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SINGLE UNIT ACTIVITY IN THE SUBICULUM OF FREELY-MOVING RATS: SPATIAL AND NON-SPATIAL CORRELATES

by

Michael Ian Anderson

A dissertation submitted for the degree of Doctor of Philosophy of the University of Dublin, Trinity College, Dublin 2, Ireland.

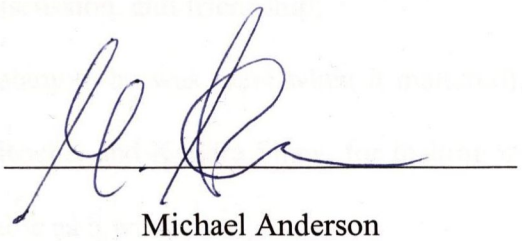
This research was conducted in the Department of Psychology.

November 2000.

Declaration

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Signed



Michael Anderson

Acknowledgements

My thanks go to:

My supervisor, Shane O'Mara, for his kindness, enthusiasm, and support;

My examiners, Sidney Wiener and Hugh Garavan, for their helpful critical comments on this work;

Sean Commins, for help with histology, and for his support, helpful discussion, and friendship;

David Delany (who was there when it mattered), Olivier Gobbo, Richard Roche, and Kendra Shaw, for making my time at Trinity as enjoyable as it was;

John Gigg, for encouragement and help;

To Mona, for keeping me sane;

And to my parents, without whose love, support, and advice I would not have had the opportunity to write this thesis.

November 2000, Dublin

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Main summary

The subiculum is a poorly understood structure, despite being only a synapse away from the intensively studied hippocampus. As such, any attempts to characterize subicular unit firing will benefit the ongoing research effort directed at elucidating the role of the hippocampal formation.

In this thesis, we have attempted to address four questions concerning the possible functions of the subiculum:

1. *What are the main subicular cell types, and how and when do they fire?*

2. *What is the nature of the spatial signal carried by subicular place cells?*

3. *Is the subiculum a structure where place information is combined with idiothetic information?*

4. *Is the firing of subicular single units correlated with non-spatial variables?*

To address these questions we have used the technique of single unit recording in the freely-moving rat. This technique is a powerful tool for

understanding the functioning of brain structures since it permits time-locked correlations to be made between the activity of the target structure (both action potentials and EEG) and ongoing behaviour of the animal. It is a technique that has come to prominence in the last 30 years and has been used to great effect, particularly in the domains of visual perception and spatial representation.

Recording subicular unit activity in four tasks (baseline recordings (Chapter 3), pellet-chasing task (Chapter 4), gradient maze task (Chapter 5), and object exploration task (Chapter 6)), we show that the role of the subiculum is best conceived of in terms of current spatial theories of hippocampal formation function, possibly as part of a path integration system (cf. Redish, 1999; Sharp, 1999b).

Abbreviations

cm	centimetre(s)
μm	micron(s)
s	second(s)
min	minute(s)
ms	millisecond(s)
μs	microsecond(s)
Hz	Hertz
μV	microvolt(s)

Chapter 1

The known anatomy, physiology, and functions of the rodent subiculum

Chapter contents

Summary

1 General introduction

2 Definition of the subiculum and the subicular complex

3 Cytoarchitectonic organisation and description of the subiculum

4 The anatomy and connections of the subiculum

5 The neurophysiology of the subiculum

6 Lesion analyses of the subiculum

7 Recordings in freely-moving animals

8 Theories of subicular function

9 Synthesis

Summary

Compared with the research effort directed at the hippocampus the subiculum has received little experimental attention despite being one synapse downstream of area CA1 and in a position to influence many cortical areas with the results of hippocampal activity. Recently, however, the subiculum has been the focus of a greater interest.

The subiculum is a pivotal structure positioned between the hippocampus proper and entorhinal and other cortices, as well as a range of subcortical structures. The subiculum has a range of electrophysiological and functional properties which are quite distinct from its input areas; given the widespread set of cortical and subcortical areas with which it interacts, it is able to influence activity in quite disparate brain regions. However, its functional properties are not well-understood; it plays an important but ill-defined role both in spatial navigation and in mnemonic processing.

General introduction

The subiculum is a cortical region adjacent to the hippocampal area CA1 layer for much of its extent. Together with the dentate gyrus (DG), the hippocampus proper (areas CA1 and CA3), and the entorhinal cortex (EC), it is considered a subdivision of the hippocampal formation (HF) (Amaral and Witter, 1995; see Figure 1.1). There are two HFs, one in each hemisphere, and together their structures resemble two letter Cs leaning together at the top and spread apart at the bottom (see Figure 1.1). The dorsal tip of the HF is called the 'septal pole' because of its proximity to the septum (Amaral and Witter, 1989). The course of each HF follows the medial aspects of the ventral floor of the lateral ventricle, ending at the more ventral tip or 'temporal pole'. The internal structure of the HF is the same throughout its length and consists of an infolded convolution of the evolutionarily older and more simple (i.e., fewer layers) archicortex or allocortex. The physiology and functions of the dentate gyrus and the hippocampus proper have been the subject of many reviews (for example Eichenbaum, 1999; O'Keefe, 1979; O'Keefe, 1999; O'Mara, 1995). The subiculum, by contrast, has received scant attention (with the exception of Witter and Groenewegen, 1990; O'Mara *et al.*, 2001).

The subiculum has traditionally been considered a part of the 'subicular complex' which is described by Amaral and Witter (1995) as 'a conglomerate of cytoarchitectonically different, relatively small cortical

fields that are located between CA1 and the entorhinal cortex, ventrally, and between CA1 and the retrosplenial cortex, dorsally.' Recent opinion, however, favours the view that for heuristic purposes the subiculum is better thought of in the context of its role in the HF (see O'Mara, Commins, Anderson, and Gigg, 2001; Amaral and Witter, 1995). In this thesis, therefore, we will treat the subiculum as properly part of the hippocampal formation; we suggest, given the major anatomical differences in terms of intrinsic and extrinsic properties that the term 'subicular complex' does not denote a useful anatomical and, by extension, functional grouping of structures. As such, the pre- and para-subiculum will not be considered in detail in this thesis.

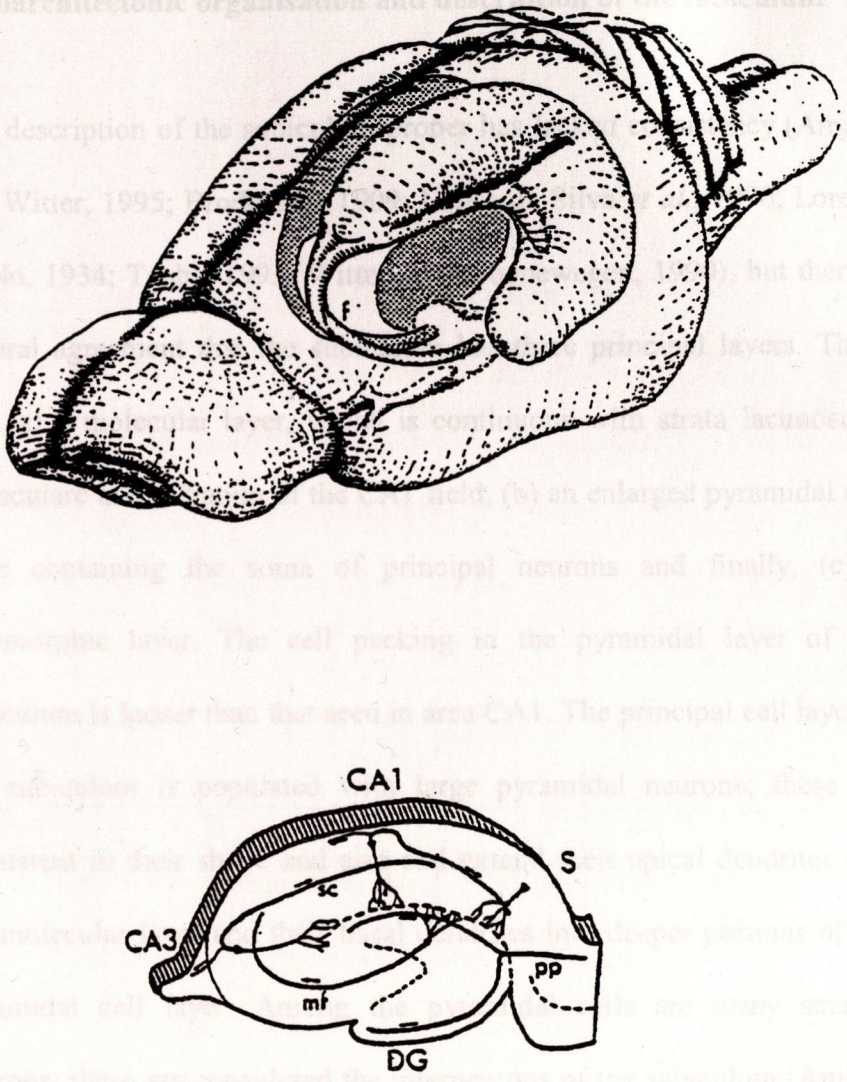


Figure 1.1. The top picture shows the two HFs in the rodent brain, one in each hemisphere: together their structures resemble two letter Cs leaning together at the top and spread apart at the bottom. The bottom picture shows a coronal section of the hippocampal formation showing the various subregions. DG, dentate gyrus; CA3 and CA1, Ammon's horn regions 1 and 2; S, subiculum; pp, perforant pathway; mf, mossy fibres; sc, Schaffer collaterals.

Cytoarchitectonic organisation and description of the subiculum

The description of the subiculum proper has lacked consistency (Amaral and Witter, 1995; Brodmann, 1909; Lopes da Silva *et al.*, 1990; Lorente de No, 1934; Taube, 1993; Witter and Groenewegen, 1990), but there is general agreement that the subiculum has three principal layers. These are: (a) a molecular layer, which is continuous with strata lacunosum-moleculare and radiatum of the CA1 field, (b) an enlarged pyramidal cell layer containing the soma of principal neurons and finally, (c) a polymorphic layer. The cell packing in the pyramidal layer of the subiculum is looser than that seen in area CA1. The principal cell layer of the subiculum is populated with large pyramidal neurons; these are consistent in their shape and size and extend their apical dendrites into the molecular layer and their basal dendrites into deeper portions of the pyramidal cell layer. Among the pyramidal cells are many smaller neurons; these are considered the interneurons of the subiculum (Amaral and Witter, 1995; Swanson *et al.*, 1987).

The anatomy and connections of the subiculum

Connections between the subiculum and the hippocampus

- The subiculum is the major output structure of the hippocampus, receiving a massive input from hippocampal area CA1 (Witter *et al.*, 1989). Amaral *et al.* (1991) suggest that the CA1 projection to the subiculum is organised in a simple pattern: cells in the proximal portion of CA1 project to the distal part of the subiculum; cells in the mid-portion of CA1 project to the middle part of the subiculum; and cells in the distal portion of CA1 project just across the CA1/subiculum border into the proximal part of the subiculum. Fibres which arise in the proximal part of CA1 travel to the subiculum mainly via the alveus and the deepest portion of the stratum oriens, whereas the fibres which originate in the mid-portion of the CA1 do not enter the alveus but project to the subiculum through the deep parts of stratum oriens. The axons of CA1 cells which are located in the distal portion travel directly to the subiculum from all parts of stratum oriens (Amaral *et al.*, 1991).

Connections between the subiculum and the pre- and parasubiculum

- There are weak projections from the presubiculum and parasubiculum to the subiculum, and there is a modest bilateral projection from presubiculum to the subiculum (Kohler, 1985).

However, the subiculum gives rise to very dense projections to the pre- and parasubiculum (Swanson and Cowan, 1977; Witter and Groenewegen, 1990).

Connections between the subiculum and cortex

- Projections from the subiculum to the EC have been described in the rat (Beckstead, 1978; Finch *et al.*, 1986; Kohler, 1985; Tamamaki and Nojyo, 1995), the guinea pig (Sorensen and Shipley, 1979), the cat (Van Groen *et al.*, 1986) and the monkey (Amaral *et al.*, 1984). Witter *et al.* (1990) report that only the proximal one-third of the subiculum projects to the EC. However, a more recent study by Tamamaki and Nojyo (1995) found that entorhinal projecting neurons exist along the entire proximodistal extent of the subiculum. The authors account for this opposing view by suggesting that the two accounts may be still compatible due to the fact that the subiculum is composed of heterogeneous projection neurons (Swanson *et al.*, 1981). Several studies suggest that the entorhinal cortex projects directly to the molecular layer of the subiculum (rat: Steward, 1976; Wyss, 1981; cat: Witter and Groenewegen, 1984; monkey: Van Hoesen and Pandya, 1975). In the rat, Witter *et al.* (1989) also showed that the subiculum receives a strong projection from the EC, where the fibres are directed towards restricted portions terminating in the outer two-thirds of the molecular layer. The entorhinal input to the

subiculum originates in both layer II and III neurons (Steward and Scoville, 1976; Witter and Amaral, 1991), with layer II neurons terminating in all layers of the subiculum (Tamamaki and Nojyo, 1993), and synapse with primary dendrites to drive postsynaptic subicular neurons. Thus, according to Tamamaki and Nojyo (1995), the entorhinal input through CA1 field to subiculum and the direct input from EC will converge in the subiculum. These combined inputs may then be fed back to the EC. The authors state that there is no evidence that the EC input to CA1 and subiculum directly contacts cells that project to the EC.

