the skull hole to provide a ground connection. (Figure 12.)

Two electrode holes of three mm diameter were drilled at the implant coordinates. (Table I.) Dura was removed from the surface of the brain exposed in the electrode holes. The microdrives were positioned over the electrode holes using a micromanipulator. The electrodes were then inserted to a depth of between 1.5 mm and 1.8 mm. The area around the electrode was packed with gelatin sponge (Spongostan Standard, Ferrosan). The bottoms of the drives, skull screws, and ground pin were joined to each other by dental acrylic (Austenal Dental). Two nylon headstage amplifier mounting posts were placed on the acrylic cap and securely cemented in place.

The wound margins were dusted with topical antibiotic (Cicatrin, Wellcome). Dental acrylic was used to cover any exposed skull and a small lip was made over the wound margins to prevent infection.

The rat was removed from the stereotaxic frame and injected intra-muscularly with 0.1 mL buprenorphine (Temgesic, Reckitt and Colman) to provide post-operative analgesia. It was placed in a bare recovery cage containing only a water bottle for one day to prevent ingestion of sawdust due to drug induced hyperphagia. It was then returned to its home cage.
5.6 Recording

After training and implantation the rats were ready for recording. However in two rats the implant failed to yield any units. Another four rats ceased being able to perform the CCE spatial memory task while connected to the recording apparatus. They appeared to be distracted by the cables swinging above them as evidenced by their looking up at the cables or repeatedly performing a "ducking" motion as though they were attempting to slip beneath a barrier. The remaining three animals were able to perform the task while being recorded from.

Amplification and Digitization

Unity gain source follower current amplifiers based on the TL074CD quad FET IC were securely mounted via alligator clips to the nylon posts cemented to the rat's head. The output of the electrodes mounted in the microdrives were fed into these headstage amplifiers. The output of the headstage amplifiers was fed along light and flexible hearing aid wire (PC Werth) supported by elastic threads out of the CCE to a second set of amplifiers based on the OP07 operational amplifier IC just outside the curtained enclosure where the signal was amplified 10 times and AC coupled to remove DC offset. The signal was then fed along ordinary copper wire to the main amplifiers. The main amplifiers were based on INA106 differential amplifier IC's and low noise OP16 operational amplifier IC's. At the main amplifiers the signal was differentially amplified between 4000 and 7000 times and passed through passband filters with a range of 400 Hz to 5.4 KHz. The amplified and filtered signal was fed to a computer (VME bus with SYS68K processor, Force Computers) via an analogue to digital converter (MPV952, Burr Brown) with a range of -5.0 to 5.0 V. The computer was programmed to continuously monitor the A to D output of all tetrode lines. When the encoded voltage exceeded 2.5 V the computer was triggered to record the digitized supra-threshold signal to disk. Two hundred voltage points were recorded across eight channels over a period of 1 ms. Thus each channel consisted of 25 voltage points for an effective sampling rate of 25 KHz per channel.
The headstage apparatus of the rat was fitted with a small 12 V incandescent bulb. A low light video camera (Sanyo) pointed downward from a hole in the center of the false ceiling. The video camera was fitted with a wide angle lens (1:1.6/5.5, Fuji Photo Optical Company) to enable coverage of the entire environment. The output of the video camera was fed to a bright spot video tracker (SP115/2, HVS Image Analyzing) that located the 12 V bulb on a 256 by 256 grid covering all four platforms of the CCE. The continuously updated position data was fed to the computer via a parallel port (APAL, Eltec) and recorded to disk every 20.48 ms.

**Unit Screening**

Prior to recording in the CCE spatial memory task the electrodes had to be lowered to Ammon's Horn stratum pyramidale and placed in the vicinity of clearly separable units. This was done while the rat was kept on the holding platform outside the CCE. During screening the electrodes in the microdrives were lowered by 25 or 50 μm increments and 30 minutes was allowed to pass to allow the electrodes to stabilize. The output of the amplifiers was displayed on an oscilloscope and assessed visually for theta, sharp waves, LIA, ripple, and actual unit spikes. When the electrodes displayed a large number of clearly separable unit spikes the rat was replaced in its home cage and left there for at least one day to insure the units isolated were stable. If on the subsequent day the same units were still clearly visible and their amplitude was unchanged then the electrode placement was deemed to be stable and recording in the CCE spatial memory task began.

**Trial Preface**

For PP and PM trials the goal location was input into the computer. The computer activated the electrically powered cues that defined the goal location via the APAL parallel port. The hanging cues were positioned manually.

During a recording session the rat would be kept in the cylinder in the CCE. The rat was continuously hooked up to all electronics. At the start of a trial
the rat was transferred from the cylinder to the CCE. The experimenter would then exit the CCE bearing the cylinder.

**Trial Running**

A trial was instigated by placing the rat on the start platform. For one animal the first phase began immediately after it was placed in the environment. For the other two animals the first phase began one minute after they were placed in the environment. However each recording phase was two minutes in duration for all animals. No difference was observed in the recorded unit responses for the two different timing paradigms. During the first phase the animal was made to cover the platform surface using the coverage paradigm described above. During all recording the experimenter manipulating the forceps was in the curtained CCE and therefore blind to all electrical activity being recorded from the rat.

Immediately after the first phase the trial manipulation was applied. If a PP trial were being run the computer would quickly pulse the electrical cues off and on. At this point the experimenter would jostle all four hanging cues to simulate the noise and disruption of cue removal. If a PM trial were being run the computer would turn the electrical cues off. At this point the experimenter would remove all four hanging cues. After the cues had been either jostled or removed the experimenter would exit the CCE.

For one animal the second recording phase began thirty seconds after the end of the first phase. For the other two animals the second recording phase began one minute after the end of the first phase. No difference was observed in the recorded unit responses for the two different timing paradigms. During the second phase the animal was made to cover the platform surface using the coverage paradigm described above for two minutes.

After the end of the second phase the animal was immediately allowed to make a choice. The experimenter would reach outside the CCE and raise the central platform via one of four switches located on the top of the false ceiling.
When the central platform had been raised the rat would select a platform. The central platform would then be lowered and the computer program recording spike and position data would be stopped. If the rat selected the goal platform it was given a food reward. If the rat had selected a non goal platform it was left there for one minute.

**Trial Verification**

After the trial was completed the cylinder was brought into the CCE and the rat was replaced within it. The recorded data was transferred from the VME data recording computer to a Unix workstation (Sun Microsystems) via the APAL parallel port and checked for integrity. Preparations were then begun for the next trial. A minimum of five minutes was allowed between trials to allow the rat to rest.

**5.7 Initial Analysis**

All data analysis was performed on Unix workstations (Sun Microsystems) using "in house" software.

**Correcting for Spatial Distortion**

In order to accurately determine the extent of place field firing and to allow manipulation of the spatial data during analysis it was necessary to correct for the spherical aberration of the video camera lens and the non-linear response of the bright spot detector used to track the rat. A procedure to do this was developed for the CCE that utilized the regular rectilinear layout of the platforms. (Figure 13.)

A light similar to the light carried by the rat was placed in the corner of each platform and the co-ordinates produced by the tracker were recorded. The recorded co-ordinate had a corresponding rectilinear co-ordinate that was the actual location of the platform corner in the environment. A vector from the actual rectilinear location of the platform corner to the recorded location of the
platform corner was calculated for each pair of recorded and rectilinear coordinates.

The vectors on the edges of the platforms were extended outward along the rectilinear horizontal lines to the edges of the environment. Vectors between two platform corners were calculated by taking the sum of the two corner vectors weighted by distance.

Using the same algorithm the vectors in the four horizontal planes were extended along the rectilinear vertical plane for all points along the horizontal line. Points between a calculated horizontal line and the environment edge were set to the vector at the horizontal line. Points between two calculated horizontal lines were set to the sum of the vectors of the two vectors weighted by the distance.

The vector field produced enabled the translation of every point in rectilinear space to a point in recorded space. The matrix was inverted so as it could be used to translate every point in recorded space to a point in rectilinear space. The result was a transform matrix that associated each location in tracker space with a location in real world space. All position data was corrected for distortion using this transform matrix prior to subsequent analysis.

### Clustering Waveforms Using Envelopes

An in house data analysis program was used to inspect the tetrode waveforms. Individual units were identified by the clustering of points representing various waveform parameters. (See Figure 14.) Each cluster was assumed to correspond to one unique unit in the locale of the electrode. Outliers were eliminated by inspection of various plots.

For each of the clusters identified the waveform "envelope" was calculated from the clustered waveforms by taking the maximum and minimum voltage at every time point on all four tetrode channels. (Figure 15.) Thus each waveform envelope defined a cluster. The set of waveform envelopes were then used to sort waveforms into the clusters automatically. Each spike
waveform recorded was compared with all the stored envelopes. If the spike waveform fell entirely within one cluster envelope the spike ID was set to that of the cluster ID. If a spike waveform fell entirely within more than one envelope then a score was assigned to each cluster ID. This score was equal to the sum of the absolute value of the difference between the spike waveform and the mean envelope waveform. The spike waveform was then assigned the cluster ID with the lowest score, that is, the ID of the envelope which it resembled the most. The advantage of this automated clustering technique is that units were sorted into clusters in an invariant fashion across trials. Experimenter bias was removed.

Verifying the Waveform Envelope

A possible consequence of the automatic cluster assignment algorithm is that it is unable to compensate for electrode drift or other phenomena that could cause a systematic shift in the waveform of an identified cluster. The result of this would be erroneous assignment of cluster ID's and this effect would become progressively more pronounced as time progressed.

The waveforms of all the clusters were checked at the start of the recording day. If the waveforms displayed changes in their morphology with respect to the previous day recording was suspended, the data collected up to that point was discarded, and the animal replaced into its home cage and left until the waveforms had stabilized. If the changes in the waveform morphology were due to electrode movement or damage to the unit then one night was normally sufficient to allow the recording to stabilize. After recording the data from one complete session of trials were checked for systematic variations in waveform morphology. Clusters were cut manually and envelopes calculated. Then data from trials from the beginning, middle, and end of the session were loaded into the cluster cutting program together and sorted based on the set of calculated envelopes. Any clusters containing spikes that showed systematic variation in their waveforms across trials were discarded.
Complex Spike Units and Theta Units

Due to the large amount of processing power and disk space necessary to accurately record high frequency theta unit firing efforts were made to reduce the number of theta units on the tetrode lines. Either recording was postponed in the hopes that the theta unit activity would be reduced or eliminated, the electrode was moved slightly in order to leave the vicinity of the theta unit, the amplifier gain on the theta unit channel was reduced so the theta spike was subthreshold, or the A to D threshold on the theta unit channel was raised so that the theta spike was subthreshold. Despite these measures some theta units were recorded, usually because they produced spikes on channels on which pyramidal spikes also occurred and therefore it was deemed necessary to maintain normal recording parameters. However due to the efforts made to minimize theta unit recording no quantitative conclusions can be drawn about the proportion of theta units verses pyramidal units observed or their detailed behavior, spatial or otherwise.

From Ranck (1973) quantitative cut off values for spike duration and mean firing rate were selected to distinguish between the two main types of hippocampal units. Pyramidal units were defined as those in which the first phase of the waveform was 0.3 to 0.5 ms in duration and that had a mean firing rate of less than 10 Hz. Theta units were defined as those in which the first phase of the waveform was 0.15 to 0.25 ms in duration and that had a mean firing rate of greater than 10 Hz. In the present study all units with a firing rate of less than 10 Hz averaged over all trials were classified as pyramidal units. All units with a firing rate of greater than 10 Hz averaged over all trials were classified as theta units. The identification based on overall rate was confirmed by inspection of the waveforms. All subsequent analysis was performed on units which could be unambiguously classified as pyramidal units based on the above criteria. Units which were either classified as theta units or which could not be unambiguously classified based on the above criteria were not analyzed further.
Creation of Event Lists

The last step in the pre-processing of the data consisted of converting the trial files with their large amounts of spike waveform data into a stream of time stamps and event identifications. Each trial with its accompanying spike waveforms and position points was loaded into a program in which position points were corrected for distortion introduced by the camera and tracker as described above and the waveforms were assigned cluster ID's where appropriate as described above. The data was then stored in a smaller file in which the only information retained was the position recorded at 20.48 ms intervals and the time of occurrence and cluster ID of spike firings. These smaller time stamped files were used in all subsequent analyses. The original spike waveform trial files were retained in case it was deemed necessary to re-cut the spikes.

5.8 Histology

At the end of recording the rats were deeply anesthetized with an intraperitoneal injection of sodium pentobarbitone (Lethobarb, Duphar) and perfused with saline followed by 4% paraformaldehyde solution. The electrodes were removed and the brain dissected out and placed in a 4% paraformaldehyde 30% sucrose solution to cross link and dehydrate the tissue. When the brain had sunk to the bottom of the solution it was then embedded in 5% gelatin 10% glycerine solution at 38°C, sliced at 50 μm on a cryostat, mounted on slides pre-coated with 0.1% chrome alum 1% gelatine solution, and stained with cresyl violet. Sections displaying electrode tracts and surrounding cytoarchitecture were traced using a camera lucida fitted to a Nikon microscope. Using the record of electrode depth and the histological determined electrode tract, units were localized to either the CA1 layer or the CA2/CA3/hilus. No attempt was made to distinguish between CA2, CA3, and hilus due to the inaccuracy inherent in determining the location of movable electrodes post-hoc.
5.9 Introduction to Results

Three animals were successfully trained, implanted, and recorded from while performing the CCE spatial memory task. Each rat was assigned a code at the time of surgery. These were R305, R331, and R408, respectively. The performance of each rat during training, upon implantation, and during recording is illustrated in Figures 16, 17, and 18. All of the rats performed at between 80 and 100% correct during recording.

Each rat was run in at least two sessions. A session consisted of twenty four trials, all twelve possible combinations of CC’s and SBC’s run as perceptual-perceptual trials and all twelve possible combinations of CC’s and SBC’s run as perceptual-memory trials. For each session the electrodes were placed in a different portion of the hippocampus, thus in each session different units were recorded.

R305 was run in five sessions, R331 was run in three sessions, and R408 was run in two sessions. Across all rats and sessions ninety two units were isolated. (See Table II.) The majority of the units isolated were classified as pyramidal units (eighty nine pyramidal units verses three theta units). The theta units were not analyzed further. An approximately equal number of complex spike units were recorded in CA1 and the CA2/CA3/hilar area (forty seven and forty two respectively).

Four analysis paradigms were applied to the data from the three animals. The first was designed to determine whether the units displayed firing rate changes in the CCE that was governed by the CC’s, the SBC’s, or the interaction between CC’s and SBC’s. The second analysis paradigm was performed on units that the first analysis had identified as being governed by the CC’s. It was designed to determine what effect, if any, cue removal had on the firing of the units recorded in the CCE spatial memory task. The third analysis paradigm was designed to determine whether place fields recorded in the CCE displayed directionality. In the last analysis the spatial firing displayed by place units during error trials was compared and contrasted with that seen in correct trials.
Chapter 6: Effects of CC's and SBC's on Hippocampal Units
6.1 Rationale

O'Keefe and Conway (1978) found that rotating the CC's in a CCE reliably rotated the place fields of complex spike units. They postulated that the CC's governed the firing of the place units. This result was replicated by O'Keefe and Speakman (1987a). Muller et al (1987) and Breese et al (1989) found that rotation of a single cue could also reliably rotate Ammon's Horn place fields. However O'Keefe and Speakman (1987a) also found some place fields in the CCE that failed to rotate when the CC's were rotated. They postulated these place units were sensitive to the SBC's.