- The perirhinal cortex projects directly to the subiculum in the rat (Kosel *et al.,* 1983), in the cat (Van Groen *et al.*, 1986) and monkey (Van Hoesen *et al.*, 1979). In recent studies, Naber *et al.* (1999) have shown that injections of an anterograde tracer in the perirhinal cortex have shown labelled fibres in the border area of CA1 and subiculum. The projections from the postrhinal cortex are much stronger to the subiculum than to area CA1, whereas the perirhinal projections are more evenly distributed (Witter *et al.,* 2000). The subiculum also projects to the perirhinal cortex in the rat, cat, guinea pig, and monkey (Kohler, 1985; Sorensen and Shipley, 1979; Swanson *et al.*, 1978; Van Groen *et al.*, 1986). In the rat, Witter *et al.* (1990) injected an anterograde tracer (PHA-L) into cells which were located in the proximal part of the dorsal subiculum, directly adjacent to the border with CA1, and found

that the labelling was located in the deep layers of the perirhinal cortex.

- An injection of an anterograde tracer in the distal part of the dorsal subiculum results in marked labelling in the retrosplenial cortex, mainly in its ventral part directly bordering the presubiculum (Witter and Groenewegen, 1990). Injections in the ventral subiculum did not result in notable labelling in the retrosplenial cortex (Witter and Groenewegen, 1990; Witter *et al.*, 1990). No portion of the retrosplenial cortex projects to the subiculum proper (Wyss and Van Groen, 1992).

- In the rodent and monkey, the subiculum projects to medial orbital areas (Jay and Witter, 1991; Carmichael and Price, 1995). In the rat, the projection arises exclusively from restricted portions of CA1 and of the subiculum. Finch (1993) suggests that ventral subiculum projects to prelimbic, infralimbic and anterior cingulate cortices. No inputs have been reported from either prelimbic or infralimbic to the subiculum.

Connections between the subiculum and subcortical structures

- The distal part of the ventral subiculum projects to the ventromedial nucleus in the hypothalamus (Witter and Groenwegen, 1990). Also, Witter *et al.* (1990) found that,

following injections in the proximal part of the dorsal subiculum, strong labelling is observed bilaterally in the mammillary nuclei.

- Canteras and Swanson (1992) suggest that the projections from the ventral subiculum course either obliquely through the angular bundle to innervate the amygdala, or follow the alveus and fimbria to the precommissural fornix and medial corticohypothalmic tract, where the major amygdalar terminal field is centred in the posterior basomedial nucleus and the posterior basolateral nucleus (see also Witter and Groenewegen, 1990).

- Both the dorsal and ventral subiculum project to the lateral septum (Witter *et al.*, 1990; Namura *et al.*, 1994). The connections of the septal area with the hippocampal formation have been studied in great detail (for example, Chandler and Crutcher, 1983 and Alonso and Kohler, 1984). In the subiculum both the molecular and the pyramidal cell layer appear to be innervated by septal projections (Lopes da Silva *et al.*, 1990).

- Witter and Groenewegen (1990) found that following injections in the proximal part of the dorsal subiculum, labelling was visible rostrolaterally in the nucleus accumbens. Aylward and Totterdell (1993) state that proximal neurons in the ventral subiculum also project to the nucleus accumbens.

- Witter and Groenewegen (1990) found labelled fibres in the nucleus reuniens after injections in the distal part of the ventral subiculum. Wouterlood *et al.* (1990), following anterograde injections in the nucleus reuniens, found terminal labelling is most dense in the molecular layer of the ventral part of the subiculum.

- Direct connections exist between dorsal subiculum and the interanteromedial nucleus of thalamus (Witter *et al.*, 1990). The ventral subiculum does not appear to innervate the anterior nuclei of the thalamus or the interanteromedial nucleus (Witter and Groenewegen, 1990). Shibata (1993) found that the anteromedial and anteroventral nuclei project to the subiculum.

The neurophysiology of the subiculum

In vitro *studies*

Electrotonic properties of subicular neurons

There have been relatively few detailed studies on the intracellular properties of subicular neurons (see Table 1 for a summary). Taube (1993) and Stewart and Wong (1993) suggest that pyramidal neurons *in vitro* can be divided into two groups based on their responses to intracellular current injection and orthodromic stimulation. The first group of neurons ('bursting' neurons) respond with a brief burst of action potentials during the initial 40ms, with each burst containing 3 - 5 action potentials embedded in a depolarising envelope, which is followed by 20 - 30ms period when neurons do not discharge. These neurons showed little spontaneous activity at the resting membrane potential. During the last 30 - 40ms neurons discharge single action potentials. The second type of neurons ('regular-firing' neurons) respond to depolarising current with firing of single action potentials throughout the current pulse.

There is a general consensus that there are more bursting than non-bursting neurons in the subiculum, although the estimated proportions vary from paper to paper (see Table 1). Taube (1993) suggests that in the rat the ratio is approximately 69 % to 31 % in favour of bursting neurons, whereas Behr *et al.* (1996) put this figure at 54% to 46% in favour of

bursting cells. Mason (1993) estimates that 74% of neurons are bursting cells. In the guinea pig, 66% of subicular neurons are thought to be bursting (Stewart and Wong, 1993). Greene and Mason (1996) suggest that bursting cells are more prominent in the deep cell layers of the subiculum, whereas regular-firing neurons are more numerous in the superficial cell layers. In addition, bursting and regular-firing cells can be localised to definite regions of the ventral subiculum: 52% of neurons in the central column were regular-firing neurons compared to 16% in the proximal portion and 10% in the distal region (Greene and Totterdell, 1997). Bursting cells may be involved in amplification of signals which might facilitate the processing of information. Bursting cells may also be involved in the generation and spread of convulsive activity (Behr *et al.*, 1996).

There are no obvious differences in either the measured membrane characteristics or cable properties of bursting and regular firing subicular neurons. Behr *et al.* (1996) found that bursting neurons are significantly different from regular firing cells with respect to their resting membrane potentials (the bursting neurons being more negative). Regular firing neurons also display both a fast and slow after-hyperpolarisation (AHP) following an action potential, whereas bursting neurons show only a slow AHP. In a comparison of CA1 and subicular neurons from the same slice, Mason (1993) found that the values for spike duration are significantly smaller in subicular neurons. No other significant difference was found between the various membrane properties assayed.

TABLE 1. Comparison of subicular cell characterisation studies

Study	Technique	Reported cell-types	Proportions*
[1]	Patch-clamp (*in vitro*)	SB,WB,RS	51%,17%,32%
[2]	Intracellular (*in vitro*)	IB,BD,FS,RS,MRS	40%,31%,2%,18%,9%
[3]	Intracellular (*in vitro*)	B,NB	79%,21%
[4]	Intracellular (*in vitro*)	B,RS	54%,46%
[5]	Freely-moving (*in vivo*)	B,NB,DB,T	34%,41%,14%,11%
[6]	Intracellular (*in vitro*)	B,NB,I	66%,31%,3%
[7]	Intracellular (*in vitro*)	B,RS	69%,31%
[8]	Intracellular (*in vitro*)	B,RS,borderline	74%,12%,14%

Studies

[1] Staff *et al.*, 2000
[2] Greene and Totterdell, 1997
[3] Stewart, 1997
[4] Behr *et al.*, 1996
[5] Sharp and Green, 1994
[6] Stewart and Wong, 1993
[7] Taube, 1993
[8] Mason, 1993

Abbreviations

B bursting
DB depolarized bursters
FS fast spiking
I interneurons
IB intrinsic burst-firing
MRS modified RS
NB non-bursting
RS regular spiking
SB strong bursting
T theta
WB weak bursting

* Proportions are presented in the same order that the cell-types are presented (i.e., in the first study, 51% of cell-types were SB)

A single-spiking mode can be induced in bursting cells as a result of a depolarising injection which shifts the membrane potential from resting potential to approximately -60mV in the guinea-pig (Stewart and Wong, 1993); in the rat, tonic firing replaces bursting modes at membrane potentials at values less negative than –55mV (Mattia *et al.*, 1997; Mason, 1993). Conversely, hyperpolarisation of non-bursting cells does

not convert them to bursting cells (Stewart and Wong, 1993). The firing pattern during a long depolarising current pulse (i.e. burst followed by single spiking) and voltage dependence of the burst discharge (i.e. the loss of burst firing with depolarisation) is not unique to the subiculum and is similar in layers IV and V of sensorimotor cortex, cingulate cortex (McCormick *et al.*, 1985) and EC (Jones and Heinemann, 1988). In the hippocampus, CA3 pyramidal cells generally exhibit a burst discharge without the subsequent single spiking action (Wong and Prince, 1978). The burst firing mode of CA3 also appears to be resistant to depolarisation requiring the membrane to reach -55mV before converting to single-firing pattern (Stewart and Wong, 1993).

The most recent of *in vitro* studies concludes that it may be most appropriate to describe the firing properties of subicular principal neurons as lying on a continuum of 'propensity to burst' (Staff *et al.*, 2000). Staff *et al.* (2000) conclude that subicular neurons belong to a homogenous group with respect to all other electrophysiological properties, and that the differences in their firing properties may be regulated by a slowly inactivating potassium current. Therefore, subicular pyramidal neurons may form a single neuronal class, sharing a burst generating mechanism that is stronger in some cells than others. Surprisingly, they did not find any bursting cells that could be made to fire single spikes, regardless of the membrane potential, a finding which is at odds with previous intracellular studies, and discussed above (Stewart and Wong, 1993; Mason, 1993). Staff *et al.* suggest that since

these studies did not observe strongly bursting cells perhaps bursting was not as robust in their preparations (and thus easier to prevent).

Responses of subicular neurons to afferent stimulation

Following stimulation of several different areas of the hippocampal slice, no differences were observed between bursting and non-bursting cells with regard to their responses (Taube, 1993). Stimulation of different areas within CA1 (stratum radiatum, stratum pyramidale, stratum oriens and alveus) evokes an field excitatory post-synaptic potential (fEPSP) in subicular neurons and this fEPSP is followed by a longer-lasting IPSP, which has a reversal potential. The IPSPs are quite often biphasic having both early and late components. Iontophoretic application of GABA could evoke hyperpolarisations similar in nature to IPSPs seen following stimulation of CA1. This suggests that these IPSPs are mediated by GABAergic transmission, possibly acting through the activation of both GABAa and GABAb postsynaptic receptors as has been suggested for other hippocampal areas. At higher levels of stimulation in any of the CA1 areas, one or more action potentials arise from an fEPSP. Multiple action potentials are also triggered at high levels of stimulation in bursting neurons. Other areas of the hippocampal formation produce different subicular responses, a small depolarising response is observed when area CA3 is stimulated; there is no response to dentate gyrus stimulation. The fEPSPs produced by CA1 or presubiculum are reduced by CNQX, thus suggesting fEPSPs are glutamatergic with distinct

AMPA and NMDA components. The depolarising envelope seen in bursting neurons is likely to be calcium-dependent as this was resistant to application of TTX (Taube, 1993).

Following intracellular and field potential recordings of rat subicular neurons *in vitro,* Stewart (1997) demonstrated that after a single stimulation of either entorhinal, presubiculum or CA1, short-latency (< 5 ms) fEPSPs were evoked in both regular and bursting subicular cells. In contrast, long-latency (> 10 ms) fEPSPs were only observed in both subicular cell types following stimulation of the presubiculum and not of CA1 or EC. Stewart (1997) suggests that the output of the two cell types may be different with bursting neurons projecting to the presubiculum and regular firing neurons projecting to the EC.

Intracellular recordings from subicular cells following stimulation of layer III of the medial EC produced a combination of short-latency excitatory and inhibitory responses (Behr *et al.,* 1998). Inhibition was blocked using GABA antagonists and the isolated AMPA or NMDA components of subicular neurons were evoked. Following simultaneous activation of alvear fibres and the layer III entorhinal projection subicular fEPSPs were augmented, while delayed stimulation of alvear fibres after activation of the EC produced a weak inhibition of fEPSPs in the subiculum (Behr *et al.,* 1998).

In vitro investigations of the plasticity of subicular synaptic transmission

There are very few studies examining synaptic plasticity in the rat subiculum *in vitro*: Boeijinga and Boddeke (1996) stimulated the CA1/subiculum border and recorded from the middle portion of the pyramidal layer in the subiculum. Application of theta-patterned stimulation induced robust long-term potentiation *in vitro* in 14/20 experiments. Dolen and Kauer (1998) recorded a combination of field EPSP and population spike responses in the subiculum following stimulation of the pyramidal layer of area CA1. Following theta-burst stimulation, LTP was induced that lasted for 20 minutes. Methot *et al.* (1997) demonstrated that tetanic stimulation of area CA1 produce long-term potentiation in dorsal subiculum, whereas the same stimulation produces long-term depression in the ventral subiculum.

In vivo *studies*

Anaesthetised preparations: field potential and single unit studies of the subiculum

Van Groen and Lopes da Silva (1986) showed that stimulation of the lateral EC elicited the largest fEPSP, of all their recorded subicular fEPSPs, in the septal part of the subiculum, whereas stimulation of the medial EC produced the largest response in the temporal part of the subiculum. Van Groen and Lopes da Silva (1986) in turn showed that

stimulation of the septal subiculum evoked the largest response in the laterocaudal EC; temporal subicular stimulation evoked the largest response in the mediocaudal EC. These physiological data support the conclusion that connections between the EC and subiculum in the cat are reciprocal.