This analysis was performed in order to determine whether Ammon's Horn complex spike units recorded in the present study displayed spatial firing that was governed by the CC's, SBC's, or the CCxSBC interaction.

6.2 Analysis

O'Keefe and Speakman (1987a) used a three way ANOVA to examine the effects of controlled cues, static background cues, within arm location, and interactions thereof on the spatial firing pattern of individual units. They divided each maze arm into a two by two grid of rotationally symmetric bins and used the spike count divided by dwell time as the dependent arm location variable. In the present study data was recorded on maze platforms (54.5 cm by 54.5 cm) instead of maze arms (5.5 cm by 61 cm or 15 cm by 38 cm). This made it impractical to calculate a three way ANOVA in the same fashion as that of O'Keefe and Speakman (1987a) as a two by two grid would be inadequate to capture the detailed spatial morphology of place fields on the larger platforms. Since the number of bins is the square of the grid size any attempt to increase grid size quickly leads to a vast increase in the number of bins. Thus any significant controlled cue or static background cue effects would be overwhelmed by the interactions between the numerous values of the arm location factor.

Initially a method was devised to characterize the spatial morphology of the firing rate map using polynomial surface fitting. Since the position data was
recorded in four different orientations corresponding to the four possible CC rotations the polynomial coefficients were made rotationally insensitive by taking the mean of the absolute value of pairs of coefficients defining x and y position (Cliff et al, 1975). Attempts were made to analyze the firing of the session maps using these rotationally insensitive polynomial coefficient combinations in a nested ANOVA design. However the variability in the polynomial values overwhelmed any spatial firing for all but the strongest of place fields.

O'Keefe and Speakman (1987a) also used a two way ANOVA consisting of the controlled cues factor, static background cues factor, and interactions thereof to examine units for which there was insufficient data to perform the three way ANOVA. Another advantage of eliminating the within arm location factor is that the firing rate data can be examined without considering the detailed morphology of the place fields. It was for this second reason the data in the present study was examined using a two way ANOVA. The fact that the morphology of the place fields within the platforms was disregarded is not a problem since subsequent analyses will examine the detailed spatial morphology of the place fields.

Each session consisted of all possible combinations of three CC defined start platforms, four SBC defined start platforms, and two trial types (PP and PM) each consisting of two phases (first and second). These were run in pseudo-random order. PP and PM trials were given in alternation. CC defined start platforms were presented in random order in blocks of three subject to the stipulation that all three CC defined start platforms had to occur within each block. The four possible start platforms with respect to the SBC's were presented in random order subject to the stipulation that the same SBC defined start platform could not occur twice in succession.

As it was felt unwise to predict in which trial type (PP or PM) or phase (first or second) place specific firing might occur all four trial type and phase combinations were deemed to be repetitions in the calculation of the ANOVA. If the spatial firing patterns in the four trial type and phase combinations were similar but weak then pooling the data in the ANOVA calculation would result
in significant F scores in instances where the individual trial type and phase combinations would not yield significant F scores. However if the spatial firing patterns differed between the four trial type and phase combinations but were consistent within each combination then pooling the data in the ANOVA calculation would result in non-significant F scores in instances where the individual combinations would yield significant F scores. It was decided that the advantages inherent in pooling the data outweighed the disadvantages.

A two factor ANOVA was calculated on the firing rate values for each cluster. The first factor was start platform location with respect to the CC's (that is, the platform nearest to the fan, the platform nearest to the light, and the platform between the ball and the card). The second factor was start platform location with respect to the SBC's, the real world framework (that is, the NW platform, the NE platform, the SE platform, and the SW platform, with the door of the experiment room to the north). The firing rate values were calculated by summing the number of spikes which occurred during the entire two minutes trial phase and divided by 120 (seconds). The ANOVA repetitions consisted of the four trial type and phase combinations (PP1, PP2, PM1, and PM2).

The formula for the estimation of the magnitude of effect in an ANOVA design (Dodd and Schultz, 1973) was used to compute the percentage of variance accounted for by the CC factor, SBC factor, and the CC by SBC interaction in the two way ANOVA. This enabled the comparison of the relative importance of the statistically significant factors.

All units isolated by cluster cutting and waveform sorting were examined in the above manner.

6.3 Results

Forty four out of the eighty nine complex spike units (49%) recorded in the hippocampus during performance of the CCE spatial memory task displayed firing that was significantly governed by the CC factor and/or the CCxSBC interaction. (Table III.)
One unit displayed firing that was significantly governed by the CC, SBC, and CCxSBC factors. In this instance the significant level and percentage of variance accounted for by the CC factor was much higher than that accounted for by the SBC and CCxSBC factors. (See Table III.)

Two units displayed firing that was significantly governed by the CC and CCxSBC factors. In the first instance the significant level of the CC and CCxSBC factors was the same and the percentage of variance accounted by the CCxSBC factor was greater. In the second instance the significant level of the CC factor was two orders of magnitude greater than that of the CCxSBC factor and the percentage of variance accounted by the CC factor was about one order of magnitude greater that accounted for by the CCxSBC factor.

Twenty four out of the forty seven units (51%) recorded in CA1 displayed firing that was significantly governed by the CC factor and/or the CCxSBC interaction. For these units the mean percentage of variance accounted for by the CC factor alone was 51%. Twenty out of the forty two units (48%) recorded in CA2/CA3/hilus displayed firing that was significantly governed by the CC factor and/or the CCxSBC interaction. For these units the mean percentage of variance accounted for by the CC factor alone was 31%.

To graphically illustrate the CC governed unit firing the rate maps were arranged by CC defined start arm by rotating the four SBC defined start arms such that the start platforms defined relative to the CC’s were superimposed. The average firing rate for each nine by nine bin was calculated across the four SBC defined start arms. An example of this CC plot for a complex spike unit with significant CC governed firing is shown in Figure 19. This unit had the largest percentage of variance (89%) accounted for by the CC factor in the CC by SBC ANOVA. The firing of this unit is highly localized to within the upper left hand corner of the fan platform.

In contrast the firing of the unit with the least percentage of variance (15%) accounted for by the CC factor but that nevertheless had significant CC governed firing is shown in Figure 20. The unit is considerably less spatially...
specific than the unit illustrated on Figure 19. It displays firing on the fan and ball platforms and failed to fire on the light platform. Thus this unit displayed firing governed by the CC’s, although to a lesser extent than the first unit.

Three units displayed firing that was significantly and solely governed by the CCxSBC interaction factor. However the significance level was never greater than 0.01 in all three cases and the mean percentage of the variance accounted for by the CCxSBC factor (26%) was less than the mean percentage of the variance accounted for by the CC factor (42%) in the other units. To graphically illustrate the SBC governed unit firing and contrast it with the CC governed firing a SBC plot was constructed. The rate maps were arranged by SBC defined start arm by rotating the three CC defined start arms such that start platforms defined relative to the SBC’s were superimposed. The average firing rate for each nine by nine bin was calculated across the three CC defined start arms.

The CC plot for a unit with firing significantly and solely governed by the CCxSBC interaction factor is shown in Figure 21. The firing rate appears to have been highest in the light platform. The SBC plot for the same unit is shown in Figure 22. The firing rate appears to have been highest in the SW platform. Thus there appeared to have been an interaction between the light platform (a CC factor) and the SW platform (an SBC factor) that gave rise to the significant p value for the CCxSBC interaction.

6.4 Discussion

Complex Spike Unit Firing is Governed by the CC’s

In the present study 49% of the complex spike units recorded during performance of the CCE spatial memory task displayed firing that was significantly governed by the CC’s. This is in qualitative accordance with the results of O’Keefe and Conway (1978), O’Keefe and Speakman (1987a), Muller and Kubie (1987), Breese et al (1989) and others in which one or more cues were found to reliably rotate place units in an environment in which access to static background cues is eliminated.
In the CCE study of O'Keefe and Speakman (1987a) 56% of complex spike units displayed place specific firing that depended predominantly on the orientation of the CC’s. This is slight higher than the 49% value obtained in the present study. This may be due to the different geometry of the CCE environments employed in the two studies. In O'Keefe and Speakman's original study the CCE maze consisted of four narrow arms. In the present study the CCE maze consisted of four square platforms. O'Keefe and Conway (1978) found place fields clustered around the edges of an elevated platform but were evenly distributed in the arms of a T maze. Thus it is possible the switch from maze arms to platforms in the present study may have reduced the number of complex spike units that displayed place fields.

For complex spike units in the present study the mean percentage of the variance accounted for by the CC factor was 42%. This is higher than that obtained by O'Keefe and Speakman (1987a). For the seven units O'Keefe and Speakman showed to be significantly governed by the CC's using a two way CC by SBC ANOVA the mean percentage of variance accounted for by the CC factor was 19%. The most likely reason for this discrepancy is the coverage paradigm employed in the present study. In the study of O'Keefe and Speakman (1987a) the rat was not made to cover the start arm in a stereotypical and invariant pattern. Thus the rat likely paused, reared, groomed and so forth while recording took place on the CCE start arm. This behavioral variability lead to heterogeneity in the spike and position data that could not be accounted for by the ANOVA factors. In the present study the rat was made to cover the start arm in a stereotypical and invariant pattern at all times. This behavioral regularity would result in less heterogeneity in the spike and position data for a given set of CC and SBC parameters. Therefore more variance could be accounted for by the ANOVA factors, chiefly the CC factor.

Complex Spike Unit Firing is Not Governed by the SBC’s

In the present study only one complex spike unit was found to display place specific firing which was significantly dependent on the SBC's as assessed by the CC by SBC ANOVA. In this instance the significant level of the CC factor
was two orders of magnitude higher than that of the SBC factor and the percentage of variance accounted for by the CC factor was over three times greater. Thus in the present study none of the complex spike units were found to display place specific firing that depended predominantly on the orientation of the SBC's as assessed by the CC by SBC ANOVA.

O'Keefe and Speakman (1987a) found four out of fifty five complex spike units (7%) recorded in the hippocampus displayed place specific firing that depended predominantly on the orientation of the SBC's. The difference is slight and likely results more from differences in the methodologies of the two studies rather than from any intrinsic firing properties of hippocampal complex spike units.

**Some Complex Spike Unit Firing is Governed by the CCxSBC Interaction**

Three units were shown by the CC by SBC ANOVA to have firing that was significantly and solely governed by the CCxSBC interaction. One of these units is illustrated in Figures 21 and 22. Upon inspection of Figure 21 the firing rate appears to have been highest in the light platform whereas upon inspection of Figure 22 the firing rate appears to have been highest in the SW platform. It is possible the firing of this unit was modulated by factors specific to the light platform as defined by the CC's and the SW platform as defined by the SBC's, for example luminosity from the light cue and sound from the recording rack located near the SW platform. (See Figures 8 and 9.) Similarly the other units that showed significant p values for the CCxSBC interaction may have responded to specific conjunctions of nearby CC and SBC cues.

**Some Complex Spike Units Fail to Display Spatial Firing**

The remaining complex spike units displayed firing that was not significantly governed by either the CC's, SBC's, or the CCxSBC interaction. Numerous workers report that complex spike units display sensory, motor, and directional firing correlates (Ranck, 1973; McNaughton et al, 1983a; Wible et al 1986; Eichenbaum et al 1987; Wiener et al, 1989; Otto and Eichenbaum 1992;
Hampson et al 1993). It is impossible using the present analysis to determine whether the firing of these unclassified complex spike units is correlated with sensory, motor, or directional parameters. This analysis does not address the question of what factors may govern the firing of these units other than the CC’s, SBC’s, and the CCxSBC interaction.

Relative Spatial Specificity of CA1 and CA2/CA3/Hilar Units

Of the units displaying CC or CCxSBC governed spatial firing 51% were located in the CA1 layer. Similarly 48% of the units located in CA2, CA3 or the hilus displayed CC or CCxSBC governed spatial firing. In contrast O'Keefe and Speakman (1987a) found 67% of CA1 units and 80% of CA3 units displayed CC governed firing in the CCE. Other results on the relative spatial specificity of CA1 verses CA3 units are similarly ambiguous. McNaughton et al (1983a) claimed that CA3 place fields are less spatially specific than CA1 place fields while recording from rats performing a forced choice radial arm maze task. Barnes et al (1990) using the same task claimed the converse, namely that CA3 place fields are more spatially specific than CA1 place fields. Muller et al (1987) claimed the spatial specificity of CA1 and CA3 place fields was essentially the same.

The mean percentage of variance accounted for by the CC factor was 51% for CA1 units and 31% for CA2/CA3/hilar units, a somewhat more convincing difference. If for a given unit the percentage of variance accounted for by the CC factor is taken as a measure of the amount of information the unit's firing contains about the CC's then it appears that the CA1 units contained more information about the CC’s than the CA2/CA3/hilar units.
Chapter 7: Effects of Cue Removal on Hippocampal Units
7.1 Rationale

O'Keefe and Speakman (1987a) extended the CCE paradigm of O'Keefe and Conway (1978) to include a memory component. Rats were placed in a CCE and allowed to observe the CC's. Half way through the trial the CC's were removed. In order to correctly choose the goal platform the rats had to remember the configuration of CC's. O'Keefe and Speakman (1987a) found that the place fields recorded during the first phase of trials in which the CC's were present persisted in the second phase of the trial in which the CC's had been removed. Thus the pattern of Ammon's Horn place unit firing appeared to contain a record of the CC constellation. O'Keefe and Speakman (1987a) hypothesized that it was this hippocampal record that guides the rat's choice of goal platform in the spatial memory task.

O'Keefe and Speakman (1987a) also noted exceptions to the simple persistence of firing seen after CC removal. They found that for some units the within field firing rate was higher in the cue absent phase of the trial than in the cue present phase. They attributed this to long latency of onset of firing for these units.

Quirk et al (1990) recorded from rat Ammon's Horn place units while the rat chased food pellets in a circular apparatus in an environment that was initially lit and then darkened. They found that when the lights were turned off the majority of the place units that displayed place specific firing in the lit phase of the trial maintained their place specific firing in the darkened phase of the trial. However a minority of place units ceased their place specific firing in the darkened phase of the trial.

Markus et al (1994) recorded place units while the rat performed a forced choice radial arm maze task. The first trial of the day was run with lights on. Thereafter the trials were run while alternating between the lights off and on. They found that place fields recorded when the environment was dark were less specific and reliable than their lit counterparts. However they also found some place fields only appeared in the trials run in darkness.
The Quirk et al (1990) and Markus et al (1994) studies differ from that of O'Keefe and Speakman (1987a) in numerous ways, chief among them being that the rat is not required to remember the position of the cues attenuated by darkness in order to successfully choose a goal. However the lights off paradigm of Quirk et al (1990) and the alternating lit and darkened environment paradigm of Markus et al (1994) may nevertheless be analogous to the change in the CCE produced by CC removal if one makes the simplistic assumption that the cues in the CCE are solely visual. The original results of O'Keefe and Speakman (1987a) also tentatively suggest that dynamic changes in place field firing may occur after CC removal.

It is hypothesized that as well as persistence of firing the place fields recorded in the CCE spatial memory task may also display decreases in or cessation of firing after cue removal whereas other units may display increases in or initiation of firing. The data was analyzed in order to determine whether Ammon's Horn complex spike units with place specific firing governed by the CC's show persistence of spatial firing, increases in spatial firing, or decreases in spatial firing after the removal of the CC's. The possibility that place specific firing simply increases as a function of time or decreases as a function of time was also tested.

7.2 Analysis

In order to test the five hypothesized effects of cue removal (persistence, cue dependent increase, cue dependent decrease, time dependent increase, and time dependent decrease) numerous comparisons had to be made among the four maps. Ideally a two by two ANOVA would have been employed to study the interaction between trial type (PP and PM) and trial phase (first and second). However a two by two ANOVA matrix has inadequate degrees of freedom to yield significant results in all but the most extreme cases. Thus it was necessary to employ a different procedure in order to classify the changes in unit firing that occurred after CC removal.

All of the units that the CC by SBC ANOVA analysis determined were governed by the CC's or CCxSBC interaction were analyzed for the presence
of place fields in each of the three CC defined start platforms. Nine by nine bin firing rate maps were calculated for the four trial type and phase combinations (PP1, PP2, PM1, and PM2), three CC defined start platforms (fan platform, light platform, and ball platform) and four SBC defined start platforms (NW, NE, SE, SW) in a given session for a total of forty eight maps.

To calculate a nine by nine bin firing rate map each unit was considered separately. The number of spikes that occurred within each grid square was calculated and divided by the dwell time within each grid square to yield the grid square firing rate in Hz. The maps were smoothed by averaging the value of each bin by that of itself and all of its neighbors. Smoothing was necessary as the firing rate maps were subtracted in a subsequent step of the analysis. If the maps were not smoothed then slight local variations in two identical place fields would be augmented by the subtraction procedure, leading to erroneously high values in the subtracted maps. (See below.)

It was decided to use the integral of the firing rate rather than the peak firing rate as a measure of the intensity of the firing rate in a given map rather than the peak firing rate. In the absence of the x and y position the peak firing rate represents a small fraction of the information about the firing rate map, just one bin's worth. However the integral of the firing rate is calculated by summatting the firing rate in all eighty one map bins. Thus it contains information about the entire firing rate map. The integral is not equivalent to spikes divided by trial length. This would be the case if the rat's coverage of the environment was perfectly even, which is essentially an impossible task.

For a given unit each CC defined start arm and trial phase was examined separately. For the fan start platform and PP1 trial phase all four SBC defined start platform maps were grouped together. The position data was then rotated about the center of the CCE such that the CC's were in register. This had the effect of discarding the positional information pertaining to the SBC's while retaining the positional information pertaining to the CC's. The average of all the bins of the four rotated SBC defined start platform maps was then calculated. Thus the four SBC defined maps were reduced to one CC defined map for each of the three CC defined start arms.
The above analysis was then repeated for the PP2 trial phase, PM1 trial phase, and PM2 trial phase. The above analysis was then repeated for the light start platform, and ball start platform. Thus for one unit all the forty eight maps in one session were reduced to three groups of CC defined start platform maps. Each group contained four maps, one for each trial type and phase combination (PP1, PP2, PM1, and PM2).

O'Keefe (1976) observed that the place units had tonic firing rates of 1 Hz or less outside their place fields. Muller et al (1987) also used 1 Hz as the cutoff value for inclusion of a bin in a place field. The same cutoff was used in the present study. If for a given CC defined start platform the peak firing rate in all four trial type and phase combinations (PP1, PP2, PM1, or PM2) was less than 1 Hz then the spatial firing for that CC defined start platform was deemed to be too weak for further analysis and the data was discarded.

Muller et al (1987) observed that complex spike unit place fields cover approximately 22% of the environment area. However in the present study very diffuse spatial firing was occasionally observed, in some cases possibly due to poor unit isolation. As the present analysis was concerned with dynamic changes in place fields in response to cue removal and not with characterizing diffuse place fields a cutoff was employed. The diffuseness of the firing map was measured by dividing the number bins greater than 1 Hz by the total number of bins. If for a given CC defined start platform the diffuseness value in any one of the four trial type and phase combinations (PP1, PP2, PM1, or PM2) was greater than 0.8 then the spatial firing for that CC defined start platform was deemed to be too diffuse for further analysis and the data was discarded.

If the hypothesis that spatial firing persists after cue removal is correct then PP1, PP2, PM1, and PM2 should all display comparable spatial firing. If the hypothesis that cue removal results in the increase or decrease of spatial firing is correct then PP1, PP2, and PM1 rate maps should display comparable spatial firing and this firing should differ from the PM2 firing. If the hypothesis that the firing rate increases or decreases as a function of time PP1 and PM1 should display comparable spatial firing, PP2 and PM2 should display
comparable spatial firing, and the PP1 and PM1 firing should differ from the PP2 and PM2 firing.

A method was devised to carry out the multiple comparisons necessary to test the five interrelated hypotheses objectively and unambiguously based on spatial subtraction. (Figure 23.) By subtracting two maps a third map can be constructed that embodies the differences between the two maps (Cliff and Ord, 1973; Unwin, 1981). Three cases are illustrated. In the first case if two maps with fields in the same location and of the same magnitude are subtracted the difference map that results is essentially flat. In the second case if two maps having fields in the same location but of different magnitudes are subtracted the difference map that results would have a field in the same location as the original maps but of intermediate magnitude. In the third case if two maps having fields in different locations are subtracted the difference map that results would have two field in different locations, one positive going and the other negative going. The effectiveness of using the integral as a measure of the similarity of two maps was verified by comparing the values of the linear correlation coefficient (Pearson’s r) of two maps to the value of the integral of the resulting subtracted map for the three cases detailed above (see Figure 23). The integral measure was in agreement with the Pearson’s r.

The four averaged maps can be paired off and subtracted a maximum of six possible ways. All six possible subtracted maps were calculated from the four averaged maps. (Figure 24.) The integral of the firing rate was used as a measure of the place field strength for the four averaged maps and six subtracted maps. For the four averaged maps all the bins contained only positive values and thus the integral was calculated in the same manner as in the CC by SBC ANOVA analysis. However in the six subtracted maps the bins could contain both positive or negative values. The purpose of the subtracted maps was to measure the similarity of the two original maps. Low firing rates, be they positive or negative, indicate the two original maps are similar whereas high firing rates indicate the two original maps are different. Since the sign of the firing rate bins in the subtracted maps did not assist in determining whether the two original maps were the same or different the
integral of the subtracted maps was calculated by summing the absolute value of the eighty one bins.

In order to automate the process of rate map comparisons and make it independent of absolute magnitude the four averaged rate maps (PP1, PP2, PM1, and PM2) and six subtracted maps (PP1-PP2, PP1-PM1, PM1-PP2, PP1-PM2, PP2-PM2, and PM1-PM2) were ordered from least to greatest integral value. The five hypothesized firing rate changes in response to cue removal lead to five separate and distinct patterns of ranks for the ten maps. (Figure 25.) Thus classification of the effect of cue removal on the place fields seen in one CC defined start platform could be carried out by matching the pattern of ranks of the four averaged rate maps and six subtracted rate maps with the expected values for the five hypotheses. Thus the analysis was both objective and independent of the absolute magnitude of the peak firing rate.

The classification procedure described above does not yield a statistical measure. In order to glean some idea as to the statistical reliability of the measure a Monte Carlo simulation was employed. The ten ranks were set to random values between one and ten using the long period random number generator "ran2" of Press et al (1992). The pattern of ranks was then matched to the patterns detailed above for groups of firing rate maps that showed persistence of firing after cue removal, increased firing after cue removal, decreased firing after cue removal, increased firing as a function of time, or decreased firing as a function of time. Thus the null hypothesis is defined by exclusion, it is correct for a given group of ten ranks when the group pattern is not in accordance with that expected for persistence of firing after cue removal, increased firing after cue removal, decreased firing after cue removal, increased firing as a function of time, or decreased firing as a function of time.

A million iterations were tested. The result was expressed as the fraction of randomly determined ranks that matched the indicated hypothesis pattern. These were persist: 0.00477; cue dependent increase: 0.00468; cue dependent decrease: 0.00471; time dependent increase: 0.00473; and time dependent decrease: 0.00486. It can be seen that in all cases the probability
of erroneously rejecting the null hypothesis was less than 0.005. Thus although the classification procedure is not based on a formal statistical derivation or known statistical distribution according to the Monte Carlo simulation it is nevertheless fairly resistant to the error of failing to reject the null hypothesis when it is false (type I error). Moreover all the probabilities are essentially equal. The classification procedure is equally likely to assign a random pattern of ranks to one of the five hypotheses. Therefore it is balanced and symmetric.

All the units that the CC by SBC ANOVA analysis determined had spatial firing which was significantly governed by the CC’s or CCxSBC interaction were examined using the analysis described above. Each CC defined start platform was examined separately to determine whether it displayed persistence of firing after cue removal, increased firing after cue removal, decreased firing after cue removal, increased firing as a function of time, or decreased firing as a function of time.

7.3 Results

The CC by SBC ANOVA analysis determined that forty four units had spatial firing that was determined by the CC’s or CCxSBC interaction for some combination of PP1, PP2, PM1, or PM2. The above analysis determined that twenty three out of the forty four units (52%) had place fields that displayed either persistence (Figure 26), cue dependent decrease (Figure 27), or cue dependent increase (Figure 28) for one or more CC defined start platforms after cue removal. (Table IV.) By exclusion the remaining twenty one units did not display one of the five responses tested. No units displayed either a time dependent increase or a time dependent decrease in place field firing after cue removal.

Twelve out of the twenty four units (50%) recorded in CA1 displayed place fields in one or more CC defined start arms as determined by the above analysis. Seven out of these twenty four units (29%) displayed only persistence of firing for one or more CC defined goal arms while the
remainder had more complex combinations of responses as a result of CC removal.

Eleven out of twenty units (55%) recorded in either CA2, CA3, or hilus displayed place fields in one or more CC defined start arms. Four out of these twenty units (20%) displayed only persistence of firing for one or more CC defined goal arms while the remainder had more complex combinations of responses as a result of CC removal.

Fifteen units displayed significant spatial firing on only one CC defined start platform as assessed by the above analysis. Of these nine displayed persistence of firing, three displayed an increase in firing, and three displayed a decrease in firing after cue removal.

Eight units displayed significant spatial firing on two CC defined start platforms as assessed by the above analysis. Three units displayed persistence of firing on one CC defined start platform and a decrease in firing on another CC defined start platform after cue removal. Two units displayed persistence of firing on two CC defined start platforms after cue removal. One unit displayed persistence of firing on one CC defined start platform and an increase in firing on another CC defined start platform after cue removal. One unit displayed an increase in firing on one CC defined start platform and a decrease in firing on another CC defined start platform after cue removal. Lastly one unit displayed a decrease in firing on all three CC defined start platforms. In general for a given unit the change in the place field firing after cue removal seen on two separate CC defined start platforms does not appear to be correlated.

Considering place fields across all units and CC defined start platforms there were seventeen instances of place fields that displayed persistence of firing, five instances of place fields that displayed increases in firing, and ten instances of place fields that displayed decreases in firing after CC removal. Upon visual inspection two units appeared to have displayed more complex changes in place field morphology after cue removal. These units were characterized by two lobed place fields. (Figures 29 and 30.)
The unit in Figure 29 was classified as displaying a decrease in firing after cue removal by the analysis on the ball platform relative to the CC's. However, upon inspection of Figure 29 it can be seen that left lobe appears to be unchanged whereas the right lobe is greatly attenuated after cue removal.

The place fields expressed by the unit in Figure 30 on the ball platform also displayed complex changes in response to cue removal. The upper lobe displayed an increase in firing whereas the firing of the lower lobe was greatly attenuated after CC removal. Since the net firing of the place field was relatively unchanged the unit was erroneously classified as displaying persistence of firing after cue removal by the analysis paradigm. However, although the net firing was unchanged the spatial distribution of the firing was altered by CC removal.

To further investigate the dynamics of place field firing in response to cue manipulations a number of three phase trials were recorded. Three phase trials resembled PM trials in that in the first two minutes the CC's were present and in the second two minutes the CC's were absent. However, after the second phase the cues were replaced within the CCE. A third two minute phase was then recorded. Five three phase trials were recorded in two animals. An example is shown in Figure 31. When the cues were removed the two lobed field of Unit 1 displayed the same change in morphology (lower lobe abolished, upper lobe strengthened) as it did in averaged two phase trials (see Figure 30). When the cues were replaced the place field was restored to its original form. For all the units that displayed place fields whose firing was altered by cue removal the characteristic alteration in place field firing was reversed when the CC's were replaced in all the three phase trials examined.

7.4 Discussion

Place Fields Display Persistence of Firing After Cue Removal

In the present study seventeen out of a total of thirty two place fields were found to display persistence of spatial firing after cue removal. O'Keefe and Speakman (1987a) also found that when the CC's were removed the majority
of the place units recorded would continue to fire in the same spatial position and orientation. O'Keefe and Speakman hypothesized that it was this stored representation of the CC configuration that enabled the rat to choose the goal platform.

Place Fields Display Decreases in Firing After Cue Removal

Ten out of thirty two place fields were classified as displaying a decrease in spatial firing after cue removal. As in the case of units displaying cue dependent increases in spatial firing this effect can not be attributed to time dependent slowing down of unit firing as this would show up as a distinct pattern in the PP1-PP2 subtraction map and the unit would have been classified as displaying a time dependent decrease in firing. No units were classified as displaying a time dependent decrease in firing.

Place Fields Display Increases in Firing After Cue Removal

Five out of thirty two place fields were classified as displaying an increase in spatial firing after cue removal. O'Keefe and Speakman (1987a) noted that for some units the within field firing rate was higher in the cue absent phase of the trial than in the cue present phase. They attributed this to the unit firing having a long latency of onset. However long latency of firing onset can not explain the increases in unit firing concomitant with cue removal that were seen in the present study. Time dependent changes in unit firing would show up in the PP1-PP2 subtraction map. If a distinct pattern of spatial firing had appeared in the PP1-PP2 subtraction map the rank would have been higher than the number permitted by the classification scheme and the unit would not have been classified as displaying a cue dependent increase in firing but rather a time dependent increase in firing. No units were classified as displaying a time dependent increase in firing.

Quantitative Analysis of Place Field Responses to Cue Removal

According to the Monte Carlo simulation the probabilities of classifying a randomly ranked group of maps as displaying either persistence, cue
dependent increase, cue dependent decrease, time dependent increase, or
time dependent decrease in firing are all approximately 0.005. Thus the
analysis paradigm is unbiased. However in the CC by SBC ANOVA analysis
all four trial type and phase combinations (PP1, PP2, PM1, and PM2) were
used as repetitions in the ANOVA matrix. Thus place fields showing
persistence after cue removal (that is, fields in PP1, PP2, PM1, PM2) would
be more likely to yield significant p values than units showing decreases in
firing after cue removal (fields in PP1, PP2, PM1) and units showing
increases in firing after cue removal (fields only in PM2) would be the least
likely of all to be detected by the CC by SBC ANOVA analysis.