Gigg *et al.*, (1997; 2000) examined subicular neuronal responses to CA1 and lateral EC activation. Stimulation of CA1 produced excitation-inhibition sequences in bursting and non-bursting principal cells and multipolar cells (presumed inhibitory neurons). The predominant subicular response to EC stimulation was weak inhibition.

In vivo investigations of the plasticity of subicular synaptic transmission

Long-term potentiation (LTP)

Commins *et al.* (1998a, 1999) examined LTP in the projection from CA1 to the subiculum *in vivo*. A rapidly-stabilising potentiation was induced in the CA1-subiculum pathway using high-frequency stimulation. Additional experiments showed that potentiation once induced remains unchanged after three hours using the above HFS protocol (Commins *et al.*, 1999). Furthermore, once the CA1 to subiculum pathway is potentiated, it seems resistant to further episodes of HFS (Commins *et al.*, 1999). Commins *et al.* (1999) were also able to induce LTP in this pathway using theta-burst stimulation (TBS).

Long-term depression (LTD)

Anderson *et al.* (2000) found no evidence of LTD induction in the CA1-subiculum pathway using two low-frequency stimulation protocols and two two-pulse stimulation protocols. However, LTD could be induced in the same pathway in rats that had been previously stressed (Commins and O'Mara, 2000).

Paired-pulse facilitation (PPF)

Commins *et al.* (1998b) found reliable and robust PPF in the CA1-subiculum pathway across a range of interstimulus intervals (ISI) from 10 ms to 500 ms; reaching a maximum at 50 ms. PPF may act to increase the reliability of synaptic transmission by ensuring that signals which occur in rapid succession are amplified, thus increasing the signal-to-noise ratio of an input, reducing the possibility that a signal is lost against a noisy background (Commins *et al.,* 1998b).

Lesion analyses of the subiculum

There have been few analyses of lesions of the subiculum. Schenk and Morris (1985) conducted the first study of the effects of lesions restricted to the subicular complex. There were two experimental groups: one group was given lesions of the EC and pre- and para-subiculum; the other experimental group was given lesions encompassing the entire subicular complex and the EC. The two groups were tested on the water maze task, a spatial memory test designed by Morris [e.g., Morris (1981)] in which rats placed into a circular pool surrounded by cues attempt to find an escape platform hidden beneath the surface of the water. Results indicated that there is a profound impairment in spatial localisation following lesions of both groups. There was a partial and selective recovery of spatial localisation during post-operative training, although larger lesions encompassing most of the subiculum, in addition to other structures, limit the extent of the recovery. Although the authors did not specify a particular role for the subiculum, it clearly plays an important part in spatial localisation.

In a follow-up study also conducted using the water maze, Morris *et al.* (1990) indicated that both hippocampal and subicular lesions cause impairment in the initial post-operative acquisition of place navigation but did not prevent eventual learning to levels of performance almost as effective as those of controls. Different strategies are deployed by hippocampal and subiculum lesioned groups: the hippocampal lesioned

group employ a circling strategy staying close to the wall, whereas rats with subicular lesions behave like naïve rats, searching the water maze in a manner similar to rats with no prior knowledge of the location of the platform. Furthermore, both hippocampal- and subicular-lesioned rats were impaired during a subsequent retention/relearning phase. Morris *et al.* (1990) suggest that hippocampal lesions may cause a dual deficit - a slower rate of learning and a separate navigational impairment. Subicular lesions they suggest, however, may cause an impairment of long-term spatial learning (because subicular lesioned rats were impaired in the postoperative learning of the hidden platform) but little impairment in spatial processing or short-term memory (because subicular lesioned rats displayed a greater and more consistent improvement in escape latency than hippocampal lesioned rats in a *delayed matching to place phase*).

Oswald and Good (2000) examined the effects of combined lesions of the subiculum and EC on performance in the Morris water maze. They extended the Schenk and Morris (1985) study by including an intramaze landmark in the water maze because a previous study had demonstrated that rats with hippocampal lesions are able to locate the platform when an intramaze landmark is placed in the pool at a fixed distance and direction from the platform (Pearce *et al.,* 1998). Both the lesioned group and control group easily acquired this task. In a second experiment in the water maze, without an intramaze cue, the subicular and EC lesioned animals were significantly impaired in finding the hidden platform. The subiculum-entorhinal group was also significantly impaired on the probe

task, where the platform was removed entirely from the water maze: the lesioned group spent less time in the platform quadrant than control animals. The authors suggest that damage to the subiculum and EC does not affect a general navigational-directional strategy (because rats with this damage could still swim to the platform with the landmark) but impairs the integration of geometric information.

Galani *et al.* (1997) examined rats with lesions of various regions of the hippocampal formation on a battery of tasks for examining locomotor activity, reactivity to novelty, spatial, working and reference memory in the Morris water maze and learning in the Hebb-Williams maze. It was found that rats with hippocampal lesions were impaired on most of the tasks, whereas the subicular-lesioned animals were only impaired in the probe trial of the watermaze task. Galani *et al.* (1998) found that rats with subicular lesions were impaired in a working memory task in the water maze (in which the position of the hidden platform was changed before each day's testing) and, interestingly, in an object exploration task (Poucet, 1989) where they failed to react to a non-spatial object change. They do not offer an interpretation of these findings.

Are there distinct roles for the dorsal versus ventral portions of the subiculum? Maren (1999) examined the effects of neurotoxic or electrolytic lesions of the ventral subiculum on Pavlovian fear conditioning. Freezing was measured in rats following conditioning by a number of tone-footshock trials in a novel chamber. Ventral subicular

lesions made prior to training produced a severe deficit in acquired freezing to the tone but modest context freezing deficits, whereas posttraining lesions produced severe deficits in freezing to both tone and context. Ventral subiculum therefore may play an important role in both the acquisition and expression of Pavlovian fear conditioning. Interestingly, lesions of the subiculum had no effect on latent inhibition (prior presentation of conditioned stimulus for a repeated number of trials before pairing of conditioned and unconditioned stimuli), whereas entorhinal lesions seem to disrupt latent inhibition (Coutureau *et al.*, 1999). Latent inhibition is considered to be an animal behaviour that is relevant to schizophrenia; the entorhinal cortex and potentially the subiculum may be considered as possible target sites for antipsychotic drugs (Greene, 1996). Lesions of the ventral subiculum also impair the acquisition of spatial tasks. Laxmi *et al.* (1999) examined the ability of rats with ibotenic lesions of the ventral subiculum to acquire a rewarded alteration test in a T-maze.They showed that subicular lesioned animals were impaired in this task compared to controls.

Recordings in freely-moving animals

Given the vast body of work which demonstrates that the hippocampus contains cells which, in the freely-moving animal, have a strongly spatially-selective firing correlate (Muller *et al.*, 1991; O'Keefe, 1979; O'Mara, 1995), it would be surprising if subicular neurons did not demonstrate some such firing correlate also. As expected, several studies have reported that subicular neurons do show spatially-selective firing (Barnes *et al.*, 1990; Sharp and Green, 1994; Sharp, 1997; Sharp, 1999a,b,c; O'Mara *et al.*, 2000).

Possibly the first study of activity in the subicular complex of the freely-moving rat was conducted by Segal (1972) who reported a relationship between subicular unit activity and the sounding of a tone paired with food reward. Spatial firing correlates of these units were not examined in this study. Barnes *et al.* (1990) and Muller *et al.* (1991) provided the first extended descriptions of the spatially-selective firing properties of subicular neurons, recording in the radial arm maze and a cylindrical open field respectively. Barnes *et al.* (1990) found that, in general, subicular cells showed spatially localised firing on the radial arm maze, though such cells displayed a rather low spatial specificity. This may be because the authors were unable to distinguish complex spike cells (which display very high spatial specificity and consistency in the hippocampus) from theta cells (which have a primary locomotor correlate and a lower spatial specificity): the subsequent grouping of complex

spike cells and theta cells may produce cell recordings with lower spatial specificities than studies which *are* able to distinguish these cell types (Barnes *et al.*, 1990). These authors argue, however, that such a grouping of CA1 or CA3 cells does not greatly reduce the spatial specificity in these cell recordings; subicular neurons therefore must have a lower spatial specificity than prior hippocampal regions. An alternative explanation is that they simply have a different firing correlate from that of place.

On the basis of their recordings in a cylindrical open field, Muller *et al.* (1991) suggest that subicular neurons can be divided into three general classes. The first class of subicular neurons resemble the head-direction cells found in the presubiculum; the firing of such neurons is controlled by the angular position of the cue card on the cylinder wall. The second class encode both head-direction and positional information; the firing of these neurons reflects position but is modulated by head direction. Interestingly, such cells may have two preferred orientations, unlike head direction cells of the dorsal presubiculum which have only one preferred direction. The primary correlate of the third class of neurons appears to be place and they are similar to those described by Barnes *et al.* (1990); these cells have a relatively noisy representation of space compared to the hippocampal representation, but less noisy than the representation in EC.

The most comprehensive analysis of the firing correlates of subicular neurons conducted to date is that of Sharp and Green (1994). In this study, most subicular cells show a robust locational signal. This pattern of firing, however, is distinct from hippocampal firing in that subicular cells tend to fire throughout the environment and show multiple peaks of activity. The authors also identified different types of cells which they classify as bursters, non-bursters, depolarized bursters, and theta units (thought to be interneurons). This classification is similar to subicular cell types identified *in vitro* (Taube, 1993 - see above). Bursting cells did not differ from non-bursting cells with respect to their spatial firing properties, suggesting that differences in membrane properties do not imply a difference in coding of environmental cues. The authors, however, did find that cells located adjacent to the hippocampus have a lower average firing rate, spatial signalling and firing field size when compared with cells located near to the presubiculum (see Figure 1.2). This is interesting because of the topographical projections of area CA1 to the subiculum (Amaral *et al.*, 1991 - also see above) and also because these two regions have different efferent connections (Amaral and Witter, 1995; Witter *et al.*, 1989). Sharp and Green (1994) and O'Mara *et al.* (2000) also reported that subicular place fields can follow rotations of a salient cue.

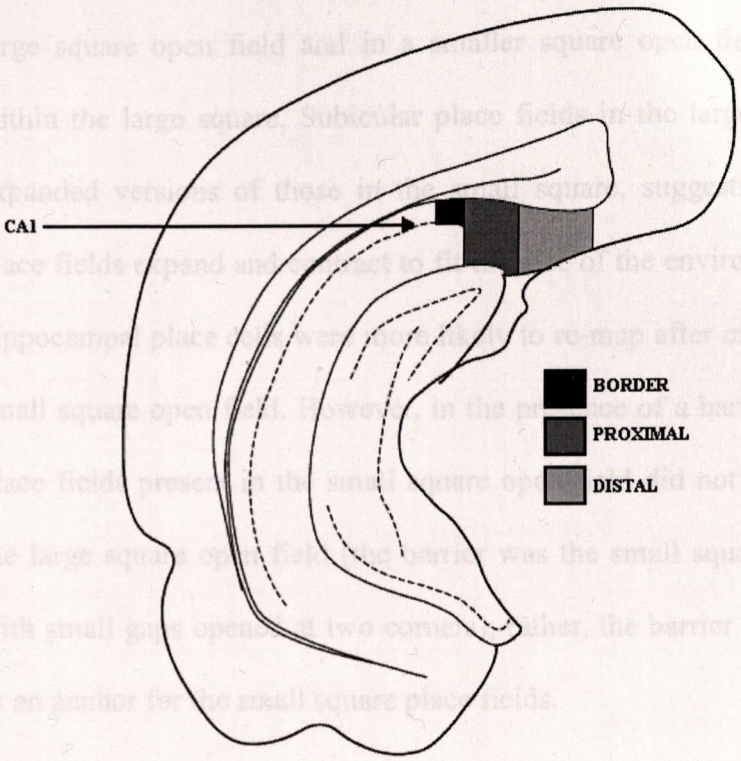


Figure 1.2. Sharp and Green (1994) divide the dorsal subiculum into three regions ('border', 'proximal', and 'distal') based on relative distance from the hippocampus. They find that subicular units located in the distal region have a lower average firing rate, spatial signalling and firing field size when compared with cells located in the proximal region. Bregma -6.04 mm (Paxinos and Watson, 1996).

Sharp (1997) compared subicular place cell firing with hippocampal place cell firing in two adjacent geometrically and visually distinctive environments (cylindrical and square open fields). Subicular place cells showed very similar patterns of firing in both environments while, in line with previous work, hippocampal place cells normally showed different patterns of firing in the two environments. This result suggests that the subiculum codes space in a qualitatively different way to the

hippocampus. Sharp (1999a) examined subicular place fields in both a large square open field and in a smaller square open field positioned within the large square. Subicular place fields in the large square were expanded versions of those in the small square, suggesting that these place fields expand and contract to fit the size of the environment; again, hippocampal place cells were more likely to re-map after exposure to the small square open field. However, in the presence of a barrier, subicular place fields present in the small square open field did not stretch to fill the large square open field (the barrier was the small square open field with small gaps opened at two corners); rather, the barrier seemed to act as an anchor for the small square place fields.