Only those units identified by the CC by SBC ANOVA analysis to have
significant CC or CCxSBC governed firing were fed into the analysis of the
effects of cue removal detailed above. Thus the biases inherent in the CC by
SBC ANOVA analysis almost definitely affected the results of the analysis of
the effects of cue removal. Therefore it is not possible to make concrete
claims about the quantitative number of fields that display one of the tested
responses to cue removal.

Effects of Cue Removal are Reversed by Cue Replacement

Five three phase trials were recorded to investigate the effects of replacing the
cues on cue dependent alterations in place unit firing. In all five trials any
alteration, be it an increase or a decrease, in place field firing rate in response
to the removal of the CC's was reversed when the CC's were replaced. Thus
it appears as though alterations in the firing rate of place units after cue
removal in the CCE are controlled by sensory inputs in a very direct and
straightforward manner since replacing the CC's leads to the reversal of the
effect of their removal.

Dynamics of Two Lobed Place Fields

In two instances the place fields recorded on a CC defined start platform
appeared to have two lobes. Moreover removal of the CC's appeared to have
different effects on each of the two lobes. In one instance cue removal did not
affect one lobe while decreasing firing in the second whereas in the second
instance cue removal increased the firing of one lobe and abolished the firing
of another.

It is possible that two separate place units give rise to each of the two lobes.
In that case the two lobed appearance of the field would be an artifact
resulting from erroneous classifying two units as one. However this is unlikely
since the units were recorded on tetrodes, and it is unlikely that two units
could display identical waveforms on all of the four electrode channels.
Moreover evidence from the studies of Muller et al (1987) and Jung and
McNaughton (1993) has demonstrated that in some instances single
hippocampal units can display double fields. Furthermore the recent study of
O'Keefe and Burgess (1996) has demonstrated that unitary fields can be
pulled apart into two separate fields by pulling apart the walls of the
environment. This appears to indicate that single units can give rise to place
fields consisting of two separate components that have a dynamic relationship
with each other.

The fact that the two lobes of double fields can display different firing rate
changes after cue removal is interesting in light of possible mechanisms of
place field formation. It is possible that hippocampal units that display double
fields receive inputs from two units that give rise to single fields. Thus the
double fields may be the union of two single fields and the different alterations
in firing rate in each of the field lobes may be due to different alterations in the
firing rates of the two parent fields. Two units that gave rise to two lobed
place fields were located in CA1 whereas the third was located in the
CA3/hilus. Thus in the first two cases the two "parent" units may have been
afferents from CA3 whereas in the third the "parent" units may have been
located in CA3, dentate gyrus, or possibly entorhinal cortex.

Postulated Mechanisms of Place Field Dynamics

The mechanism that gives rise to changes in place unit firing after cue
removal is unknown. It could be argued that the firing rate changes may be a
result of the perception on the rat's part that the old environment has been
exited and a new one entered. The place units specific to the dark situation are simply coding for a new environment. This appears unlikely as other units recorded concurrently do not change their spatial firing. Alternatively there may be an inhibitory network between the pyramidal neurons that mediates place fields. Recurrent inhibitory loops between pyramidal and basket cells are known to exist in Ammon's Horn (Andersen et al, 1963). Furthermore the axons of basket cells are thought to ramify widely throughout the area they are located in, forming inhibitory synapses on the soma of numerous pyramidal neurons (Sik et al, 1995).

If pyramidal units receive inputs from sensory structures whose activity is modulated by the presence or absence of cues then the removal of these inputs in the cue absent phase of the trial could cause some units to cease firing. These units would display decreases in firing after cue removal. The decrease in unit firing would result in a reduction in excitatory inputs to basket cells, resulting in a reduction of the inhibition on adjacent pyramidal units. These other units would display increases in firing after cue removal. Pyramidal units are known to receive direct or indirect inputs from all the subdivisions of the hippocampal formation as well as extra-hippocampal structures.

Alternatively basket cells may receive inputs from sensory structures with activity that is modulated by the presence or absence of cues. If these inputs modulated basket cells firing then the basket cells would in turn modulate pyramidal unit firing. This could give rise to the cue dependent increases and decreases in spatial firing. Basket cells are known to receive external inputs from the entorhinal cortex via the perforant path (Seress and Ribak, 1985; Schwartzkroin and Mathers, 1978). These inputs may mediate the cue dependent modulation of basket cell firing and thus give rise to increases and decreases in pyramidal unit spatial firing as a result of the removal of the CC's.

There appears to be no topographic organization of responses to cue removal within the hippocampus. Units displaying persistence after CC removal were recorded on the same electrodes as units displaying increases and decreases
in spatial firing. In some cases single units displayed place fields in two
different CC defined start platforms. These fields often displayed different
types of firing rate changes after CC removal. This argues against any sort of
response topography within the hippocampus. The distribution of units
displaying the various responses to CC removal is random, as would be
expected if it arose as a result of a "push pull" network of inhibitory and
excitatory connections.

Place Fields in CA1 and CA2/CA3/Hilus

According to the above analysis 50% of CA1 units and 55% of CA2, CA3, or
hilar units displayed place fields on one or more CC defined start arms. Thus
the probability of observing place fields is slightly higher in the CA3 layer.

In order to compare the relative proportions of units in CA1 and CA2/CA3/hilus
that display one or more of the three observed responses to CC removal a
"simple" category was created. Units that displayed place fields that only
showed persistence of firing after CC removal in one or more CC defined start
arms were grouped together into the "simple" category. Applying this
categorization procedure to the data it was found that 29% of CA1 units were
of the "simple" type whereas 20% of CA2/CA3/hilar units were "simple". Thus
the probability of observing "simple" units is also slightly higher in the CA1
layer.
Chapter 8: Effects of the Direction of Travel on Hippocampal Units
8.1 Rationale

O'Keefe (1976) first reported directional correlates of place unit firing while running rats on an elevated maze. This result was confirmed by numerous others (McNaughton et al, 1983a; O'Keefe and Recce, 1993; Muller et al, 1994; Markus et al, 1995). However the directional specificity in place unit firing may be due to the geometric constraints of the narrow arms and not to any intrinsic place field directionality. Muller et al (1987) found no directional specificity in place unit firing while recording from rats that were confined in a high walled cylinder and chasing scattered food pellets.

Muller et al (1994) compared place fields recorded in a high walled cylinder while the rats chased scattered food pellets and place fields recorded when rats ran on eight arm maze. Using a mathematical model that accounted for directionality due to geometric constraints they found all of the apparent place field directionality in the cylinder could be accounted for by the geometric constraints imposed by the edges of the cylinder. However only some of the place field directionality on the eight arm maze be accounted for by the geometric constraints imposed by the edges of the arms. Thus it appears that when rats move randomly in open fields their place fields do not possess any intrinsic directionality but when they traverse radial arm mazes in stereotypical paths that are constrained by the arms of the maze their place fields do possess intrinsic directionality.

Markus et al (1995) also found that place units display directionality when recorded on a radial arm maze and moreover that they displayed little to no directionality when recorded in an open field. However they observed that shaping a rat to traverse a circular open field between reward sites in a stereotypical and invariant manner caused place field to show directionality. Thus it is possible that the intrinsic place field directionality observed by Muller et al (1994) and others was not dependent on the geometry of the environment but rather the rat's behavior. Place field directionality appears to result when a rat traverses an environment in a stereotypical and invariant manner either as a result of geometric constrains or behavioral shaping.
One major difference between the CCE employed by O'Keefe and Speakman (1987a) and the one employed in the present study is the arm geometry. The CCE of O'Keefe and Speakman (1987a) had narrow arms either 5.5 cm wide and 61 cm long or 15 cm wide and 38 cm long. The evidence from previous studies in which place fields were recorded on narrow arms would suggest that the fields observed by O'Keefe and Speakman (1987a) had some directional specificity. The published paper does not make any mention of place field directionality although some fields did indeed display directional specific firing (O'Keefe, personal communication).

In the present study the arms were replaced by square platforms with 54.5 cm sides. The results of Muller et al (1987, 1994) in which place fields were recorded in open field environments would suggest that fields observed on the CCE platforms would not display significant directionality. However the rats were made to follow a baited forceps in an invariant stereotypical fashion. (Figure 10.) Therefore according to the results of Markus et al (1995) the place fields recorded in the present study would be expected to show directionality.

The data from the current study were analyzed in order to determine whether Ammon's Horn pyramidal units that demonstrate place specific firing governed by the CC's showed directionally specific firing.

8.2 Analysis

The CC by SBC ANOVA determined that for place units in which significant spatial firing occurred the spatial firing was governed by the CC's or the CCxSBC interaction. Therefore directionality was assessed with respect to the CC's. The CC orientation with the goal in the NE with respect to the SBC was selected as the standard. (Figure 32.)

The platform coverage paradigm ensured that the rat ran in the 0° direction (towards the cage) and the 90° (towards the card) in alternation approximately six times, followed by the 180° direction (towards the ball) and the 270° direction (towards the towel) in alternation approximately six times, whereupon
the pattern would be repeated for the duration of the trial. (Figure 10.) This restricted movement constrained the number of possible directional analysis procedures that could be employed. It was clearly inappropriate to calculate the directionality for a full 360° as there would inevitably be four peaks corresponding to the platform coverage paradigm's four directions of travel.

All the position data was rotated about the center of the CCE such that the CC's were in register as in the cue removal analyses. Therefore all directions were with respect to the CC's. Then for each unit the spike data was partitioned into four groups based on the direction the rat was travelling when the spike occurred. For the four groups the permitted range of direction of travel was from 315° to 45°, 45° to 135°, 135° to 225°, and 225° to 315° respectively. Thus each group corresponding to a 90° wedge centered on one of the four directions of the platform coverage paradigm. For brevity these were referred to as up, right, down, and left, respectively.

For each trial type and phase combination (PP1, PP2, PM1, and PM2) four separate nine by nine bin firing rate maps were calculated in the same manner as in the analysis of the effects of cue removal, with the one change being the data was first divided into four directionally specific groups. For each directionally specific group the spike count and dwell time data was binned. The values of the spike count bins were divided by the dwell time bins to yield a bin firing rate. The binned rates were then smoothed. For each CC defined start arm the four SBC defined start platform firing rate maps were averaged. Thus the session data of a given unit was initially split into four non-overlapping directionally specific groups. Each of the four directionally specific unit data sets was then reduced to four directionally specific firing rate maps, one for each trial type and phase combination. For each of the four directionally specific firing rate maps four integral values were calculated, one for each trial type and phase combination.

A one way ANOVA was performed on the travel direction integral values. The only factor was travel direction. Since it is known from the results of the analysis of the effects of cue removal that some units display increases or decreases in spatial firing after cue removal only the first three out of the four
trial type and phase combinations (PP1, PP2, and PM1) were used as repetitions in the ANOVA. The last trial type and phase combination (PM2) in which the cues were absent was discarded, only trial type and phase combinations in which the cues were present were considered by the directional analysis. Thus the variance due to alterations in place field firing as a result of cue removal was eliminated. However by using only the first three out of the four trial type and phase combinations the analysis was biased towards detecting directionality in units with place fields that showed persistence or decreases in firing after cue removal at the expense of those showing increases in firing.

In common with the analysis of the effects of cue removal the data to be fed into the directionality analysis was checked against a cutoff minimum firing rate before the one way ANOVA was calculated. Since the unit data was partitioned into four directionally specified groups the possibility of their being too few samples in a given bin leading to spurious high firing rates was increased. Therefore the rate cutoff was raised from 1 Hz to 3 Hz. If for a given CC defined start platform all four directionally specific firing rate maps (up, down, left, and right) by three trial type and phase combinations (PP1, PP2, and PM1) had peak firing rates that were never in excess of 3 Hz then the firing in that CC defined start platform was deemed to be too weak for further analysis and the data was discarded. The PM2 trial type and phase combination was not considered.

If the one way ANOVA was significant at the 5% level then post-hoc Scheffé tests were performed (Keppel, 1982). For each travel direction the mean of the integral values for the first three trial type and phase combinations (PP1, PP2, and PM1) was computed. In order to determine if any of the directions of travel produced significantly higher firing rates six comparisons based on the mean integral values were tested. (Figure 33.) In the first four comparisons the mean integral value of one direction was compared to the averaged mean integral values of the other three directions. In the fifth comparison the mean integral values of the two adjacent directions above a positive 45° line were compared to those below the line. In the sixth
comparison the mean integral values of the two adjacent directions above a negative 45° line were compared to those below the line.

The resulting six Scheffé F scores were tabulated and the greatest one selected. If it was not significant at the 5% level then the unit firing was deemed not to have displayed directionality. If the greatest Scheffé F score was significant at the 5% level or less the comparison of mean integral values that generated it was examined.

If the greatest Scheffé F score corresponded to the first comparison then the integral value of the up wedge was compared to the mean integral value of the three other three wedges. If the first value was greater than the second then the maximum unit firing was deemed to have occurred when the rat travelled in the up direction. If the first value was less than the second then maximum unit firing was deemed to have occurred when the rat travelled in the left, down, and right directions. The analogous calculations were performed if the greatest Scheffé F score corresponded to the second, third, or fourth comparisons.

If the greatest Scheffé F score corresponded to the fifth combination then the mean integral values of the two wedges above the positive 45° line (left and up) were averaged and compared to the average of the two below the line (right and down). If the first average was greater than the second then the maximum unit firing was deemed to have occurred when the rat ran left and up relative to the CC's. If the second average was the greater then the maximum unit firing was deemed to have occurred when the rat travelled right and down relative to the CC's. Similarly if the highest Scheffé F score corresponded to the sixth combination then the mean integral values of the two wedges above the negative 45° line (up and right) were averaged and compared to the average of the two below the line (down and left) to determine whether maximum unit firing occurred when the rat travelled up and left or down and right.
The above analysis was also carried out with the position data rotated such that the SBC's were in register, that is, all directions were with respect to the SBC's. No significant place field directionality was found. This data is not shown.

8.3 Results

Sixteen out of the forty four units recorded (36%) displayed firing that was significantly directional as assessed by the one way ANOVA and post-hoc Scheffé tests. (Table V.) An example of a unit displaying directionally specific firing on one CC defined start platform is shown in Figure 34.

Eleven out of the twenty four units (46%) recorded in CA1 displayed firing that was significantly directional. Five out of the twenty units (25%) recorded in CA2/CA3/hilus displayed firing that was significantly directional.

Twelve units displayed directionality on only one CC defined start platform, three units displayed directionality on two CC defined start platforms, and only one unit displayed directionality on all three CC defined start platforms. For three out of the four units that displayed directionality on more than one CC defined start platform the directionality appeared to be correlated such that one wedge was common to all the start platforms. For example, the unit that displayed on all three start platforms displayed maximal firing in the right wedge on the fan platform, light platform, and ball platform. (See Table V.)

Across all sixteen directional units there were a total of twenty one CC defined start platforms on which the unit firing displayed significant directionality. Out of the twenty one CC defined start platforms on which the unit firing displayed significant directionality sixteen displayed maximal firing for one 90° wedge (e.g. Figure 34), four displayed maximal firing for two adjacent 90° wedges (e.g. Figure 35), and only one displayed maximal firing for three adjacent 90° wedges. Thus across all CC defined start platforms on which the unit firing displayed significant directionality there were a total of twenty seven 90° wedges in which units showed maximal firing.
The number of up, down, left, and right directional wedges was not uniform. Out of the twenty seven directional wedges in which units showed maximal firing there were thirteen right wedges (48%), seven left wedges (26%), five down wedges (19%), and only two up wedges (7%).