Sharp (1999b) proposes that the qualitative differences between the hippocampus and the subiculum in the representation of space lend support to a path-integration model of spatial behaviour. Specifically, subicular place cells appear to transfer a single, universal locational firing pattern from one environment to the next, changing the pattern's size and shape to fit into the current environmental boundaries. The model proposes that the subicular / entorhinal spatial representation 'assist[s] the hippocampal layer to rapidly form new environment and context specific "maps" for each new environment / temporal context ("episode") the animal experiences.' (Sharp, 1999b). Support for this model comes from Sharp (1999c) who demonstrated that subicular place cells appear to anticipate future locations by approximately 50 to 70 ms, while hippocampal place cells were best correlated with positions about 30 to

40 ms in the future, showing that the subicular signal is apparently generated earlier than the hippocampal signal and as a result cannot be the result of simple transmission of spatial information from the hippocampus alone.

Theories of subicular function

The rodent hippocampal formation (HF) is believed to play a crucial role in spatial navigation (*e.g.*, O'Keefe and Nadel, 1978). Compelling evidence of its role in spatial navigation comes from lesion studies which show that damage to the HF, or its efferent projections, can disrupt performance in various spatial tasks such as the Morris water maze and the radial arm maze (Morris *et al.*, 1982; Walker and Olton, 1979). More evidence comes from single-unit recordings in freely-moving rats which have shown that the firing of some HF units ('place cells') is correlated with discrete portions of the animal's current environment ('place fields') (O'Keefe, 1979; O'Mara, 1995; Wiener, 1996). These findings led to the development of a number of HF-centred theories of rodent navigation of which the best known is the cognitive mapping theory (O'Keefe and Nadel, 1978).

Further work has extended the spatial theory of the HF to encompass path integration (e.g., McNaughton, 1989; Samsonovich and McNaughton, 1997; Sharp, 1997). Path integration, also called 'dead reckoning', is the ability to return directly to a starting point from any location in an environment, even in the dark or after a long circuitous route (Barlow, 1964; Gallistel, 1990). Mittelstaedt and Mittelstaedt (1980) examined path integration in an elegant experiment in which they showed that a female gerbil searching for a missing pup could return along a straight-line, or 'geodesic', to the nest once the pup had been

found. If the animal was rotated during its search at speeds below the vestibular detection threshold, the return path was offset by a comparable amount, eliminating the possibility that auditory or olfactory cues had guided the animal.

The specific role of the subiculum is addressed in a number of the 'spatial' theories in relation to the structure that represents the 'anatomical instantiation' of path integration. As discussed above, Sharp (1999b), based on her work showing that subicular place fields are similar in different environments (Sharp, 1997), suggests that the subiculum and entorhinal cortex work together as the key anatomical structures in instantiating path integration (see p. 37). Meanwhile, Redish and Touretzky (1997) have suggested instead a three-stage loop of subiculum, parasubiculum, and superficial entorhinal cortex: they base their claim on five criteria that they say the structures involved in the path integration system must meet. These are:

1. *They must collectively be able to represent the position of the animal* (because in order to compute the return to the starting point the path integration system must have a representation of the current location)
2. *They must receive input from the head direction system* (because to update the representation of location the path integration system must have a representation of the direction in which the animal is moving)

3. *They must receive information about self-motion from the motor and vestibular systems* (because the path integration system requires information about the speed at which the animal is travelling)
4. *They must update the representation as the animal moves around the environment* (because path integration must occur *as* the animal moves)
5. *They must send output to the area associated with the place code* (because the representation of location is updated even in the dark [Quirk *et al.*, 1990])

(See Redish [1999], p. 77)

Redish (1999), in his comprehensive review of the spatial theory of HF function, explains the Redish and Touretzky (1997) model by claiming that Sharp's model does not meet criterion five: that is, the subiculum alone cannot be the anatomical instantiation of the path integration system because it does not feed directly into the hippocampus; rather, they claim that their model *does* meet all five criteria since the entorhinal cortex feeds into the hippocampus. However, given that Sharp's model, as well as including the subiculum, also includes the entorhinal cortex (which sends a strong projection to the hippocampus) it is difficult to see why Redish states that this model does not meet his five criteria.

If the subiculum is indeed a component of a spatial navigation system or a path integration system then lesion studies will help to provide

evidence. Unfortunately, the picture at the moment is not clear. While the lesion studies discussed above do provide strong evidence of a general role of the subiculum in spatial navigation, no lesion study has as yet addressed the role of the subiculum specifically in path integration processes.

A different school of thought suggests that the role of the rodent HF in spatial navigation is only a specific example of what is in fact a wider mnemonic role of the HF in rodents, primates, and humans (e.g., Cohen and Eichenbaum 1993; Rolls, 1996). This represents an attempt to construct a coherent theory of HF function from disparate cross-species empirical evidence. Given, however, that no model in this domain suggests an explicit role for the subiculum we cannot discuss their relative merits further.

Finally, Gray (1982) stated that the subiculum occupies a central position in the processing of information in the hippocampal memory system: it receives inputs of information at different levels of processing, and it is able to transfer information to different brain structures. The pivotal position of the subiculum between the hippocampus and cortex suggests that it is a likely site for comparing the incoming and outgoing information (Gray, 1982). Gray therefore stated that the subiculum most likely performs a comparator-like function (in Gray's original postulation the model was first developed as a theory of anxiety). It is difficult to see how this hypothesis could be tested.

Synthesis

The dorsal subiculum sends a strong location-related signal to each of the areas to which it projects. This signal is transmitted via both bursting and non-bursting spike train patterns (the majority are bursting patterns which may indicate the importance of the subicular signal – bursting is a reliable means of communicating between neurons). The subiculum thus provides a way for hippocampal information to reach other brain areas. These areas include structures thought to be involved in a wide variety of learning and memory functions, such as instrumental reward learning (nucleus accumbens), aversive learning (cingulate cortex), and working memory (prefrontal areas). Thus, one role of the subiculum may be to transmit information about the animal's current location in space so that it may be used for various navigation-related functions, such as path integration, and the storage of information about the spatial location of rewarding and aversive stimuli.

The aim of this thesis work is to further our understanding of subicular function by examining single unit responses in the subiculum under a variety of conditions: from the 'baseline' responses of single units to their 'high-level' representations.

Chapter 2

Main methods used in this thesis

Chapter contents

2.1 Summary

2.2 Methods

2.1 Summary

Male adult Wistar rats were implanted with eight microelectrodes in the most dorsal part of the subiculum at specific stereotaxic coordinates (see each 'Chapter methods' for specific coordinates and hemisphere). The electrodes were mounted on a microdrive to permit dorsal-to-ventral movement of the electrodes through the target structure.

Before every experimental session, electrodes was scanned for neuronal activity (action potentials and EEG). If suitable activity was present on an electrode, the activity was recorded while the rat performed a task, specifically designed to try to engage the subiculum. During each task, the activity of each recorded unit was correlated with both the simultaneous behaviour and location of the rat. At the end of the experimental session off-line analyses of the activity were conducted to examine possible subicular unit correlates with aspects of that task.

After every experimental session all eight electrodes were moved ventrally by approximately 50 μm in an effort to encounter more units. In such a way a large sample of subicular units was amassed for each task. Finally, after euthanizing each rat, brains were sectioned and electrode paths were examined under a microscope to check for correct placement.

Note

This chapter gives details of the procedures common to all the experiments in this thesis. For the specific procedures used in each experiment, the separate 'Chapter methods' sections should be consulted.

Subjects

In all experiments presented in this thesis, a total of ten adult male Wistar rats (Bio Resources Unit, University of Dublin), weighing 350 – 500 g upon arrival in the laboratory, were used as subjects. Before surgery, rats were housed in pairs in a temperature controlled laminar airflow housing unit, and maintained on a 12:12 light-dark cycle (0800-2000hrs). All testing was carried out during the light phase. After surgery, rats were housed singly in the housing unit.

Microdrive

The microdrive assembly was based on a design by O'Keefe (e.g. O'Keefe and Speakman, 1987; Speakman and O'Keefe 1990). In brief, it consists of a vertical post (17 gauge) which carries a hollow screw which, when turned, allows the vertical movement of an acrylic 'nut'. The nut itself is prevented from turning by a post mounted on, and parallel to, the screw post. The nut holds a 14-pin connecting plug behind and a hollow guide cannula (25 gauge) in front which contains the electrode wires. The

screw can be turned by a small metal turner which makes contact with flanges on the top of the screw. In order to ensure accuracy in the movement, a spring maintains the contact of the lower screw bearing surface against a fixed bush with sufficient pressure to prevent vertical displacement. The electrodes can be lowered in steps of approximately 50 μm by making one-eighth turns.

Electrodes

The recording electrodes consisted of eight Nichrome wires (80 % nickel / 20 % chromium alloy; FORMVAR insulated; 25 μm bare wire diameter; Advent Research Materials Ltd., Halesworth, Suffolk, UK) twisted together to form two bundles of four electrodes. The bundles were cut flat to expose the tips at approximately the same level. Both bundles were inserted into one stainless steel guide cannula (25 gauge) fixed to the microdrive. Occasionally the electrode tips would not stay together; in these instances glue was applied to the insulated part of the electrodes. The microdrive was originally designed with the principles of tetrode unit separation in mind. *It should be noted, however, that all units in this thesis were recorded using single electrode techniques (operating in differential mode to reduce noise common to both channels [a quiet electrode was used as the differential electrode]).* Multiple electrode techniques (using tetrodes and stereotrodes) have been shown not to improve the quality of subicular unit separations (Sharp, 1997). Stereotrodes were tested briefly in this laboratory, and, similarly, no

improvement in the ability to separate subicular units was noted (for a comprehensive discussion of unit separation see Appendix 1).

Surgical implantation of electrodes

Rats were anaesthetised with intraperitoneal injections of sodium pentobarbital (60 mg/kg Sagatal, Rhone-Merieux, Essex, UK), followed by atropine 0.5ml/50kg, (Bimeda, Dublin, Ireland), with supplementary doses of sodium pentobarbital administered if necessary. They were placed in a stereotaxic apparatus (Kopf Instruments, Tujunga, CA, USA), the skull was exposed, and a small burr hole was drilled over the electrode implantation site so as to expose the brain. Seven bone screws were inserted at strategic sites around the exposed skull so as to anchor the lightweight (~1g) microdrive assembly. One of these screws also acted as the ground connection. The microdrive electrode bundles were aimed at the dorsal part of the subiculum using stereotaxic methods (Paxinos and Watson, 1996. For exact co-ordinates see the separate methods sections in the relevant experiment chapter). The dorsal part of the subiculum was chosen for two reasons: (1) a study of the dorsal subiculum would be more readily interpreted in light of the greater understanding of and research effort directed at the dorsal part of the hippocampal formation in general; and (2) from their initial position the electrodes would pass through the greatest extent of the subiculum.

Once the electrodes had been lowered to the required depth, the small exposed area between the cannula and the surrounding skull was packed with sterile gel (Granugel, Convatec, Uxbridge, UK) and the entire apparatus was cemented to the skull with dental acrylic (Associated Dental, Swindon, UK). After surgery, the wound area was dusted with antibiotic powder (Cicatrin, Wellcome Ireland Ltd., Dublin, Ireland) and the rat was kept under observation for several hours. The animal was allowed to recover for at least six days before experimentation began.

Recording techniques

A headstage (NB Labs, Texas, USA) containing field effect transistors (FETs), one for each channel, was plugged into the microdrive connector for recording purposes. The FETs are necessary for lowering output impedance so as to prevent electrical artifacts from obscuring the low amplitude extracellular neuronal signals (artifacts produced, for example, by movements of the recording cable). The headstage also contained a small LED which could be detected by the overhead video tracking system and is used to follow the location of the rat in the maze. The camera operated in one-spot (monochrome) tracking mode, and was fitted with a wide-angle lens. The tracking system (DataWave Technologies, Colorado, USA) split the environment into 256 x 256 pixels and sent time-stamped positional data (x-y coordinates) to the acquisition system (see below).

The output from the headstage was fed via a long cable to a filter-amplifier (A-M Systems Inc. Model 1700, Carlsborg, WA, USA) where each spike channel signal was amplified (x 10,000) and filtered (bandpass 300 Hz – 5 kHz). EEG channels were amplified (x 1,000) and filtered (bandpass 1 Hz – 500 Hz).

The amplifier output was split three ways and fed into a loudspeaker (for auditory feedback), an oscilloscope, and a computerized analogue-to-digital conversion system (DataWave Technologies, Colorado, USA). The DataWave system captured and stored only the neuronal events whose amplitude exceeds a user-defined threshold. The waveforms of each event (spikes) were digitized by the program according to a user-defined sampling rate (typically 33 kHz at 32 data points per spike) and produced 1 ms of activity for each spike. The DataWave system also received the EEG output from the amplifier and stored this information as a continuous record; the sampling rate for EEG is much lower than for spikes (approximately 150 Hz) and may be recorded continuously since it places much less demand on computer memory. The DataWave system also enables the experimenter to record the time of any behavioural or other event by pressing a key on the keyboard when the event occurs. These 'event flags' are also saved to file and can be processed off-line.

In summary, the DataWave acquisition system stored action potential, EEG, positional and event flag data, all time-stamped to enable off-line analysis.