The interaction between place field location as assessed by the analysis of the effects of cue removal and the place field directionality as assessed by the directionality analysis was examined by combining the information in Tables IV and V to form Table VI. From Table VI it can be seen that seventeen CC defined start platforms displayed place fields that persisted after cue removal, ten displayed place fields that decreased in firing rate after cue removal, and five displayed place fields that increased in firing rate after cue removal. Moreover out of the seventeen persisting place fields nine displayed directionality (53%), out of the ten decreasing place fields three displayed directionality (33%), and out of the five increasing place fields one displayed directionality (20%).

Two units that displayed persistence of firing after cue removal and significant directionality are shown in Figures 34 and 35. Upon inspection of Figures 34 and 35 it can be seen that the directional specificity of the place field firing remained unchanged after cue removal. In Figure 34 the PM2 firing rate map is comparable to the PP1, PP2, and PM1 firing rate maps in the wedge that displayed maximal firing and similarly for Figure 35.

The directional specificity of the units observed in the present study did not appear to be topographically organized within the hippocampus. Units recorded on the same tetrode bundle showed maximal firing for different directions.
8.4 Discussion

Place Fields Display Directionality in the CCE Spatial Memory Task

Previous work has shown that place fields show directionality when a rat traversed an area in a restricted stereotypical manner (McNaughton et al, 1983a; Marcus et al, 1995). Rats trained on the CCE spatial memory task were constrained to run in a stereotypical raster pattern to insure uniform platform coverage. The place fields recorded in this situation were found to display directionality. This in itself is not a novel finding. However the use of a CCE enables one to investigate the interaction between directionality and place field dynamics in response to cue removal as well as the cue dependence of directionality. These issues are discussed below.

Directionality Persists after Cue Removal

Units on CC defined start arms that displayed directionality and that had place fields make it possible to determine the effect of the cue removal on directionality. In all cases the directionality of the place fields appeared unaffected by cue removal. Place field directionality appears to be initially set by the CC's but is not dependent on them.

Place field directionality can exist in association with all three observed place field rate changes in response to cue removal. The relative number of directional place fields displaying persisting, decreasing, and increasing firing rates after cue removal (53%, 30%, and 20%) differ. However since only the first three trial type and phase combinations (PP1, PP2, and PM1) were used in the calculation of the one way ANOVA the actual quantitative values are likely biased in favor of fields showing persistence or decreases in firing rate in response to cue removal at the expense of fields showing increases in firing rate. This may account for all the variation in the three percentages. Thus fields displaying directionality are equally likely to display persistence, increases, or decreases in firing rate in response to cue removal.
Peak Directional Firing Occurs in Multiple Adjacent Directions

Muller et al (1994) have suggested that the place field directionality observed on radial arm mazes is orientated with respect to the long axis of the arms. Similarly Markus et al (1995) observed directionality in goal directed traversal of an open field that was specific for one of two possible traversal directions. In the present study there were four possible traversal directions through place fields corresponding to the four directions the rat was constrained to travel in by the coverage paradigm. The majority of the units observed showed maximal firing for one 90° wedge centered on one of these four directions. This follows from the results of Muller et al (1994) and Markus et al (1995) in which a greater firing rate was observed when the rat traversed a place field in one of two possible directions. However a minority of units showed maximal firing in two adjacent 90° wedges. It is possible that the preferred direction of these units is not limited to the horizontal or vertical traversal directions of the platform coverage paradigm planes but rather lies somewhere in between, in a direction in which the rat did not run. One unit showed directionality for three adjacent 90° wedges on one CC defined start arm. In this case it is possible that directional specificity was coded for by low firing rate as opposed to a high firing rate. The unit is conveying information about the environment configuration by firing less rather than more.

Peak Direction of Unit Firing is not Uniformly Distributed

Out of twenty one CC defined start platforms displaying directionality twenty six directional wedges were observed. This is because unit firing in some start platforms displayed significantly higher rates for more than one 90° wedge. Out of the twenty six directional wedges 48% were specific for the right direction, 26% were specific for the left direction, 19% were specific for the down direction, and 7% were specific for the up direction relative to the CC's. The percentage of right wedges is fully half of the total number, whereas the percentage of left wedges is one quarter. Thus it appears the units fired more vigorously when the rat ran right or left with respect to the CC's in comparison to when it ran up or down. Upon inspection of Table VI it
appears the bias towards right and left directionality is found in all three animals.

The explanation for the non-uniform distribution of directionality is unknown. It is possibly due to the fact that the CC's in the CCE are not symmetrically distributed. (See Figure 9.) If one or more of the CC's are stronger than the others the resulting inhomogeneity in cue strength for different angular positions in the CCE may cause inhomogeneity in the distribution of peak firing rate directions.

Unit Directionality May be Correlated Across Start Platforms

Four units displayed directionality for more than one CC defined start platform. In three out of four of these cases there was partial overlap between the preferred direction of the two or three CC defined start platforms. For example in one unit that firing on the fan platform displayed was maximal in the right direction whereas on the ball platform it was maximal in the right and down directions. This it appears that in the rare instances in which units display directionality in more than one start platform this directionality may be correlated.

Directionality is not Due to Geometric Constraints

In the present analysis no effort was made to separate the unit directionality due to the geometric constraints of the environment from any true directionality. Therefore directionality observed in place fields located near the platform edges may have been due to constraints in the rat’s movements due to these edges. However this could not have occurred if the place field was located in the center of a platform. A number of place fields located approximately in the center of a platform displayed clear directionality (e.g. Figure 34). Thus it can be concluded that for at least some of the CC defined start platforms in which directionality was detected this directionality was not due to geometric constraints.
Directionality is not Due to Behavioral Constraints

In addition to mechanical constraints the appearance of place field directionality could result from behavioral regularity. This possibility was controlled for by only examining the four directions in which the rat was constrained to traverse via the coverage paradigm. Despite limiting the analysis to these four directions, significant directional firing was observed for some units.

Postulated Mechanisms of Place Field Directionality

Sharp (1991) presented a model of place unit formation in which inputs to the simulated units contain both distance and direction information. Input units are associated with each other on the basis of similarity in "winner take all" groups. As units are associated with each other on the basis of the similarity of the cue views, adjacent areas of the environment come to be coded for by single units. The simulated place fields did not display directionality when training was conducted with a "rat" traversing an environment in a pseudo-random fashion. However when the model was trained on a simulated radial arm maze in which a "rat" traversed an environment in a linear bi-directional fashion the resulting place fields displayed directionality. It appears that when a model "winner take all" algorithm has access to only a limited subset of distance and direction cues the result is unit directional specificity. An analogous process may be responsible for the production of directional place fields in rats that are constrained or shaped to run in a stereotypical fashion on an open field.

Relative Directionality of CA1 and CA2/CA3/Hilar Units

In CA1 46% of the units displayed directional firing whereas only 25% of the units recorded in CA2/CA3/hilus displayed directionality. Thus it appears the probability of observing directionality in CA1 units is approximately two fold higher in CA1 than in CA2, CA3, or the hilus.
Chapter 9: Examination of Spatial Firing Seen During Error Trials
9.1 Rationale

O'Keefe and Speakman (1987a) ran control trials in which the CC's were removed before the rat was placed in the environment. That is, a goal arm was defined relative to the CC's as normal but the rat had no access to the CC information. This goal arm could be termed the experimenter's goal as it is defined by and known to the experimenter but not the rat. As expected the rat performed at chance levels in this control condition, selecting the experimenter's goal in approximately one third of the trials. The arm the rat selected could be termed the rat's goal since insofar as the rat's behavior is concerned it is the goal.

O'Keefe and Speakman observed that the spatial firing of hippocampal place units in control trials in which the rat failed to chose the experimenter's goal (error trials) was not the same as that seen in normal trials in which the rat chose the experimenter's goal (correct trials). However they found that if the place fields seen in error trials were rotated about the center of the environment such that the rat's goal was superimposed upon the experimenter's goal the error trial place field constellation matched that of the correct trials. The spatial firing displayed by the rat's hippocampus during error trials was consistent with the spatial firing seen when it ran in correct trials, the only difference being that the place fields were rotated in the same direction and by the same amount as the rotation between the experimenter's goal and the rat's goal. Thus the hippocampal place field firing could be used to predicted the choice the rat made, regardless of whether it was correct or not.

No cue absent control trials were run in the present study. Nevertheless in a minority of trials run the rat chose a non-goal platform. These error trials are analogous to the cue absent control trials of O'Keefe and Speakman (1987a) in that there exists two distinct goals, the experimenter's goal as defined by the CC's and the rat's goal defined by the platform the rat selected.

It is possible that during the error trials in the present study the hippocampal place units recorded demonstrate the same alterations in their place field
constellation as seen by O'Keefe and Speakman (1987a). That is, the fields seen in error trials are identical to those seen in correct trials when rotated by the angle between the rat's goal and experimenter's goal. In this case it could be said that the fields were consistent with the "rat's" goal.

Alternately the hippocampal units recorded in the present study may display different alterations in their place field constellation as compared to those observed by O'Keefe and Speakman (1987a). In the present study the cues are present in either one (in PM trials) or both (in PP trials) phases of the error trials, unlike the case in O'Keefe and Speakman in which the cues were not present at any point. The presence of the cues in one or both phases of the error trials in the present study may cause the hippocampal place fields to be identical in all respects to that seen in correct trials. In this case it could be said that the fields were consistent with the "experimenter's" goal.

Lastly the hippocampal units recorded in the present study may have displayed alterations in their place fields that were not consistent with the spatial firing seen in correct trials, regardless of orientation. The place fields may move to completely random locations or the units may cease displaying spatial firing altogether. In this case it could be said that the fields were not consistent either the rat's goal nor the experimenter's goal.

This analysis was designed to investigate whether hippocampal spatial firing recorded during individual error trials were consistent with the rat's goal, the experimenter's goal, or neither the rat's goal nor the experimenter's goal.

9.2 Analysis

Ideally CC by SBC ANOVA analysis would have been applied to the error trial data in order to first determine whether spatial firing was present and whether it was governed by the CC's or SBC's. However there were too few error trials to construct a balanced ANOVA. In order to analyze the data it was necessary to assume that any spatial firing shown by the units during error trials was governed by the CC's.
Each session was assessed for units that gave rise to strong clear place fields governed by the CC's via the CC by SBC ANOVA analysis and the analysis of the effects of cue removal. Only those error trials that occurred in sessions with an adequate baseline of units displaying clear place fields (minimum of two) were examined. The unit firing in the error trial was compared to that seen in the averaged correct trials in order to determine if it matched the pattern expected if the firing was consistent with the rat's goal, the experimenter's goal, or neither the rat's goal nor the experimenter's goal.

Each error trial was examined separately. For those units shown by the analysis of the effects of cue removal to display place fields the error trial data was used to construct a firing rate map in the same manner as in the analysis and the analysis of the effects of cue removal. Two maps were generated, one for each phase. PP error trials would yield a PP1 and a PP2 firing rate maps and PM error trials would yield a PM1 and a PM2 firing rate map. Thus the two maps could be examined separately to see whether the firing in either the first, second, or both first and second phases matched that seen in correct trials.

The three CC defined start platform firing rate maps were calculated from all the correct trials in the session in the same manner as in the analysis of the effects of cue removal to yield the four firing rate maps, one for each trial type and phase combination (PP1, PP2, PM1, and PM2).

The hypothesis that the error trial rate map was consistent with the experimenter's goal was tested by simply plotting the error trial rate map in the orientation it was recorded and comparing it by eye with the corresponding averaged firing rate map calculated from all the correct trials in the session.

The hypothesis that the error trial unit firing was consistent with the rat's goal was tested by rotating the error trial firing rate map such that the rat's goal and experimenter's goal were superimposed. Thus the start platform of the error trial was effectively changed. For example, if the rat was started from the fan platform and went to the light platform instead of the goal platform the firing rate map was rotated -90° about the center of the environment. This
would have the effect of re-positioning the firing rate map on the light platform. If this rotated situation were viewed from above during the actual trial the rat would appear to be choosing the correct goal platform. The rotated map was then simply compared by eye with the corresponding averaged firing rate map calculated from all the correct trials in the session.

The firing of all the units in one error trial was graphically summarized by plotting all relevant maps together on one figure (e.g. Figures 36, 37, and 38). The error trial rate map oriented by the experimenter's goal was plotted on the left. The error trial rate map oriented by the rat's goal was plotted on the right. The three CC defined averaged firing rate maps calculated from all the correct trials in the session were plotted in the middle. An arc was drawn between the error trial rate map oriented by the rat's goal and its corresponding averaged correct rate map as a visual aid to assist in comparing the two maps. Another arc was drawn between the error trial rate map oriented by the experimenter's goal and its corresponding averaged correct rate map.

For a given session all the units with significant place field firing were plotted on successive rows of the corresponding figure. The resulting figure was assessed by eye to determine whether the unit firing in the error trial plotted with respect to the experimenter's goal, rat's goal, or neither the experimenter's goal nor the rat's goal resembled that seen in correct trials.

9.3 Results

Across all three rats twenty four error trials were recorded in eight out of the ten sessions. Of these nine were recorded in sessions in which only one unit was shown upon subsequent analysis to display a clear place field. Since one unit was deemed to yield an inadequate amount of information about the place field constellation of correct trials these trials were not analyzed further. Of the remaining fifteen eight were recorded during PP trials and seven were recorded during PM trials. (Table VII.)

Nine out of the fifteen error trials were classified as displaying firing that failed to resemble that seen in correct trials regardless of orientation (see Figure 36).
Three were classified as displaying spatial firing that when oriented by the rat's goal resembled that seen in correct trials (see Figure 37). All three of these were PP type trials. Three were classified as displaying spatial firing that when oriented by the experimenter's goal resembled that seen in correct trials (see Figure 38). All three of these were PM type trials.

9.4 Discussion

Hippocampal Error Trial Firing Can Predict the Rat's Choice

O'Keefe and Speakman (1987a) found that hippocampal place fields recorded during error trials could predict the choice of the rat. During the trial the rat's hippocampus displayed place field firing consistent with a maze arm that was not the goal arm as defined by the CC's. When the central platform was raised the rat would select this predicted arm. Both the hippocampal firing and the eventual choice behavior were independent of the sensory inputs provided by the CC's. Moreover the hippocampal firing could be used to predict the eventual choice of the rat.

In the present study three error trials displayed spatial firing that when oriented by the rat's goal resembled that seen in correct trials. In these trials that hippocampal firing is in accordance with the choice the rat made and not the position of the CC's defining the experimenter's goal. Moreover if the unit firing had been observed as the trial was being run it would have be possible to predict the erroneous choice the rat was going to make. Thus these four error trials are in accordance with the previous results of O'Keefe and Speakman.

All three of the error trials with "rat's goal" spatial firing were of the PP trial type. Thus despite having access to the cues for the entire trial the rat's hippocampus was nevertheless able to display firing which was inconsistent with the CC configuration.
Hippocampal Error Trial Firing Can Reflect the CC's

Three error trials displayed spatial firing that when oriented by the experimenter's goal resembled that seen in correct trials. The error trial place field constellation was in accordance with the experimenter's goal, as in the correct trials. Nevertheless, the rat chose a non-goal platform. Thus it could be said that the rat "knew" in some sense on which platform it was on and by extension where the goal platform was and yet failed to go to the goal platform when the center was raised. It appears that in some instances the CC's appear capable of controlling place field firing in the normal manner and yet the eventual choice made by the rat is erroneous. The hippocampal firing and the eventual choice behavior are not in agreement.