After data collection ASCII files were exported from DataWave: these files contained unit waveform data with time-stamps, positional data with time-stamps, event-flag data with time-stamps, and continuous EEG records with time-stamps. All ASCII files were imported into and analysed with custom-written software (M. Anderson) using Matlab, a C-based high-performance language for technical computing (Matlab, Version 5, The MathWorks Inc., Massachusetts, USA). Matlab is particularly suited to this purpose as it allows rapid manipulation of large data sets (see Appendix 2 for sample screen print-outs).

Data analysis usually proceeded in the following order:

(i) *Unit separation.* All waveform files were separated into single units using a spike-sorting program.

(ii) *Spatial analysis.* Firing rate maps, when appropriate, were plotted.

(iii) *Electrophysiological analysis.* Electrophysiological characteristics of units were calculated, including plotting inter-spike interval histograms (ISIHs) and autocorrelation histograms (ACHs).

(iv) *Behavioural analysis.* Event flags were processed by plotting peri-event time histograms (PETHs).

(v) *EEG analysis.* Simultaneous EEG traces and action potential data were analysed using a data replay plotter.

(vi) *Histological analysis.* Brain sections were examined to verify correct electrode placement.

(vii) *Statistical analysis.* Statistical analyses appropriate to each experiment were then conducted.

Occasionally other analyses were used, as each study required, including a measure of the 'information content' of each unit's firing (Skaggs *et al.*, 1993). Briefly, this measure treats the firing of a unit as the output of an information channel, and bins the firing of that unit around any parameter of interest (e.g., a location-related parameter would bin the firing in pixels), and gives a figure, measured in bits of information per spike (bits / spike), which represents the amount of information about that parameter signalled by that unit. The information content, I, is calculated according to the formula

$$I = \sum_i (\lambda_i / \lambda) \log_2 (\lambda_i / \lambda) P_i ,$$

where λ_i is the mean firing rate in bin i, λ is the overall mean firing rate, and P_i is the occupancy probability of bin i (which is simply the number of spikes in bin i divided by the total number of spikes). Further

explanation of this measure is given in the experimental chapters in which it is used.

(i) Unit separation

Unit separation is a topic of singular importance in all electrophysiological recordings. Subicular unit separation, however, appears to present its own difficulties: even with commercially available software satisfactory unit separation is difficult to achieve owing, we believe, to the number of simultaneously active neurons in the subiculum. A detailed description of the inherent problems of unit separation in the subiculum are presented in Appendix 1. Details of the separation techniques used in this thesis are given below.

All unit separation in this thesis was conducted using a custom-written template matching program (Matlab program; M. Anderson). This technique was chosen because '[no] linearly derived feature set can outperform template matching, provided that the [background] noise is white and uncorrelated with spike class' (Wheeler, 1998). Our program uses a template matching algorithm which measures the so-called 'City block distance (CBD)' between each spike and a template constructed from a sample of spikes. This measure is expressed mathematically as,

$$d_j^{CBD} = \sum_k \left| f_k - m_{jk} \right|,$$

where d_j^{CBD} represents the sum of the absolute distances between each sampled point on the template and spike. If the value of the d_j^{CBD} is less than a pre-defined value then the current spike is assigned to that template class. The templates are constructed automatically, based on a random selection of a user-defined number of waveforms from the file (typically 20%). The template production algorithm attempts a sequential learning of spike classes. The first spike in each file becomes the first class then each new spike is compared to all previously defined classes. If a match is made, the spike is assigned to that group and the features of the class are updated; if there is no match a new class is created. The number of templates may be reduced at the end by removing all groups with less than a user-defined number of matches.

To be accepted for further analysis units had to meet the following separation criteria:

(1) initial visual inspection of overlaid unit waveforms confirmed or disconfirmed similarity of waveform shape. Units with dissimilar waveforms were rejected (although template matching is designed to eliminate such units);

(2) visual inspection of averaged unit waveform shapes revealed any 'noise spikes', waveforms containing impossible components which are occasionally acquired by the recording system and can be produced by nearby electrical equipment being turned on or off, mobile phones etc.;

(3) autocorrelation histograms (ACHs) were plotted immediately after separation and particular attention was paid to the 0 – 1 ms bin in these plots. Since we know that typical neurons have refractory periods of *at least* 1 ms and that this holds for subicular neurons also (Taube, 1993), any units whose histograms showed counts in the 0 – 1 ms bin were rejected since this represents a contravention of the refractory period constraint;

(4) ACHs also highlighted any irregularities in unit firing patterns sometimes caused by noise contamination, artifacts which were not eliminated under criterion (2) above.

(iii) Spatial analysis

Firing rate maps enable easy visualisation of location-specific firing. They are constructed by dividing the video image of the open-field environment into pixels. The number of spikes occurring when the rat occupied a particular pixel is divided by the total time the rat spent in that pixel (in seconds) to produce a firing rate for that pixel (in Hz). The firing rate in each pixel is mapped as a contour plot using Matlab's contouring algorithm. This contouring algorithm treats an input matrix Z as a regularly spaced grid, with each element connected to its nearest neighbours. The algorithm scans this matrix comparing the values of each block of four neighbouring elements (i.e., a cell) in the matrix to

pre-determined contour level values. If a contour level falls within a cell, the algorithm performs a linear interpolation to locate the point at which the contour crosses the edges of the cell. The algorithm connects these points to produce a segment of a contour line. The maps are colour-coded in increments of 20% of the peak firing rate for each individual map.

The 'peak rate' displayed at the bottom of each firing rate map is equal to the rate of the pixel with the highest firing rate in each map. The 'non-zero pixel rate' was also calculated. This is the mean of all the pixels in a firing rate map displaying a firing rate above 0 Hz. This measure controls for the possibility that units would not fire in all areas of the task environment but only in discrete locations, thus biasing the simple mean rate.

(iv) Electrophysiological analysis

ISIHs show the timing relationships between spikes fired by the same unit. They are constructed by summing (or 'binning') the time intervals between consecutive spikes: the x-axis displays the length of the time interval (typically ranging from 0 ms to 500 ms) and the y-axis shows the number of intervals that occurred within each bin.

ACHs are constructed by summing the number of times a spike occurs within a user-defined time period (again typically ranging from 0 ms to

500 ms) *given the occurrence of a spike at time 0 ms.* Both histograms can indicate rhythmicity in the firing of a unit and can assist with unit classification (e.g., Sharp and Green, 1994).

The amplitude (in μV) and duration (in ms) of each unit was calculated. Also, simple mean rate (in Hz) was calculated for each unit by dividing the total number of spikes in a session generated by that unit by the session time (s).

(v) Behavioural analysis

PETHs represent a time-locked and averaged distribution of unit firing with respect to one of the event flag-coded behaviours. PETHs are constructed by binning the unit activity with respect to the event flag time (which is used as a zero reference), within a user-defined time range (typically from 1 to 20 s before and after the event flag). Bin-widths are selected to emphasise any differences in firing between pre- and post-event periods (typically 500 – 1000 ms). This process continues for the same event flags in the file to produce a cumulative histogram; the x-axis represents the time range, and the y-axis represents the number of spikes occurring during each bin in that time range. PETHs help to detect correlations between unit discharge and behaviour.

(vi) EEG analysis

EEG was visualized using a data replay plotter (Matlab program; M. Anderson) which also allowed the simultaneous viewing of spike times and event flags; as such, portions of the EEG trace could be cropped for further analysis. Continuous records of unit and EEG activity were also visualized.

EEG was analysed using discrete Fourier transforms. Fourier analysis is extremely useful for EEG analysis as it breaks down the signal into constituent sinusoids of different frequencies. Using this data power spectra were plotted, and dominant frequencies present in the EEG appear as peaks in the plot.

(vii) Statistical analysis

Where appropriate, differences between firing rates were analysed parametrically, depending on the task design, with one-way or two-way analyses of variance (ANOVA). For analyses of firing rates before and after the onset of a particular behaviour (as recorded with the event flags and displayed in the PETHs), paired t-tests were used (a plot of the differences pre-and post-flag showed them to be normally distributed). All statistics were calculated using the Statistical Package for the Social Sciences (SPSS) software.

(viii) Histological analysis

At the end of each experiment, rats were deeply anaesthetized using sodium pentobarbitol then perfused. Brains were removed and stored in 4% formaldehyde for several days. Brains were subsequently sliced on a vibrotome and all electrode placements were viewed under a microscope to verify correct positioning.

Chapter 3

Baseline recordings of subicular unit firing and EEG in freely-moving rats

Chapter contents

3.1 Summary

3.2 Introduction

3.3 Method

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3.1 Summary

In an effort to characterize the in vivo *electrophysiological characteristics of the subiculum, we recorded subicular unit activity and the subicular electroencephalogram (EEG) while rats occupied their home cages. As well as affording the opportunity to compare fast neuronal activity (action potentials) with slow neuronal activity (EEG), we were able to correlate both types of activity with the ongoing behaviour of the animal.*

Following consideration of electrophysiological characteristics, units were separated into four classes: bursting units, regular spiking units, theta-modulated units, and fast spiking units. The bursting and regular spiking unit classes are well-known cell types in the hippocampal formation, while the fast spiking units appear to be interneurons. Interestingly, the bursting unit class showed much variation, with some units bursting strongly while others appeared to burst very little; this result is interpreted in light of a recent in vitro *study which reports similar findings (Staff et al., 2000). The theta-modulated class, however, is novel compared with previous classifications. These units were similar to regular spiking units in all respects except that they increased their firing significantly when theta oscillations were present in the simultaneous EEG record.*

The EEG showed familiar patterns to hippocampal EEG (e.g., O'Keefe and Nadel [1978]), with theta oscillations dominating 'alert, moving' behaviours, while large amplitude irregular activity (LIA), which included sharp waves, predominated when theta oscillations were not present, mainly during 'alert, still' and 'quiet' behaviours.

3.2 Introduction

To describe the precise nature of the subiculum's interaction with other structures, it will be crucial to characterize subicular neurons, and what role each neuronal type plays in subicular function. To this end, a number of studies have attempted to classify cell types within the subiculum, using different techniques, such as intracellular recording (Greene and Totterdell, 1997; Stewart, 1997; Stewart and Wong, 1993; Taube, 1993; Mason, 1993), patch-clamping recording (Staff *et al.*, 2000), and unit recording in the freely-moving animal (Sharp and Green, 1994). Taken together, these studies have generated a confusing nomenclature of putative subicular cell types (see Table 1). There is, however, broad agreement between studies in the distinction between bursting cells (usually defined as cells which fire 2-6 fast action potentials with, approximately, 5 ms interspike intervals riding, on a slow potential [Stewart, 1997]) and regular spiking cells (which typically fire only single action potentials with interspike intervals of 64.5 ± 37.5 ms [for the first interval] and 159.0 ± 89.3 ms [for the last interval] during intracellular current injections [Behr *et al.*, 1996] although Stewart [1997] reports that with high-frequency stimulation regular spiking cells fire with the highest probability at 10 ms interspike intervals). There is also general agreement that subicular bursting is an intrinsic membrane property of subicular pyramidal cells, since it is not abolished by blocking excitatory synaptic transmission (Mason, 1993). However, a number of the intracellular studies report that bursting cells can be made

to fire single action potentials by shifting the cell membrane potential to more depolarized levels (Stewart and Wong, 1993; Mason, 1993; classification also used by Sharp and Green, 1994). Surprisingly, Staff *et al.* (2000), in their patch-clamp study, did not find any bursting cells that could be made to fire single spikes regardless of the membrane potential.

The most recent of these studies, however, concludes that it may be most appropriate to describe the firing properties of subicular principal neurons as lying on a continuum of 'propensity to burst' (Staff *et al.*, 2000). While this study initially categorizes cells as strong bursting (SB), weak bursting (WB), and regular spiking (RS), Staff *et al.* state that neurons displayed 'a wide assortment of firing properties, ranging from neurons that never burst to those that would burst repetitively (7 times in 1 s, with nearly all varieties in between (an example being a neuron that burst twice and then spiked regularly, which was classified as SB).' Staff *et al.* (2000) conclude that subicular neurons belong to a homogenous group with respect to all other electrophysiological properties, and that the differences in their firing properties may be regulated by a slowly inactivating potassium current. Therefore, subicular pyramidal neurons may form a single neuronal class, sharing a burst generating mechanism that is stronger in some cells than others. Surprisingly, they did not find any bursting cells that could be made to fire single spikes, regardless of the membrane potential, a finding which is at odds with previous intracellular studies, and discussed above (Stewart and Wong, 1993; Mason, 1993). They suggest that this finding may be explained by the

TABLE 1. Comparison of subicular cell characterisation studies

Study	Technique	Reported cell-types	Proportions*
[1]	Patch-clamp (*in vitro*)	SB,WB,RS	51%,17%,32%
[2]	Intracellular (*in vitro*)	IB,BD,FS,RS,MRS	40%,31%,2%,18%,9%
[3]	Intracellular (*in vitro*)	B,NB	79%,21%
[4]	Intracellular (*in vitro*)	B,RS	54%,46%
[5]	Freely-moving (*in vivo*)	B,NB,DB,T	34%,41%,14%,11%
[6]	Intracellular (*in vitro*)	B,NB,I	66%,31%,3%
[7]	Intracellular (*in vitro*)	B,RS	69%,31%
[8]	Intracellular (*in vitro*)	B,RS,borderline	74%,12%,14%

Studies

[1] Staff *et al.*, 2000
[2] Greene and Totterdell, 1997
[3] Stewart, 1997
[4] Behr *et al.*, 1996
[5] Sharp and Green, 1994
[6] Stewart and Wong, 1993
[7] Taube, 1993
[8] Mason, 1993

Abbreviations

B bursting
DB depolarized bursters
FS fast spiking
I interneurons
IB intrinsic burst-firing
MRS modified RS
NB non-bursting
RS regular spiking
SB strong bursting
T theta
WB weak bursting

* Proportions are presented in the same order that the cell-types are presented (i.e., in the first study, 51% of cell-types were SB)

differences in preparations, i.e., given that these studies did not observe SB neurons, it is possible that bursting was not as robust in these preparations and, as such, was easier to prevent.

Hippocampal EEG has been well-studied and many attempts have been made to implicate the distinctive patterns of EEG activity in various

functions (for reviews of the EEG literature, see O'Keefe and Nadel, 1978, and Buzsaki and Vanderwolf, 1985). Hippocampal EEG is broadly divided into theta activity (or rhythmic slow activity [RSA]) and irregular activity. Theta has been well-known and well-described for many years (e.g., Saul and Davis, 1933; Green and Arduini, 1954); briefly, theta oscillations appear in EEG traces as an approximately sinusoidal waveform of regular amplitude with a frequency of somewhere between 4 and 15 Hz, though most theta in the awake behaving rat occurs in the 6 - 9 Hz range. Irregular activity is typically slower than theta (1 – 4 Hz) and has been subdivided into large amplitude irregular activity (LIA) and small amplitude irregular activity (SIA) (Vanderwolf *et al.*, 1975). Much of the LIA appears to be sharp wave activity (large amplitude irregular waves which appear in the EEG trace believed to reflect an increase in the excitability of intrahippocampal circuity as a result of temporary disinhibition from afferent control - Buzsaki, 1986).

Most hypotheses concerning the generation of theta oscillations in the hippocampus have implicated interneurons in the driving or pacing of the firing of principal neurons, and that the summed post-synaptic potentials of populations of principal neurons produce theta rhythms in the EEG (Bland and Colom, 1993; Vanderwolf, 1988; Buzsaki *et al.*, 1983). Sharp waves, on the other hand, which occur in the absence of theta oscillations, are believed to be triggered by population bursts of CA3 pyramidal cells as a result of their temporary disinhibition from afferent control (Buzsaki, 1986).

The relationship of single unit activity to these distinctive hippocampal EEG patterns has also been studied (e.g., O'Keefe and Nadel, 1978; Buzsaki, 1986; O'Keefe and Recce, 1993; Skaggs *et al.*, 1996). Putative interneurons increase their firing rates when theta oscillations appear in the EEG (e.g., Feder and Ranck, 1973); principal neurons can fire in phase with the theta oscillations, though 'theta precession', where units fire at progressively earlier times in relation to the ongoing theta oscillations, has been implicated in hippocampal function (e.g., O'Keefe and Recce, 1993). Sharp waves are associated with increased activity in all cell types (Buszaki, 1986).

In an attempt to clarify the *in vivo* classification of subicular cells, and to describe subicular EEG, here, we have recorded both unit activity and EEG in freely-moving rats in a simple setting (rats occupied their home cages). Both types of activity were also correlated with the simultaneous behaviour and state of the animal.

3.3 Method

Subjects

Five adult male Wistar rats (Bio Resources Unit, University of Dublin), weighing approximately 300 - 400 g on arrival in the laboratory were used as subjects. Rats were housed singly in a temperature controlled laminar airflow cupboard, and maintained on a 12:12 hour light-dark cycle (light: 0800 – 2000 hours). All testing was carried out during the light phase. All subjects were well-handled prior to the experiment.

The rats used in the current study were also used in the gradient maze and object exploration experiments in later chapters of this thesis. In a typical recording session, if unit activity was present on an electrode, a baseline recording session was conducted prior to the gradient maze or object exploration recording session.

Apparatus

All recordings for this experiment were conducted while the rats occupied a small square chamber in the experimental room (side 25 cm, height 30 cm). All rats were very familiar with this chamber as it was used to bring the rats from the housing unit to the testing room. The chamber rested on the floor of the experimental room, close to the experimenter so that the ongoing behaviour of the rat could be easily

observed; it occupied the same position for all recording sessions. The experimental room was illuminated as usual by two 12 volt bulbs attached to the ceiling (for full details of the experimental room see Chapter 2).

Surgery

Electrodes were implanted using the standard method (see Chapter 2). They were aimed at the following coordinates in dorsal subiculum: AP – 6.8 mm (relative to bregma), L 4.0 mm, DV 2.4 mm (Paxinos and Watson, 1996).

Following the experiment, rats were deeply anaesthetized using sodium pentobarbitol then perfused. Brains were removed and stored in 4 % formaldehyde for several days. Brains were subsequently sliced on a vibrotome and all electrode placements were verified as being in dorsal subiculum.

Recording techniques

Two channels of neuronal activity were recorded during each session: the first channel acquired unit activity and was filtered with bandpass 300 – 5000 Hz and amplified by a factor of 10,000; the second channel acquired EEG and was filtered with bandpass 1 – 500 Hz and amplified by a factor of 1,000. Both channels were fed into the analogue-to-digital

conversion system (DataWave Technologies, Colorado, USA) described in Chapter 2.

After each session the electrodes were driven ventrally by approximately 50 μm with the hope of encountering new units.

Protocol

Each session typically lasted approximately 20 mins. During this time the rat was not required to perform any task. Usually rats were very active for the first few minutes, exploring the chamber rapidly, sniffing and rearing frequently. By the end of the session this very active behaviour usually subsided to 'alert, still' or 'quiet' behaviours, punctuated regularly by brief 'alert, moving' periods. Occasionally a rat would not settle for a long period. To ensure a good sample of different behaviours, in these cases the recording was halted; once the rat did settle recording was resumed.

The experimenter recorded the ongoing behaviour of the rat by pressing a key on the keyboard which was saved to file as an event-flag together with a time-stamp. Event flags used in this experiment were: A = 'alert, moving'; S = 'alert, still'; Q = 'quiet'; and R = 'rearing'. It is presumed that during the 'quiet' period, at least for some of the time, the rat was asleep.

Data analysis

Output ASCII files were exported from the DataWave system into custom-written analysis software (M.Anderson using Matlab; see Chapter 2 for a full discussion of all analysis software and techniques). Spike sorting was conducted using a template-matching algorithm with conservative criteria for acceptance. EEG was visualized using a data plotter (Matlab program; M. Anderson) which also showed simultaneous spike and event flag data; as such, portions of the EEG trace could be cropped for further analysis.

After spike sorting, individual unit data were further processed to display inter-spike interval histograms (ISIHs), autocorrelation histograms (ACHs), peri-event time histograms (PETHs) based on the event flags, and raster plots (see Chapter 2). ISIHs, ACHs, and raster plots were used, together with the electrophysiological characteristics of the units (spike duration and spike amplitude) to classify each unit according to known cell subicular cell types. Simple mean rate (Hz) was also calculated for each unit by dividing the total number of spikes by the session time.

EEG was analysed using discrete Fourier transforms. Fourier analysis is extremely useful for EEG analysis as it breaks down the signal into constituent sinusoids of different frequencies. Periodograms were plotted using this data; any dominant frequencies present in the EEG appear as peaks in the plot.

Statistical analysis

Where appropriate, differences between firing rates were analysed parametrically with one-way analyses of variance (ANOVA) or t-tests for independent samples. For analyses of firing rates before and after the onset of a particular behaviour (as recorded with the event flags and displayed in the PETHs), paired t-tests were used since a plot of the differences pre-and post-flag showed them to be normally distributed.

All statistics were calculated using the Statistical Package for the Social Sciences (SPSS) software.

3.4 Results

130 units in total were recorded in the five rats during 30 sessions. The tracks of electrode bundles were reconstructed by visual inspection after histological processing (see Figure 3.1). The recording electrodes from all rats were positioned in dorsal subiculum (one bordered hippocampal area CA1 and was included because it recorded units with similar electrophysiological characteristics to the other recorded units).

Unit classification

Following consideration of each unit's electrophysiological and firing characteristics, four unit classes were defined: bursting units, regular spiking units, theta-modulated units, and fast spiking units. Working definitions were formulated for each class:

Bursting units were defined as units which had clear peaks in their ISIHs and ACHs *in the small ISI range* (typical ISIs ranged from 1 - 9 ms), and whose ISIH and ACH did not differ greatly in overall appearance;

Regular spiking units were defined as units which had a large spread of ISIs with no marked peaks in the ISIH and ACH, which showed no increases in firing when a theta rhythm was present in the EEG, and which fired at a much lower rate compared with fast spiking units (though rate was not used as a criterion for distinguishing regular spiking

LEFT HEMISPHERE RIGHT HEMISPHERE

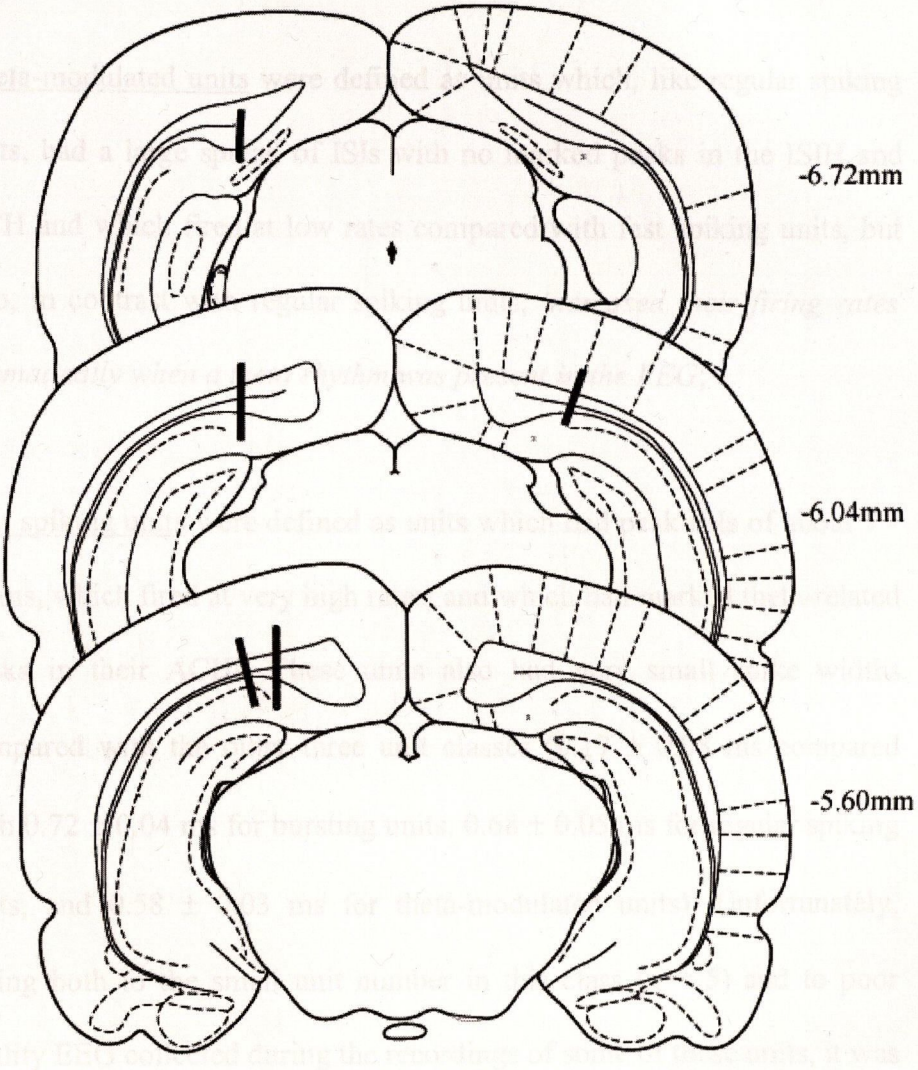


Figure 3.1. Results of the histological analysis showing the reconstructed electrode tracks from all five animals. The heavy black line in each section represents the electrode track. Slice measurements are posterior to bregma (Paxinos and Watson, 1996).

units from fast spiking units since the latter were always distinguished by the small width of their spikes);

Theta-modulated units were defined as units which, like regular spiking units, had a large spread of ISIs with no marked peaks in the ISIH and ACH and which fired at low rates compared with fast spiking units, but also, in contrast with regular spiking units, *increased their firing rates dramatically when a theta rhythm was present in the EEG*;

Fast spiking units were defined as units which had peak ISIs of about 7 - 10 ms, which fired at very high rates, and which had marked theta-related peaks in their ACHs. These units also had very small spike widths compared with the other three unit classes (0.17 ± 0.08 ms compared with 0.72 ± 0.04 ms for bursting units, 0.68 ± 0.05 ms for regular spiking units, and 0.58 ± 0.03 ms for theta-modulated units). Unfortunately, owing both to the small unit number in this class (n = 5) and to poor quality EEG collected during the recordings of some of these units, it was not possible to determine whether these units increased their firing rates when theta rhythms were present in the EEG, a known property of putative interneurons.