O'Keefe and Speakman (1987a) did not report observing error trials in which the spatial firing oriented by the experimenter's goal resembled that seen in correct trials. However, in the trials they examined the cues were removed before placing the rat in the environment. The rat never had access to the CC's defining the experimenter's goal. Therefore, it appears less likely to observe place fields oriented with respect to the experimenter's goal as the goal defining CC's were absent throughout the entire trial.

All three of the error trials with "experimenter's goal" spatial firing were of the PM trial type. Thus during the first two minute phase of the trial the rat had access to the cues and its hippocampus displayed firing which was consistent with these cues. Moreover, after cue removal the place fields recorded during the error trial displayed the same alterations in firing rate as in correct trials. Nevertheless, the rat still chose an incorrect goal platform, despite having the "correct" hippocampal representation of the CC's.
Hippocampal Error Trial Firing May Reflect a Separate Environment

In the majority of error trials (nine out of the fifteen) the spatial firing oriented by both the experimenter's goal and rat's goal does not resemble that seen in correct trials. O'Keefe and Speakman (1987a) did not report whether they observed error trials in which the firing did not resemble that seen in correct trials regardless of orientation.

Numerous studies have demonstrated that a given hippocampal unit can participate in the representation of different environments by firing in a different location in each environment (O'Keefe and Conway, 1978; Muller and Kubie, 1987; Wilson and McNaughton, 1993). The same unit can participate in the representation of multiple environments. It is possible that in those instances in which error trial unit firing was unrelated to that seen in correct trials the rat failed to realize it was in the CCE. Consequently the hippocampus coded for a complete separate environment. The firing seen in the error trial may be very spatially specific, robustly coding for an environment. However the environment it is coding for is not the one the rat is currently in.

Correlation Between Trial Type and Error Trial Firing

All three of the error trials with "rat's goal" spatial firing were of the PP trial type. Conversely all three of the error trials with "experimenter's goal" spatial firing were of the PM trial type. However the small sample size is small and classification was performed by eye. Thus too much importance can not be ascribed to the observed correlation.
Chapter 10: Conclusions
10.1 Summary of Findings

Place Field Dynamics in a Spatial Memory Task

Units were observed in the hippocampus that display place fields in the CCE spatial memory task. When the CC's were removed in the presence of the rat the majority of these place units continued to fire with the same intensity and in the same location. The presence of units in the hippocampus that continue to fire upon CC removal is in complete agreement with the main result of the study of O'Keefe and Speakman (1987a).

The main novel result of the present study is the observation of place field dynamics in a spatial memory task. Place fields were observed that increased or decreased firing after removal of the CC's. This finding has important implications for models of hippocampal function and place field formation.

Rotation of cues in the CCE was employed in an effort to eliminate the effects of external environmental cues on place field firing. By rotation it was hoped that the information content of the external environmental cues would be rendered meaningless. Thus the external environmental cues would effectively be eliminated. The fact that the all the place units recorded in the present study were responsive to the CC's suggests this strategy was effective.

Since all the units were responsive to the CC's, their removal deletes the majority of the cues that could potentially polarize the environment. The presence of hippocampal units that continue to fire in the same place specific manner after removal of the CC's was expected on the basis of previous research in the CCE spatial memory task. The observation that some place units increase or decrease their firing after cue removal was unexpected.

The units that decreased or increased firing after cue removal appear to signal both spatial location by virtue of their localized place specific firing and also the presence or absence of spatial cues by virtue of the changes in their firing rate. Units displaying place fields that decreased firing rate after cue removal
to the extent that they virtually "turned off" can be thought of as pure perceptual units, operational only when the spatial situation included a perceptual component. Conversely, units that increased firing rate after cue removal to the extent that they virtually "turned on" can be thought of as pure memory units, operational only when the spatial situation includes a memory component. Lastly units displaying place fields both before and after the cues are removed can be thought of as perceptual-memory units.

A scheme can be envisioned whereby the three populations of hippocampal units co-operate to produce spatial memory. Perceptual units may process sensory inputs and set up the initial spatial map in the hippocampus. Memory units may utilize the output of perceptual units to set up a representation of the cue configuration that is suppressed as long as the cues are present but that endures and is expressed after they are removed. Perceptual-memory units may be responsible for integrating the output of the perceptual and memory units. Motor and other downstream systems may preferentially read the output of the perceptual units in order to determine the location of the rat regardless of the presence or absence of sensory cues and plan appropriate behaviors. However this account is likely an oversimplification as many units failed to display pure "turn on" or "turn off" responses but rather displayed graded alterations in firing rate after cue removal. Moreover two units displayed two lobed fields, the two lobes of which displayed different alterations in firing rate after cue removal.

**Place Field Directionality in a Spatial Memory Task**

In the present study the rats were constrained to travel in specific directions on the start platform via the coverage paradigm. The place fields recorded on the start platform displayed directionality. The majority of directional units also displayed persistence of firing after the cues were removed. Upon inspection of the directional firing rate maps it was found that the directional specificity of these units was also maintained in the absence of the CC's.

The observation of place field directionality in an open field environment when rats are shaped to travel in a stereotypical fashion is in itself not a novel
result. Markus et al (1995) observed place field directionality when a rat traversed an open field from one reward site to another in an open field. The observation that place field directionality can be maintained in the absence of spatial cues is a novel finding that could only have been observed in a spatial memory task. Thus for units showing persistence of firing after cue removal the place field location and place field directionality appear to share the property of cue independence. Once the location and directionality of a place field is set up by a cue set, removal of the cue set does not affect either property of the place field firing. The unit retains a record of its preferred location and directionality.

Place Fields and Error Trials

In the present study it was found that place field firing can both predict and fail to predict the rat's choice on different trials. This is in contrast to the results of O'Keefe and Speakman (1987a) who only reported control trials in which recorded place fields always predicted the rat's choice.

If the hippocampus was a straightforward sensory processor it would simply reflect the current cue configuration regardless of the eventual choice behavior. However this was not observed to always be the case. In three trials the sensory configuration of CC's and the hippocampal firing were not in agreement.

Moreover if the hippocampus was a straightforward motor system that stored the eventual choice behavior to be displayed the firing it displayed would invariable correlate with the eventual choice made. This was also not observed. In three trials the hippocampal firing was in agreement with the spatial configuration of CC's as seen in the averaged correct trials and yet the rat nevertheless made an erroneous choice.

The fact that in some cases the hippocampal fails to reflect the sensory inputs and in others it fails to reflect the behavioral outputs may be taken as evidence that the hippocampus processes abstract information rather then simply responding to sensory cues or processing motor outputs.
Differences in Spatial Firing Between CA1 and CA2/CA3/Hilus

In the present study 51% of the units in the CA1 layer and 48% percent of the units located in CA2/CA3/hilus displayed CC or CCxSBC governed spatial firing. Thus according to the CC by SBC ANOVA analysis essentially the same proportion of CA1 and CA2/CA3/hilar units displayed CC or CCxSBC governed firing.

The mean percentage of variance accounted for by the CC factor was 51% for the CA1 units whereas for CA2/CA3/hilar units it was 31%. Thus a larger proportion of the variance was accounted for by the CC factor for CA1 units as compared to CA2/CA3/hilar units. It can be said that the CA1 units more accurately reflected the CC configuration that CA2/CA3/hilar units, that is, they contained more information about the CC's.

According to the analysis of the effects of cue removal 50% of CA1 units and 55% of CA2, CA3, or hilar units displayed place fields for one or more CC defined start arms. These two values are essentially the same. Thus according to this analysis essentially the same proportion of CA1 units and CA2/CA3/hilar units display place fields. This is in agreement with the results of the CC by SBC ANOVA analysis.

According to the analysis of the effects of cue removal 29% of CA1 units were of the "simple" type (only displaying persistence on one or more CC defined start arm) whereas 20% of CA2/CA3/hilar units were "simple". Thus according to the analysis of the effects of cue removal a slightly higher proportion of CA1 units displayed place fields and the CA1 fields were less likely to display dynamic changes in response to cue removal.

The CC by SBC ANOVA analysis and the analysis of the effects of cue removal examine different but related aspects of location specific spatial firing. The CC by SBC ANOVA analysis is design to determine whether units fire in response to the CC's, SBC's, or CCxSBC interaction. The analysis of the effects of cue removal endeavors to determine whether a given unit displays dynamic changes in firing rate after cue removal. Both analyses suggest that
the same proportion of CA1 and CA2/CA3/hilar units display place fields. However both analyses also suggest the CA1 units display firing which is both more closely tied to the CC configuration but less affected by CC removal.

The connections in the hippocampus are predominantly unidirectional. Thus units in CA2/CA3/hilar area project to those in the CA1 layer via the third and last synapse of the trisynaptic pathway. This has led some workers to postulate that a crude spatial map is formed by the first synaptic levels of the trisynaptic pathway and is subsequently refined by subsequent levels (McNaughton and Nadel, 1990; O’Keefe, 1990).

The results of the present study suggest the CA1 units display firing which is both more closely tied to the CC configuration but less affected by CC removal. This can be taken as evidence that a relatively cruder spatial map exists in CA2/CA3/hilus that is progressively refined as it is processed by the feedforward connections of the trisynaptic pathway. The map in CA1 is more closely tied to the CC’s than that in CA2/CA3/hilus. It is also more stable than the map in CA2/CA3/hilus, being relatively less affected by CC removal.

In CA1 46% of the units displayed directional firing whereas only 25% of the units recorded in CA2/CA3/hilus displayed directionality. Thus it appears the probability of observing directionality in CA1 units is approximately two fold higher in CA1 than in CA2, CA3, or the hilus. The postulate that a relatively cruder spatial map exists in CA2/CA3/hilus that is progressively refined as it passes to the CA1 layer is either supported or weakened depending on how one views directionality in the context of "crude" and "refined" spatial firing. If the directionality of place fields is considered to be an additional layer of information then the postulate that spatial information is refined as it travels from the CA2/CA3/hilar area to CA1 is supported. However if one holds the view that the most refined place fields are those that fire purely in response to a particular location and are insensitive to other spatial parameters such as direction of travel then the postulate is weakened.
10.2 Implications for the Spatial Map Hypothesis

The CCE spatial memory task was expressly designed to test the spatial map hypothesis. As proposed by O'Keefe and Nadel (1978) and O'Keefe (1990, 1991, 1993) the hippocampal spatial map is based on the computation of a "centroid" defined by the geometric center of the cues within an environment and an "eccentricity" defined by direction of the vector average of all pairs of cues. The centroid and eccentricity are fixed and unvarying as long as the cues remain stationary. The rat is postulated to calculate the environment centroid and eccentricity and to use this information to construct an allocentric spatial map that it then uses to navigate through the environment.

The place field firing of Ammon's Horn, entorhinal cortex, and subicular formation units, the head direction firing of postsubicular units, and the movement firing of Ammon's Horn theta units are postulated to be electrophysiological manifestation of the spatial map located within the hippocampal formation (O'Keefe, 1990; O'Keefe, 1991; O'Keefe, 1993).

According to the spatial map hypothesis the centroid is located in the center of the CCE and the eccentricity is in a fixed direction. When the controlled cues are rotated the eccentricity is rotated by the same amount but the location of the centroid remains unchanged. The majority of the place fields recorded in the CCE were found to be maximally sensitive to the CC's. Thus it can be assumed that all the polarizing cues the rat attends to are the CC's. Thus rotation of the CC's leads to rotation of the CCE place fields.

According to the spatial map hypothesis, place fields are the electrophysiological manifestation of the relationship between the rat's current and the environment centroid and eccentricity. Thus rotation of the eccentricity by rotating the CC's should lead to concomitant rotation of the place fields. The observation that place fields actually rotate upon rotation of the CC's is compatible with the spatial map hypothesis, although not limited to it.
Whereas the spatial map hypothesis provides an account of how the rat hippocampus could use environmental cues to create a spatial map it does not specify any mechanism by which this firing could be maintained in the absence of all the environment cues. However it is possible that removal of the CC’s in the CCE does not constitute removal of all the environment cues. Evidence points to hippocampal place fields being sensitive to environment outlines as well as the cues within an environment. Muller and Kubie (1987) found that dilating an environment led to a concomitant increase in the size of place fields recorded within the environment. O’Keefe and Burgess (1996) found that place fields could be stretched in either the horizontal or vertical plane by moving the walls of a rectangular enclosure. Thus in the CCE it is possible that after the removal of the CC’s the rat may still be attending to the platform edges and the environment outline. According to the spatial map hypothesis individual place fields can be calculated using a fraction of the cues within an environment. Therefore removal of a subset of the environment cues should not affect the majority of place fields.

The platform and environment outlines are rotationally symmetrical about the center of the CCE. If after training in the CCE the rat learns to attend to the CC’s and disregard the SBC’s then after removal of the CC’s no cues remain that could polarize the environment. According to the spatial map hypothesis in this situation the environment centroid would still be able to be calculated from the outlines but there would be insufficient information to derive the environment eccentricity.

In the seminal studies of Ranck (1984) and Taube et al (1990a, 1990b) units were found in the postsubiculum of rat that fired as a function of head direction and were essentially insensitive to location. The dependence of postsubicular head direction units on environment cues was measured by placing a single white card in the environment. Taube et al (1990b) found that rotation of the card between trials would reliably rotate the preferred direction of postsubicular head direction units when the rat was replaced within the environment. On the basis of this evidence O’Keefe (1990) has suggested that calculation of environment eccentricity takes place in the postsubiculum. If this eccentricity information can be maintained in the absence of polarizing
cues by some mechanism and the centroid can be calculated based solely on
the environment outline then the above explanation for the maintenance of
hippocampal place fields after CC removal is tenable. Unfortunately to date
no study has attempted to ascertain what changes occur in the preferred
direction of postsubicular head direction units if all environment polarizing cues
are removed while the rat is in the environment. The postsubicular head
direction units may continue to display firing in the same preferred direction
after the removal of polarizing cues. Alternatively they may cease firing in the
same preferred direction after cue removal.

Some units show alterations in place field firing after CC removal. The finding
that some place units show increases or decreases in place specific firing after
cue removal may be a functional demonstration of the connections between
inhibitory basket cells and pyramidal neurons in Ammon's Horn that previously
have been demonstrated anatomically and physiologically. O'Keefe (1979)
established that place units are Ammon's Horn pyramidal neurons. Andersen
et al (1963) established that inhibitory basket unit interneurons are present in
Ammon's Horn layer of the hippocampus. These interneurons have axons
that ramify throughout a large area and contact numerous pyramidal neurons
(Sik et al, 1995). According to the spatial map hypothesis upon entry into a
novel environment the cues within the environment are observed by egocentric
sensory systems and this information is fed to the hippocampus and used to
derive the allocentric spatial map. Hippocampal pyramidal neurons are
postulated to be the locus of much of the calculation necessary to create the
spatial map. It is possible although not explicitly stated that in calculating the
spatial map the pyramidal neurons excite basket cell inhibitory interneurons
that ramify widely and provide recurrent inhibition. As time goes on the firing
of some pyramidal neurons ceases as a result of basket cell inhibition
whereas the others, by virtue of their stronger initial activation, continue to fire.
Eventually a "winner" emerges in each group by virtue of the recurrent
inhibitory architecture of the hippocampus. The firing of the "winning"
pyramidal neuron in each group remains stable as long as the sensory inputs
available to the hippocampus remain static.
The directionality of place fields in situations in which the rat is constrained to follow stereotypical paths is not explained by the spatial map hypothesis. O'Keefe and Burgess (1996) observed omni-directional place fields in a rectangular enclosure that could be pulled apart into two unidirectional place fields by pulling apart the enclosure walls. This seems to suggest that the omni-directional place fields recorded in the open field are composed of superimposed uni-direction place fields whereas in situations in which the rat's path is constrained the unidirectional place fields predominate. How the calculation of numerous unidirectional place fields and their superposition in certain situations could be accomplished by a spatial mapping system that computes environment centroids and eccentricities is unclear.

During nine error trials hippocampus displayed firing that was not consistent with the place field firing seen in any of the three start platforms defined with respect to the CC's. If the postsubicular eccentricity system produces output that is rotated by multiples of 90° regardless of the current orientation of the CC's it is possible that these six trials represent the place fields that result when the rat is on or believes itself to be on the goal platform. Unfortunately it is impossible to verify or disprove this postulate since no place field data was collected on the goal platform.

In three error trials the hippocampus displayed firing that was consistent with the goal the rat eventually chose. Both the goal chosen and the hippocampal firing were at variance with the current orientation of the CC's. According to the above account of the spatial map hypothesis, after CC removal the hippocampus system can construct and maintain place fields based only on the environment outlines and the output of the postsubicular head direction system. If in some cases the eccentricity system produced output that was rotated by a multiple of 90° relative to the correct eccentricity and if the CC independent mechanism operated from the outset of a trial even when the CC's were present then the hippocampal place fields would be rotated by the same multiple of 90° regardless of the current orientation of the CC's. However this still leaves an open question, namely how can the postsubicular eccentricity system produce output that is rotated by multiples of 90° regardless of the current orientation of the CC's.
In three other error trial the hippocampal unit firing was consistent with the actual goal platform. If the hippocampal spatial map is indeed what guides the rat’s eventual choice and if the information in the spatial map is correct than the rat’s choice should have been correct. However it is possible that the error in the rat’s choice was introduced somewhere between the hippocampal spatial map system and some unspecified "choice behavior" system elsewhere in the rat brain.

10.3 Implications for the Working Memory Hypothesis

The CCE spatial memory task could be classed as a working memory task. The working memory hypothesis states that the hippocampus stores information necessary for the performance of individual trials. Thus during the first phase of PM trials the orientation of the CC’s would be held in hippocampal "working memory". After CC removal the hippocampus would retain this information. At the end of the trial the information contained in the hippocampus would be used by the rat in order to correctly choose the goal platform.

In light of the working memory hypothesis the CC’s in the CCE could be said to create a pattern of activation in the hippocampus in which is stored the current configuration of the CC’s. The fact that the pattern of activation is spatial such that individual active units fire in spatially contiguous areas of the environment is considered incidental, an artifact of the operation of the working memory system. According to the working memory hypothesis during the performance of non-spatial tasks different patterns of hippocampal activation would be observed that would be consistent with the mnemonic demands of the task.

The rotation of the firing fields of hippocampal units due to the rotation of the CC’s is also in accordance with the working memory hypothesis. Since the hippocampal units code for information necessary for the correct performance of an individual trial when the CC’s are in a different orientation the altered pattern of activation would reflects the storage of the altered information the rat requires to make the correct choice at the end of the trial.
Olton et al (1979) stated that if the working memory hypothesis is correct then one would expect to see alterations in the activity of hippocampal units during a working memory trial. At the start of the task there is nothing in working memory and therefore the units will reflect this. Later on working memory begins to become activated as trial specific information is gathered, leading to a change in the pattern of unit activity. Finally at the end of the trial the working memory information is discarded, leading to another change in single unit activity. The presence of units whose firing either increases or decreases after CC removal is compatible with this interpretation of the working memory hypothesis.

Olton (1989) gives a taxonomy of unit firing behavior that can be displayed during a working memory task. Within the context of the CCE task, units that display firing during the presentation of the CC's but cease firing once the cues are removed can be classified as being involved in either perception or registration. Units that do not display firing when the CC's are present but begin to fire once the CC's are removed can be classified as pure memory units that are not involved in perception. In tasks used to examine the electrophysiological manifestation of the working memory system, it is postulated that the changes in firing rate of the different units denote their participation in either perception, registration, recall, and so forth. The fact that the hippocampus displays firing in both the CC removal "working memory" trials and the CC present "reference memory" trials would seem to indicate that the postulated hippocampal working memory system is always functioning. This is to be expected in light of the fact that the CCE task is designed such that rat is unable to know in advance whether the trial it is currently performing is to be a "working memory" or "reference memory" trial until it is halfway over.

The directionality of place fields in the CCE working memory task is accounted for by the working memory hypothesis if one equates "working memory" with "dead-reckoning". Thus the information necessary for performance in the current trial is the continuously updated spatial position of the rat. Some evidence for the involvement of dead-reckoning in directionality is provided by the study of O'Keefe and Burgess (1996). They found fields whose location
appeared dependent on one wall in an environment since when the wall was moved the field remained at a constant location with respect to it. Moreover some of these fields were also directional, displaying peak firing when the rat ran away from the wall. Thus it is possible the place field was maintained using dead reckoning, that is, the rat remembered how far he had travelled from the wall.

During nine error trials the hippocampus was found to display firing that was not consistent with the firing seen in correct trials. Thus it could be postulated that the hippocampal working memory system had broken down and was storing information that was irrelevant to the task. Consistent with this postulate is the observation that the rat made erroneous choices at the end of these trials.

In three error trials the hippocampus was found to display firing that was consistent with the choice the rat made as opposed to the choice the rat was supposed to make. This is good evidence for the working memory hypothesis. According to the hypothesis the hippocampus stores the information the rat requires to correctly choose the goal platform. Thus the hippocampus guides the eventual choice the rat makes. When the information is consistent with a non-goal platform than according to the working memory hypothesis the rat should chose this non-goal platform.

In three other error trials the hippocampal unit firing was consistent with the actual goal platform. The fact that the rat made an incorrect choice despite the fact that the hippocampus was storing the correct information for the performance of the working memory task is evidence against the simplest version of the working memory hypothesis. If the information stored in the hippocampus is indeed what guides the rat’s eventual choice and if this information corresponds to the correct choice than the rat should have chosen correctly. However it is possible that the error in the rat’s choice was introduced somewhere between the working memory system of the hippocampus and some unspecified “choice behavior” system elsewhere in the rat brain. However this explanation, with some alterations in terminology, is the same as the one used to reconcile the data from this error trial with the
spatial map hypothesis. Thus it is equally consistent, or inconsistent, with both hypotheses.

10.4 Implications for the Relational Memory Hypothesis

According to the relational memory hypothesis the CC's in the CCE set up a pattern of activation in the hippocampus in which is stored the current relational configuration of the CC's. According to the relational memory hypothesis during the performance of non-spatial tasks different patterns of hippocampal activation would be observed that would be consistent with the mnemonic demands of the task. Thus during spatial tasks the pattern one would expect to be set up in the hippocampus would be spatial. Similarly the rotation of the firing fields of hippocampal units due to the rotation of the CC's could be said to be in accordance with the relational memory hypothesis. By rotating the relationship the cues have to each other a concomitant rotation of the unit firing correlates should result.

O'Keefe and Conway (1978) found that removal of a subset of the cues in the CCE does not affect the place specific firing of the majority of hippocampal units. O'Keefe and Speakman (1987a) later extended this finding. They found that the place specific firing of the majority of hippocampal units was unaffected when all of the CC's in the CCE were removed. Cohen and Eichenbaum (1993) postulate that the pattern of converging sensory inputs are synchronized by the theta rhythm and stored in the hippocampus using LTP in much the same manner as proposed by Teyler and DiScenna (1986). The hippocampus then acts as an index to retrieve memories from neocortex. Thus according to the relational memory hypothesis when the cues are present the appropriate hippocampal "index" pattern becomes active. When the cues are removed this pattern of activation persists by some unspecified mechanism. It is this stored hippocampal index in conjunction with neocortical processing that is postulated to enable the rat to chose the correct platform when the center platform is raised.

Cohen and Eichenbaum (1993) claim place field directionality is further evidence in favor of the relational memory hypothesis. According to their
hypothesis direction is a second order spatial variable. The fact that on some occasions hippocampal units are sensitive to these second order spatial variables is taken as evidence that the hippocampus is not exclusively involved in the creation of a spatial map.

The relational memory hypothesis does not make any straightforward predictions as to the expected single unit behavior under cue present and cue absent conditions. Thus the finding that some hippocampal units increase or decrease their firing after CC removal can not be construed as evidence in favor for or against the hypothesis. Similarly the hypothesis makes no clear predictions as to what firing one would expect during error trials. Thus the hippocampus firing seen during error trials can not be considered in light of the relational memory hypothesis.

10.5 Future Directions

The main assumption made upon considering the data contained in this thesis and the spatial map hypothesis is that the preferred direction of postsubicular head direction units is unaltered when the CC’s are removed from the CCE. This postulate is readily testable by recording from postsubicular head direction units in the CCE and removing the CC’s.

If the postsubicular head direction units maintain their preferred direction after cue removal then it may be possible to determine the mechanism by which this is accomplished. It is possible that the rat hippocampus calculates the angle between the polarizing CC and some external environmental cues. Thus when the CC’s are removed the preferred direction of the head direction units can be maintained with reference to these unspecified external environmental cues. Alternately the rat may only attend to the polarizing CC’s such that when they are removed head direction firing is maintained using idiothetic sensory systems (Goodridge and Taube, 1995; Taube and Burton, 1995).

Furthermore according to the spatial map hypothesis during error trials the orientation of the postsubicular head direction units will be consistent with the
orientation of the place fields seen in the hippocampus. Again this postulate is readily testable by recording from postsubicular head direction units in the CCE while the rat performs error trials.

A weaker prediction based on the limited error trial data is that the hippocampal spatial map does not directly guide behavior but is at least one step removed. The rationale for this assumption are those error trials in which the hippocampal firing matches the experimenter's goal but the rat nevertheless makes an incorrect choice. An attempt could be made to elucidate the breakdown between the hippocampal spatial map in the rat hippocampus and the postulated choice center elsewhere in the rat brain from which originates the eventual choice behavior by recording from motor or premotor structures that may be responsible for the locomotory behavior involved in making a choice, for example the nucleus accumbens (Mogenson and Nielsen, 1984; Annett et al, 1989; Schacter et al, 1989; Brown and Sharp, 1995).
References


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Recce ML, Speakman A, O'Keefe J (1991) Place fields of single hippocampal cells are smaller and more spatially localized than you thought. Soc Neurosci Abstr 17: 484.


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Whishaw IQ, Vanderwolf CH (1973) Hippocampal EEG and behavior; changes in amplitude and frequency of RSA (theta rhythm) associated with spontaneous and learned movement patterns in rats and cats. Behav Biol 8: 461-484.


Wigström H, Gustafsson B, Huang YY, Abraham WC (1986) Hippocampal long-term potentiation is induced by pairing single afferent volleys with


Illustrations
Table I. Bilateral microdrive implant co-ordinates for the three animals used in the present study in mm. (AP: anterior posterior location relative to bregma; ML: medial lateral location relative to the midline.)

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Table II. Summary of the units recorded from the three animals used in the present study. (A) The number of complex spike and theta units recorded. The theta units were not analysed further. (B) The number of complex spike units recorded from the CA1 layer and the combined number recorded from the CA2, CA3, and hilar layers.

(A)

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(B)

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Table III. Results of the CC by SBC ANOVA analysis. The values tabulated are the percentages of variance accounted for by the indicated factors as calculated using the formula of Dodd and Schultz (1973). Asterisks denote the significance level. (**: p<0.001, ***: p<0.0001, *: p<0.01.)

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Table IV. Results of the cue removal analysis for the three CC defined start platforms. The entries denote either the change in place field firing after cue removal (per: persistence; inc: increase; dec: decrease) or the reason the firing was not analysed (<: rates too low; >: firing too diffuse).

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Table V. Results of the directionality analysis. The characters denote directions (U: up; D: down; L: left; R: right) which showed statistically significant higher firing rates as assessed by Scheffé tests. (***: p<0.0001; **: p<0.001; *: p<0.01; §: p<0.05; <: rates too low.)

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Table VI. Interaction between place field directionality and the response to cue removal. Entries denote the response to cue removal (per: persist; inc: increase; dec: decrease) for place fields which displayed significant directionality. (?: directional firing was present but there was no place field.)

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Table VII. The goal specificity of error trial firing. (exps: error trial firing was consistent with the experimenter's goal; rats: error trial firing was consistent with the rat's goal; unkn: error trial firing was not consistent with either the experimenter's goal or the rat's goal.)