For examples of the ACHs of all unit classes, see Figures 3.2(a-e) and 3.3.

From the definition above, the fast-spiking units appear to be interneurons, and the small spike width of these units (< 0.2 ms) would appear to confirm this. It might appear that what we call theta-modulated units should better be classified with the fast-spiking units in a general 'theta unit' class, made up of putative interneurons. However, the theta-modulated class was created for theta-modulated units which had much lower rates than would be expected of interneurons (which typically display a mean firing rate of > 5 Hz), and which had much larger spike widths than would be expected of interneurons (a mean width which was not significantly different from bursting unit or regular spiking unit spike widths but which was highly significantly different from fast spiking unit spike widths).

The fact that theta-modulated units increase their firing rates when theta rhythms are present in the EEG was sometimes used to distinguish theta-modulated units and regular spiking units, particularly when spike numbers were small. To achieve this, the simultaneous spike and EEG traces were divided into 4 s sections, and the EEG sections were analysed using Fourier techniques as described in the chapter *Methods* (and see Figure 3.3). EEG sections were then separated into 'theta' (dominant frequencies in the range of 4 - 10 Hz) and 'non-theta' (other dominant frequencies) groups, and the mean firing rates of the units recorded during these times were calculated. Some units showed much higher firing rates in the theta than the non-theta EEG sections, and so were assigned to the theta-modulated unit class; t-tests for independent

samples were conducted on those units which showed similar firing rates during theta and non-theta EEG. Fast spiking units could always be distinguished on the basis of their small spike width and high rates.

The bursting unit class showed much variation in ACH appearance (see Figure 3.2a-c). The decision to form only one class from these units was influenced by the difficulty in establishing clear boundaries between bursting unit types, and by the findings of Staff *et al.* (2000), who suggest that subicular bursting cells may be more appropriately classified according to their firing properties, which they state lie on a continuum of 'propensity to burst' (see chapter *Introduction* and *Discussion* for more details). In relation to the difficulty in establishing clear boundaries between bursting unit types, a comparison of the ISIHs and ACHs for each unit failed to clearly distinguish bursting units (Greene and Totterdell, 1997; Stewart, 1997; Stewart and Wong, 1993; Taube, 1993; Mason, 1993; Sharp and Green, 1994) from depolarized bursters (Sharp and Green, 1994, based on Stewart and Wong, 1993). This may result from the Staff *et al.* (2000) 'continuum of firing' idea expressed above, and hence all units showing evidence of burst firing were assigned to a single bursting unit class.

Of the 130 subicular units recorded here, only 61 (47 %) could unequivocally be assigned to a class. The large majority of the remaining units (86 %) had too few spikes to be classified confidently, rather than different firing characteristics (ISIHs and ACHs become impossible to

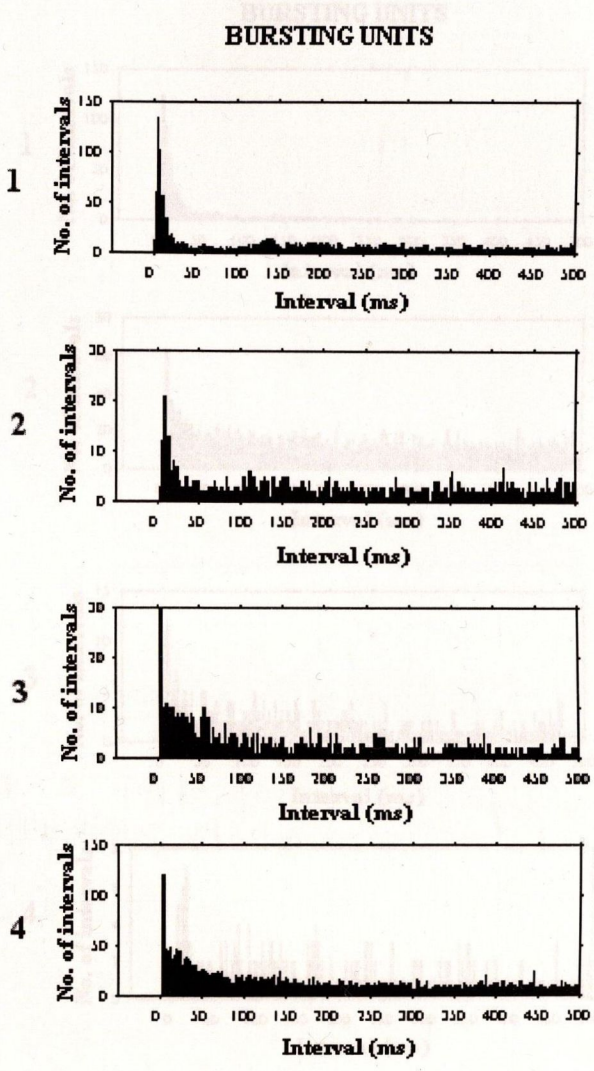


Figure 3.2(a). Non-normalized ACHs of four bursting units (bursting unit examples are continued overleaf) are displayed to illustrate the firing characteristics of this class. The top two ACHs are very similar to each other and display some theta rhythmicity as shown by the slight hump at around 150 ms. The bottom two ACHs are similar to each other but markedly different from the top two, with a pronounced hump immediately after the peak and no theta rhythmicity. See text for discussion.

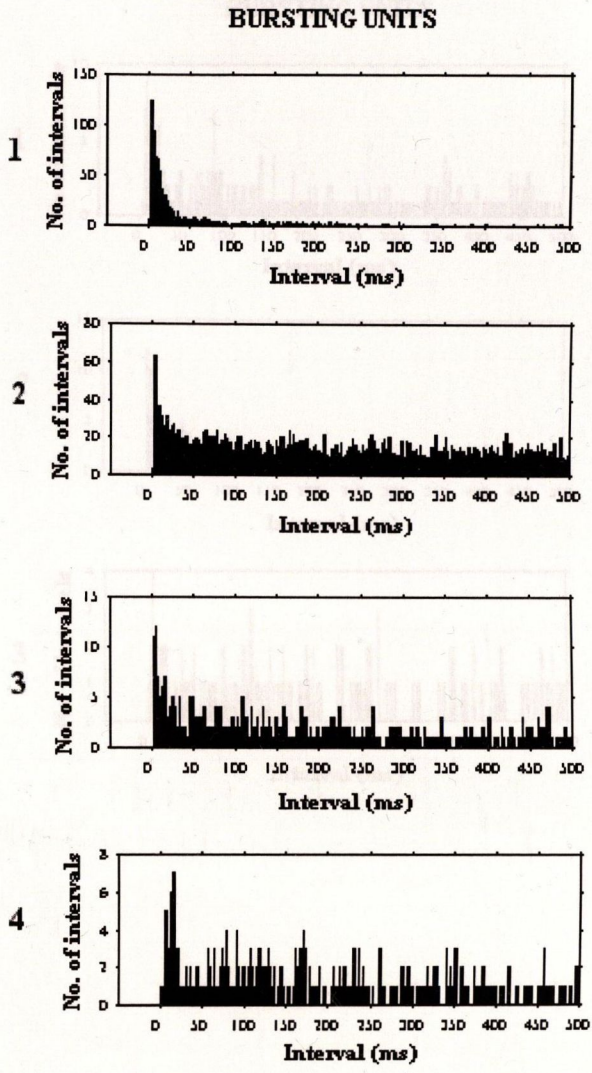


Figure 3.2(b). Further examples of non-normalized ACHs of bursting units (bursting unit examples are continued overleaf) are displayed to illustrate the firing characteristics of this class. The top ACH shows no theta rhythmicity. The second ACH shows many ISIs at intervals longer than the burst ISIs, with slight theta rhythmicity. The bottom two ACHs are constructed from units firing fewer spikes. See text for discussion.

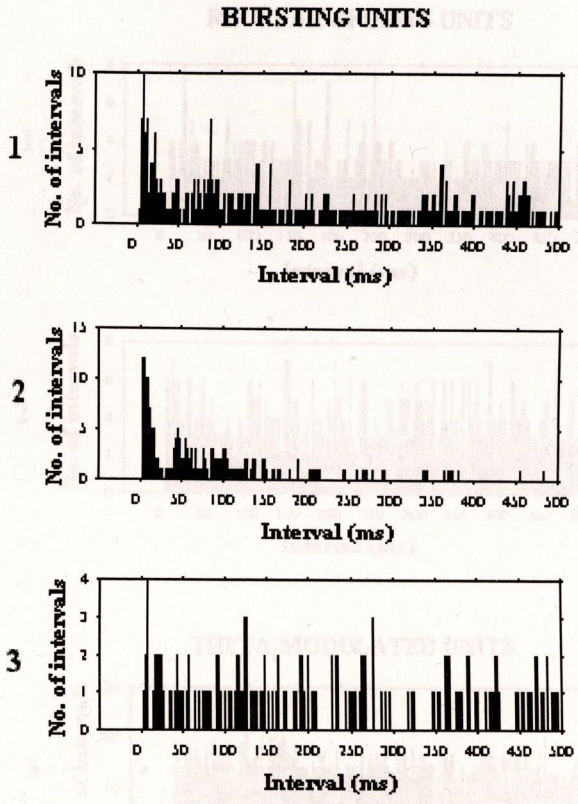


Figure 3.2(c). Further examples of non-normalized ACHs of bursting units are displayed to illustrate the firing characteristics of this class. The top two ACHs, though from units firing fewer spikes, show humps at around both 50 ms and 100 ms which would indicate non-theta rhythmicity, possibly beta or gamma rhythmicity (beta 12 - 20 Hz; gamma 30 - 70 Hz). The bottom ACH is shown as the poorly defined example of the bursting unit class. Despite having few ISIs, a peak at bursting ISIs and later theta-rhythmic peaks are evident. See text for discussion.

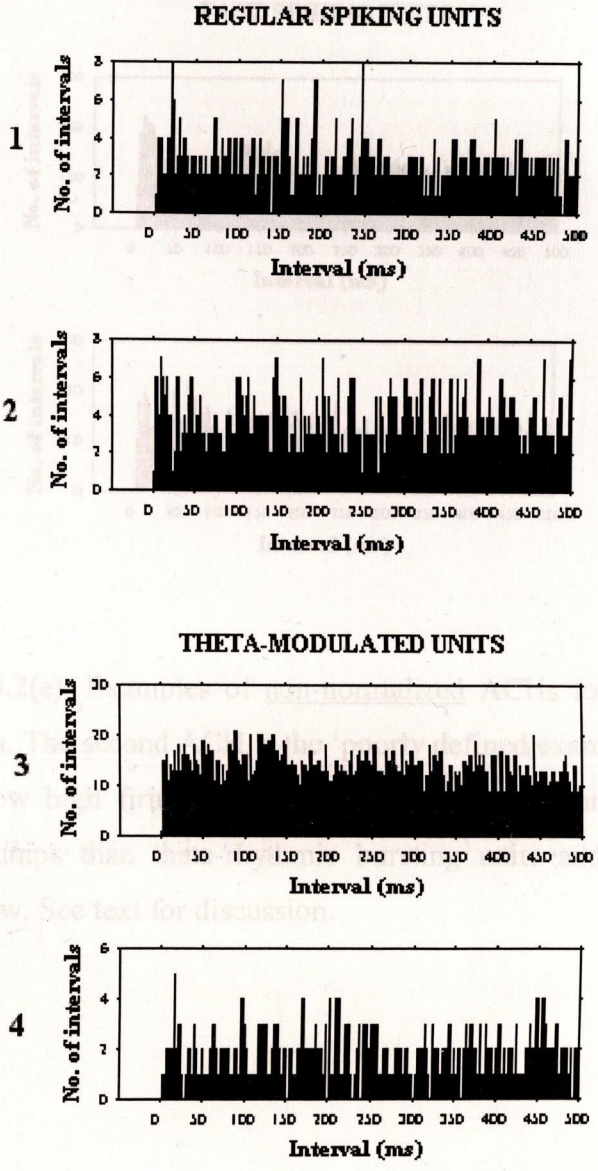


Figure 3.2(d). Examples of non-normalized ACHs for the regular spiking unit class (1 and 2) and theta-modulated unit class (3 and 4). The second ACH in each pair is the 'poorly defined example' for that class. The regular spiking units all had poorly defined ACHs but could be distinguished from poorly defined theta-modulated units by using the EEG records as described in *Results.* See text for discussion.

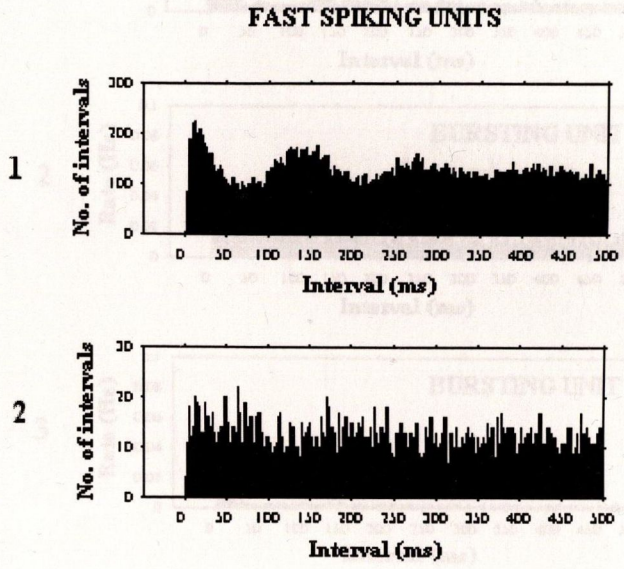


Figure 3.2(e). Examples of non-normalized ACHs for the fast spiking unit class. The second ACH is the 'poorly defined example'. Fast spiking units show high firing rates and marked theta rhythmicity with much larger humps than theta-rhythmic bursting units and theta-modulated units show. See text for discussion.