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Figure 1. Comparison of the rat and human hippocampus. (A) Drawing of the rat hippocampus showing its location in the brain. (B) Photo of a rat brain with the hippocampus dissected out. (C) Drawing of the human hippocampus showing its location in the brain. (D) Photo of a human brain with the hippocampus dissected out. (Modified from West (1990), p 16.)
Figure 2. Diagram of the rat hippocampus showing its curvature from the anterior dorsal, to the posterior, to the anterior ventral position. The septal pole is located at the septum or midline and the temporal pole is located at the ventral temporal tip. (Modified from O'Keefe and Nadel (1978), p 103.)
Figure 3. Reproduction of a photomicrograph showing the components of the hippocampal formation (mouse). The inset shows at what level of the hippocampus the section was taken. (CA1, CA3: subdivisions of Ammon’s Horn; DG: dentate gyrus; HIL: hilus; SUB: subiculum; PRES: presubiculum; PARA: parasubiculum; mENT: medial entorhinal cortex; lENT: lateral entorhinal cortex; I-VI: the six layers of the entorhinal cortex; PERI: perirhinal cortex; the postsubiculum does not appear at this level; modified from Swanson (1979), p 10.)
Figure 4. The layers of the Ammon’s Horn (AL: alveus, OR: striatum oriens, PY: striatum pyrimidale, RA: striatum radiatum, LM: stratum lacunosum-moleculare) and the dentate gyrus (MO: molecular layer, GR: granule layer, HI: hilus) in schematic form. (Modified from Nafstad (1967), p 533.)
Figure 5. A sampling of the major cell types found in the hippocampal formation. (A) Pyramidal cell. (B) Dentate granule cell. (C) Basket cell. (D) Mossy cell. (E) Horizontal cell. (F) Stellate cell. (G) Multipolar cell. (A, B: modified from Ylinen et al (1995b), p 82, 84; C: modified from O'Keefe and Nadel (1978), p 110; D: modified from Ribak et al (1985), p 839; E, F, G: modified from Schwerdtfeger et al (1990), pp 166-167.)
Figure 6. Illustration of the mechanism of recurrent inhibition of pyramidal cell discharge by basket cells. Pyramidal cell axon collaterals make excitatory synapses on basket cells. Basket cell feedback to pyramidal cells and form inhibitory synapses. Thus when the pyramidal cell fires it causes the basket cells to fire, which then recurrently inhibits firing in all the pyramidal cells which its afferents contact. (Modified from Andersen et al (1963), p 541.)
Figure 7. Comparison of the firing properties of hippocampal complex spike units and theta units. (A) An extracelluarly recorded burst of spikes of progressively decreasing amplitude characteristic of complex spike units. (B) A series of theta spikes recorded extracelluarly. The amplitude is constant. (C) Extracelluarly recorded waveforms from a complex spike unit and a theta unit displayed on a short time base. The complex spike unit is of higher amplitude and longer duration than the theta unit. The first phase of the complex spike wave is substantially larger than the second whereas the first and second phases of the theta unit spike are of comparable amplitude. (Up is positive; modified from Christian and Deadwyler (1986), p 335.)
Figure 8. Diagram of the experiment room as seen from above detailing the location of the cue controlled environment (CCE) in the static background cue (SBC) reference frame. By convention north is defined as the door of the experiment room. The SBC defined platforms are labelled in accordance with the convention north. Magnetic north is indicated by an arrow.
Figure 9. Four diagrams of the experiment room containing the cue controlled environment (CCE) showing the location of the goal arm with respect to the static background cues (SBC's) as defined by the four possible rotations of the controlled cues (CC's). (A) With the CC's in this orientation the goal is on the NE platform relative to the SBC's. The CC's and all four CC defined platforms are labelled. (B) Goal on the NW platform relative to the SBC's. (C) Goal on the SW platform relative to the SBC's. (D) Goal on the SE platform relative to the SBC's.
Figure 10. Schematic of the paradigm used to insure uniform coverage of the start platform during recording. The experimenter is shown leading the rat using forceps baited with a pellet of rat chow. The lines indicate the previous path of the rat, i.e. alternately moving up down and left right.
Figure 11. An exploded view of the microdrive apparatus used to isolate single units in the rat hippocampus. On left side of the figure is the basic frame and on the right are the fittings. The complete microdrive loaded with tetrodes is shown in the middle. (Modified from an unpublished figure drawn by Ainsworth.)
Figure 13. Calculation of the spatial position correction matrix. (A) The vectors from the rectilinear spatial positions to the observed spatial positions calculated for the four horizontal planes. The rectilinear outline of the platform covers are shown as four large squares. The calibration points recorded by placing lights in the corners of the platforms are shown as small circles. (B) The vectors from the recorded spatial positions to the real spatial positions extended throughout the vertical plane. (C) The reverse of B. Now the vectors are from the observed spatial positions to the rectilinear spatial positions. (D) Testing the spatial correction matrix. The vector from each calibration point to the corresponding rectilinear spatial position according to the matrix is shown. The corrected positions are almost directly over the maze corners.
Figure 14. Cluster cutting procedure. (A) The max less min voltage plot. The four numbered axes correspond to the four tetrode channels. The red polygon encircles a cluster that has been isolated and cut. (B) The waveform of the cluster in A (clipped on channel 1 by the A to D converter). (C) The remaining clusters are cut. (D) All the waveforms of the clusters in C.
Figure 15. Calculation of the waveform envelope. (A) A group of spikes clustered manually. The four traces correspond to the four lines of the tetrode. (B) The envelope calculated from the group of spikes. (C) The calculated envelope shown alone.
Figure 16. Performance of rat R305 in the CCE spatial memory task expressed as the percentage of correct choices in successive blocks of ten trials. Dashed lines denote the onset of potentially disruptive procedures (PMs: start of perceptual-memory trials; IMP: implantation; REC: start of recording).
Figure 17. Performance of rat R331 in the CCE spatial memory task expressed as the percentage of correct choices in successive blocks of ten trials. Dashed lines denote the onset of potentially disruptive procedures (PMs: start of perceptual-memory trials; IMP: implantation; REC: start of recording).
Figure 18. Performance of rat R408 in the CCE spatial memory task expressed as the percentage of correct choices in successive blocks of ten trials. Dashed lines denote the onset of potentially disruptive procedures (PMs: start of perceptual-memory trials; IMP: implantation; REC: start of recording).
Figure 19. The firing rate maps for all three CC defined start platforms for a single pyramidal unit for which 89% of the variance was explained by the CC factor in the CC by SBC ANOVA analysis. The firing was highly spatially specific, with a strong place field in the fan platform. (Rat: 408; session: 950817; unit 15; PP1: first phase of perceptual–perceptual trials; PP2: second phase of perceptual–perceptual trials; PM1: first phase of perceptual–memory trials; PM2: second phase of perceptual–memory trials; contour interval: 1.0 Hz)
Figure 20. The firing rate maps for all three CC defined start platforms for a single pyramidal unit for which 15% of the variance was explained by the CC factor in the CC by SBC ANOVA analysis. The firing was moderately spatially specific, being relatively higher in the light and ball platforms relative to the fan platform. (Rat: 408; session: 950817; unit 4; PP1: first phase of perceptual–perceptual trials; PP2: second phase of perceptual–perceptual trials; PM1: first phase of perceptual–memory trials; PM2: second phase of perceptual–memory trials; contour interval: 1.0 Hz)
Figure 21. The firing rate maps for all three CC defined start platforms for a single pyramidal unit whose firing was significantly governed by the CCxSBC interaction according to the CC by SBC ANOVA analysis. The firing is highest in the light platform relative to the CC's. (Rat: 305; session: 940601; unit 02; PP1: first phase of perceptual–perceptual trials; PP2: second phase of perceptual–perceptual trials; PM1: first phase of perceptual–memory trials; PM2: second phase of perceptual–memory trials; contour interval: 1.0 Hz)
Figure 22. The firing rate maps for all four SBC defined start platforms for a single pyramidal unit whose firing was significantly governed by the CCxSBC interaction according to the CC by SBC ANOVA analysis. The firing is highest in the SW platform relative to the SBC's. (Rat: 305; session: 940601; unit 02; PP1: first phase of perceptual–perceptual trials; PP2: second phase of perceptual–perceptual trials; PM1: first phase of perceptual–memory trials; PM2: second phase of perceptual–memory trials; contour interval: 1.0 Hz)
Figure 23. Comparison of firing rate maps by subtraction. The linear correlation coefficient (Pearson’s r) was calculated for maps X and Y and compared to the integral calculated for map X-Y to determine whether subtraction can be used to compare maps. (The r ranges from -1 to 1, with values close to 0 denoting uncorrelated maps. The integral ranges from 0 on up, with values close to 0 denoting similar maps.) (A) The subtraction of two maps with fields in the same location and of the same magnitude leads to a difference map which is essentially flat. (Pearson’s r: 1.0; integral: 0.0.) (B) The subtraction of two maps with fields in the same location but of different magnitudes leads to a difference map with a field in the same location but of intermediate magnitude. (Pearson’s r: 1.0; integral: 29.0.) (C) The subtraction of two maps with fields in different locations leads to a difference map which displays the two original fields, one of which is inverted. (Pearson’s r: -0.1; integral: 116.0.) (Steps shaded grey are negative; contour steps are arbitrary.)
Figure 24. The six possible pairwise subtractions (PP1-PP2, PM1-PM2, PP1-PM1, PP2-PM2, PP1-PM2, and PM1-PP2) for the four trial type and phase combination maps (PP1, PP2, PM1, and PM2).
Figure 25. The pattern of rankings for the four averaged maps (PP1, PP2, PM1, and PM2) and six subtracted maps (PP1-PP2, PP1-PM1, PM1-PP2, PP1-PM2, PP2-PM2, and PM1-PM2) expected for place fields that show one of the five hypothesized changes in firing after cue removal. (A) Persistence of firing. The PP1, PP2, PM1, and PM2 maps all have higher ranks than the PP1-PP2, PP1-PM1, PM1-PP2, PP1-PM2, PP2-PM2, and PM1-PM2 maps. (B) Cue dependent increase in firing. The PM2, PP1-PM2, PP2-PM2, and PM1-PM2 all have higher ranks than the PP1, PP2, PM1, PP1-PP2, PP1-PM1, and PM1-PP2. (C) Cue dependent decreased in firing. The PP1, PP2, PM1, PP1-PM2, PP2-PM2, and PM1-PM2 maps all have higher ranks than the PM2, PP1-PP2, PP1-PM1, and PM1-PP2 maps. (D) Time dependent increase in firing. The PP2, PM2, PP1-PP2, PM1-PP2, PP1-PM2, PM1-PM2 maps all have higher ranks than the PP1, PM1, PP1-PP2, PP1-PM1, and PP2-PM2 maps. (E) Time dependent decrease in firing. The PP1, PM1, PP1-PP2, PM1-PP2, PP1-PM2, PM1-PM2 maps all have higher ranks than the PP2, PM2, PP1-PM1, and PP2-PM2 maps.
Figure 26. A unit which displayed persistence of spatial firing after cue removal when recorded from on the fan platform relative to the CC’s. The four averaged and six subtracted firing rate maps are shown with their corresponding ranks in parentheses. (Rat 331: session 941027; unit 06; PP1: first phase of perceptual–perceptual trials; PP2: second phase of perceptual–perceptual trials; PM1: first phase of perceptual–memory trials; PM2: second phase of perceptual–memory trials; CP: center platform; contour interval: 1.0 Hz; grey denotes negative contour intervals.)
Figure 27. A unit which displayed an decrease in spatial firing after cue removal when recorded from on the light platform relative to the CC’s. The four averaged and six subtracted firing rate maps are shown with their corresponding ranks in parentheses. (Rat 331: session 941027; unit 06; PP1: first phase of perceptual-perceptual trials; PP2: second phase of perceptual-perceptual trials; PM1: first phase of perceptual-memory trials; PM2: second phase of perceptual-memory trials; CP: center platform; contour interval: 1.0 Hz; grey denotes negative contour intervals.)
Figure 28. A unit which displayed an increase in spatial firing after cue removal when recorded from on the fan platform relative to the CC's. The four averaged and six subtracted firing rate maps are shown with their corresponding ranks in parentheses. (Rat 305: session 940607; unit 01; PP1: first phase of perceptual–perceptual trials; PP2: second phase of perceptual–perceptual trials; PM1: first phase of perceptual–memory trials; PM2: second phase of perceptual–memory trials; CP: center platform; contour interval: 1.0 Hz; grey denotes negative contour intervals.)
Figure 29. A unit which displayed a two lobed place field when recorded from on the ball platform relative to the CC’s. After cue removal the left lobe was unchanged while the right lobe displayed a decrease in firing. The cue removal analysis classified this set of place fields as displaying a decrease in firing after cue removal. (Rat 408: session 950817; unit 16; PP1: first phase of perceptual–perceptual trials; PP2: second phase of perceptual–perceptual trials; PM1: first phase of perceptual–memory trials; PM2: second phase of perceptual–memory trials; CP: center platform; contour interval: 1.0 Hz; grey denotes negative contour intervals.)
Figure 30. A unit which displayed a two lobed place field when recorded from on the ball platform relative to the CC's. After cue removal the lower lobe was abolished while the upper lobe displayed an increase in firing. The cue removal analysis erroneously classified this set of place fields as displaying persistence of firing after cue removal. (Rat 331: session 941110; unit 01; PP1: first phase of perceptual-perceptual trials; PP2: second phase of perceptual-perceptual trials; PM1: first phase of perceptual-memory trials; PM2: second phase of perceptual-memory trials; CP: center platform; contour interval: 1.0 Hz; grey denotes negative contour intervals.)
Figure 31. Illustration of a three phase trial showing the effects of cue removal and cue replacement. Firing rate maps recorded on the ball platform relative to the CC's are shown for the three trial phases. The response of the two lobed place field to cue removal in the second phase of the three phase trial is the same as the response to cue removal seen in averaged two phase trials (lower lobe abolished, upper lobe strengthened, see Figure 30). In the third phase the change in place field morphology caused by cue removal is reversed after cue replacement. (Rat 331: session 941110; unit 01; CP: center platform; contour interval: 1.0 Hz.)
Figure 32. Four diagrams of the experiment room containing the cue controlled environment (CCE) showing direction with respect to the static background cues (SBC's) as defined by the four possible rotations of the controlled cues (CC's). (A) With the CC's in this orientation up is towards the door. (B) With the CC's in this orientation up is towards the door. (C) With the CC's in this orientation up is towards the door. (D) With the CC's in this orientation up is towards the door.
Figure 33. The six comparisons used in the post-hoc Scheffé directionality analysis. (A) 315° to 45° compared to the other three directions. (B) 135° to 225° compared to the other three directions. (C) 45° to 135° compared to the other three directions. (D) 225° to 315° compared to the other three directions. (E) The two adjacent directions above a positive 45° line compared to the other two directions. (F) The two adjacent directions above a negative 45° line compared to the other two directions.
Figure 34. A place unit which displayed maximal firing when the rat travelled between 45° and 135° relative to the CC's (i.e. one 90° "wedge"). This directionally dependent variation in firing was maintained even in the absence of the CC's. (Rat 305; session 940607; unit 07; PP1: first phase of perceptual-perceptual trials; PP2: second phase of perceptual-perceptual trials; PM1: first phase of perceptual-memory trials; PM2: second phase of perceptual-memory trials; contour interval: 1.0 Hz.)
Figure 35. A place unit which displayed maximal firing when the rat travelled between 315° and 135° relative to the CC's (i.e. two adjacent 90° “wedges”). This directionally dependent variation in firing was maintained even in the absence of the CC's. (Rat 408; session 950817; unit 17; PP1: first phase of perceptual-perceptual trials; PP2: second phase of perceptual-perceptual trials; PM1: first phase of perceptual-memory trials; PM2: second phase of perceptual-memory trials; contour interval: 1.0 Hz.)
Figure 36. Firing rate maps for the first phase of a PP error trial in which the unit firing failed to resemble that seen in correct trials. On the left are the error trial firing rate maps oriented by experimenter's goal and on the right are the same maps oriented by rat's goal. Averaged firing rate maps calculated for all the correct trials in the session are plotted in the middle and arcs connect the error trial maps with their corresponding "correct" map. The firing in the second phase of the trial was comparable to that seen in the first phase. (Rat: 331; session: 941110; error trial 255; G: goal platform; contour interval: 1.0 Hz.)
Figure 37. Firing rate maps for the first phase of a PP error trial in which the unit firing oriented by the rat's goal resembled that seen in correct trials. On the left are the error trial firing rate maps oriented by experimenter's goal and on the right are the same maps oriented by rat's goal. Averaged firing rate maps calculated for all the correct trials in the session are plotted in the middle and arcs connect the error trial maps with their corresponding "correct" map. The firing in the second phase of the trial was comparable to that seen in the first phase. (Rat: 305; session: 940607; error trial 343; G: goal platform; contour interval: 1.0 Hz.)
Figure 38. Firing rate maps for the first (cues present) and second (cues absent) phases of a PM error trial in which the unit firing oriented by the experimenter’s goal resembled that seen in correct trials. On the left are the error trial firing rate maps oriented by experimenter’s goal and on the right are the same maps oriented by rat’s goal. Averaged firing rate maps calculated for all the correct trials in the session are plotted in the middle and arcs connect the error trial maps with their corresponding “correct” map. In the second cues absent phase of the trial the firing of unit 5 ceased in the error trial, the same response as it displayed in the averaged correct trials. (Rat: 331; session: 941110; error trial 257; G: goal platform; contour interval: 1.0 Hz.)