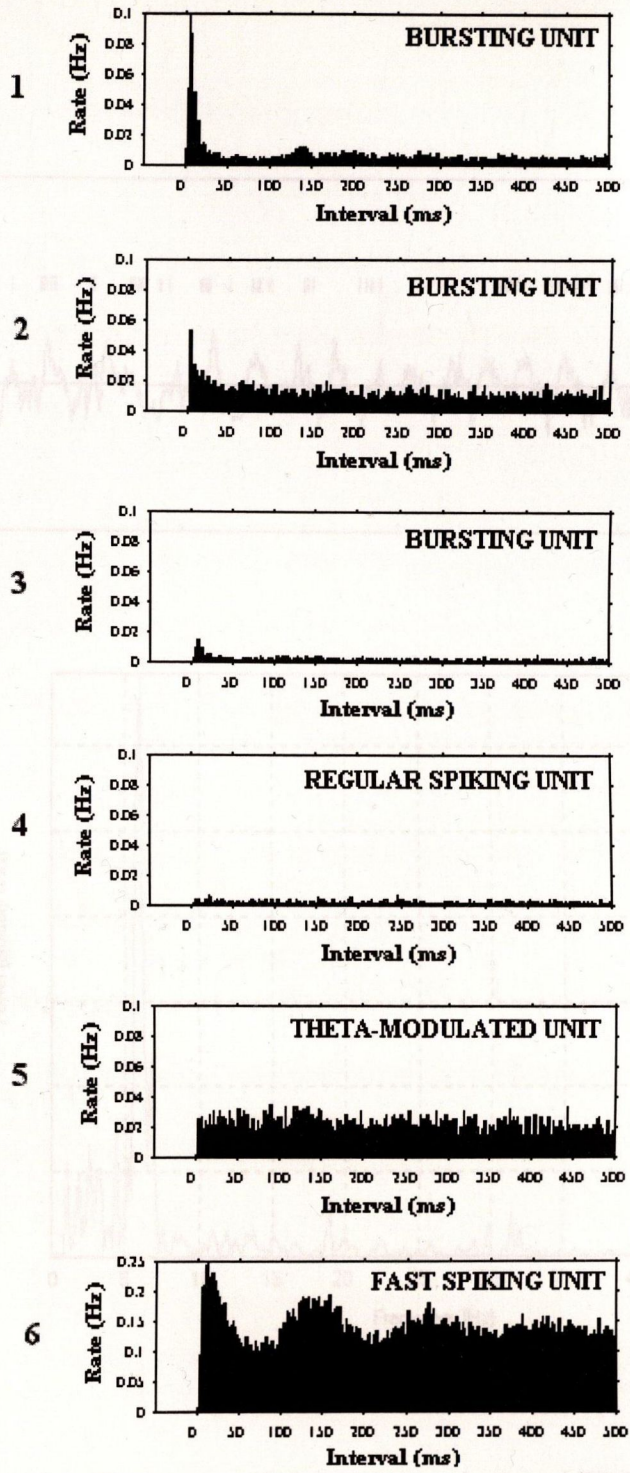


Figure 3.3. Examples of normalized ACHs for three bursting units (1-3), a regular spiking unit (4), a theta modulated unit (5), and a fast spiking unit (6). Note the different scale for the fast spiking unit y-axis. See text for discussion.

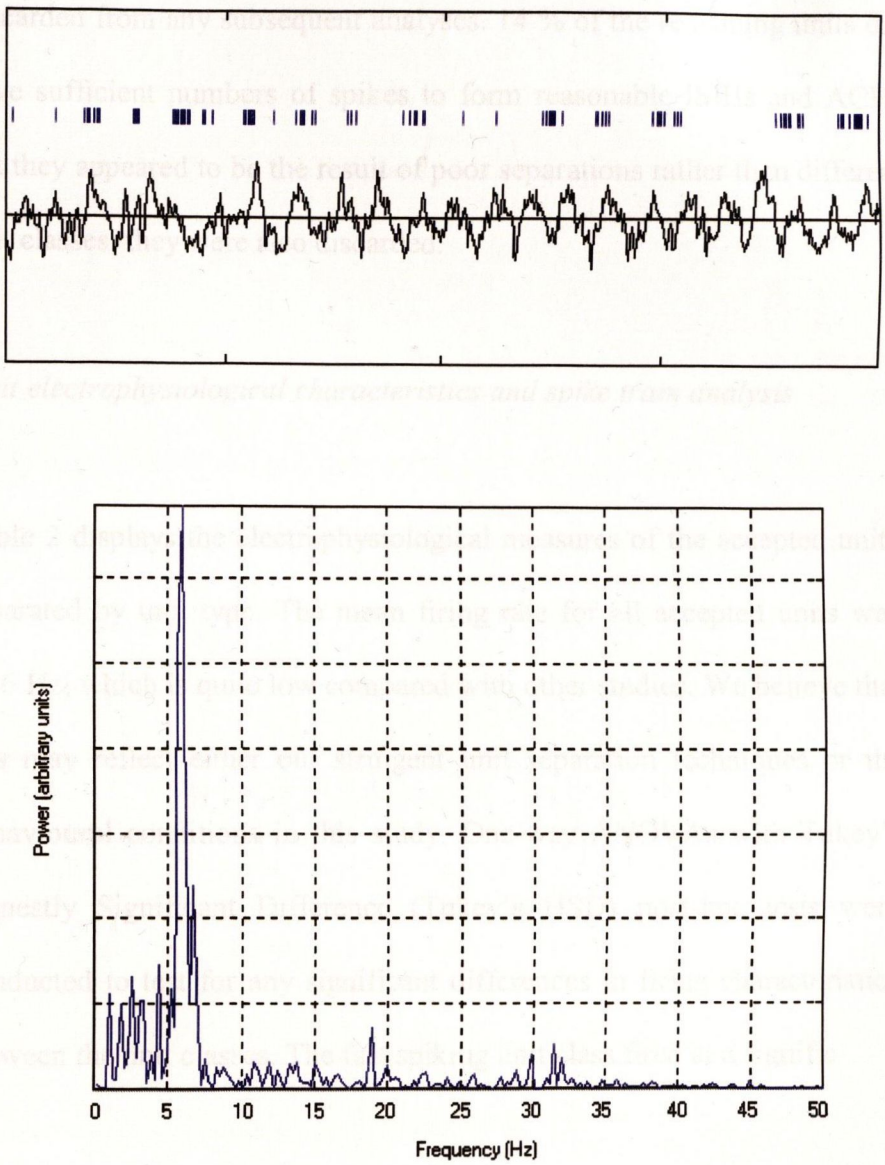


Figure 3.4. The top panel shows a sample 4 s of EEG together with the simultaneously recorded spikes (represented by a blue vertical line). The bottom panel shows the periodogram constructed using the EEG data in the top panel. A clear dominant peak appears at around 6 Hz, indicating that this EEG section contains theta rhythmic patterns. This figure illustrates the method of sectioning data files and using Fourier analysis on the EEG to assist in the classification of unit types (see *Results* for details).

interpret with fewer than approximately 200 spikes), and these were discarded from any subsequent analyses. 14 % of the remaining units did have sufficient numbers of spikes to form reasonable ISIHs and ACHs but they appeared to be the result of poor separations rather than different unit classes; they were also discarded.

Unit electrophysiological characteristics and spike train analysis

Table 2 displays the electrophysiological measures of the accepted units separated by unit type. The mean firing rate for all accepted units was 1.36 Hz, which is quite low compared with other studies. We believe that this may reflect either our stringent unit separation techniques or the behavioural conditions in this study. One-way ANOVAs with Tukey's Honestly Significant Difference (Tukey's HSD) post-hoc tests were conducted to test for any significant differences in firing characteristics between the unit classes. The fast spiking unit class fired at a signific-

TABLE 2. Electrophysiological measures of subicular units separated by class

Unit class	Bursting	Regular	θ-mod	Fast spike
N	25	12	19	5
Rate (Hz)	0.83 ± 0.12	0.84 ± 0.12	1.60 ± 0.28	4.17 ± 1.48
Height (μV)	265 ± 19	266 ± 26	173 ± 8	197 ± 15
Width (ms)	0.72 ± 0.04	0.68 ± 0.05	0.58 ± 0.03	0.17 ± 0.08

-antly higher rate and had a significantly smaller spike width than all the other unit classes (both $p < 0.0001$). The theta-modulated unit class had a significantly smaller spike height than the bursting unit and regular spiking unit classes ($p < 0.001$ and $p < 0.01$ respectively). There were no other significant differences.

Figure 3.2 (a-e) illustrates, with examples of ACHs, the four unit classes. Many more ACHs for bursting units are displayed because of the large variation in firing characteristics. For each unit class a poorly defined example is also presented to illustrate the limits of confident classification (except for the regular spiking units for which, inexplicably, most ISIH and ACHs were rather poorly defined). Note that all the ACHs in Figure 3.2 are not normalized. Figure 3.3 shows examples of *normalized* ACHs for all three classes. The non-normalized ACHs are presented to illustrate more clearly each unit's firing characteristics, while the normalized ACHs are presented to show the differences in rate between unit classes.

The appearances of the ACHs for the bursting unit class, the regular spiking class, and the fast spiking class are very similar to published ACHs for units collected in one of the few previous freely-moving studies to examine subicular unit firing characteristics (Sharp and Green, 1994). Interestingly, the bursting units here show much more variation than in the Sharp and Green (1994) study. It is possible that the type of unit that Sharp and Green called a 'depolarized burster' is classified here

as a bursting unit. Sharp and Green (1994) do not report theta-modulated units but they did not record EEG in their recordings so these units may have been assigned to their 'non-bursting class'. As stated above, the bursting unit class showed much more variation in firing characteristics, so in an effort to describe this class further, and influenced by the Staff *et al.* (2000) continuum idea, an elementary measure of 'propensity to burst' was calculated for each bursting unit. This was simply *the percentage of intervals which occurred in the burst ISI range* (1 - 9 ms). For example, a bursting unit containing 2000 spikes of which the number occurring in the burst ISI range is 250 would give a propensity to burst value (PtB) of (250/1999)*100, or approximately 12.5 %. PtBs ranged between 1.5 % and 33 %, where PtBs close to 0 % mean 'little bursting' and PtBs above 10 - 20 % mean 'much bursting'. Figure 3.5 displays a histogram of PtB values for the 25 bursting units. Although the majority of PtBs are small, there is a wide spread of values across the range.

EEG analysis

EEG traces from 26 of the 30 sessions (EEG was not recorded during four sessions) were analysed using fast Fourier transforms. A periodogram of power against frequency was then plotted to reveal the dominant frequencies in the trace. Examples of these periodograms are shown in Figure 3.6. By far the most common pattern of EEG activity during a session (20 / 26 traces) is represented by the bimodal

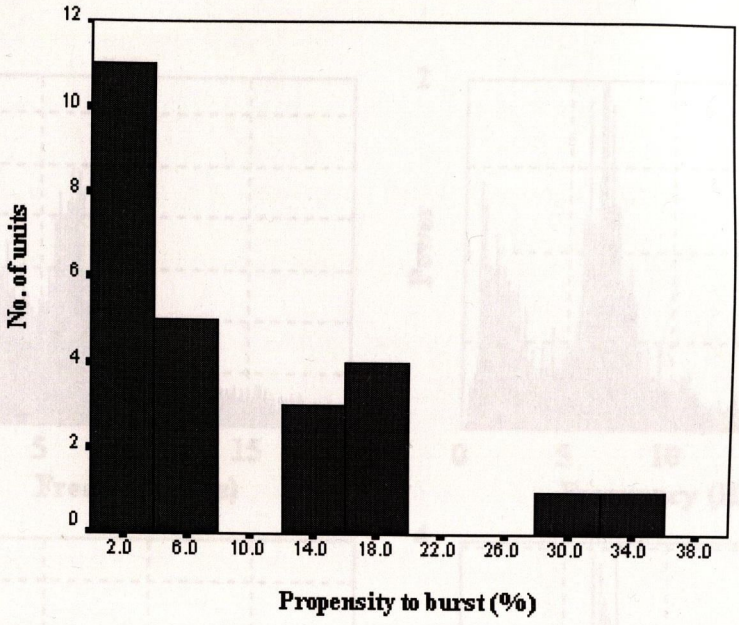


Figure 3.5. A histogram of the propensity to burst measure calculated for the 25 bursting units. Most units show smaller PtB values, but there is a spread of values across the range 1.5 % - 33 %.

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distribution shown in Figure 3.6 [1] to [4]. Two peaks occur, the lower frequency peak occurring between 1 - 3 Hz and probably reflecting LIA, and the higher frequency peak occurring in the 6 - 10 Hz and reflecting theta rhythms. The ratio of the size of the 'LIA peak' and the size of the 'theta peak' varied, so that in some traces very little LIA or theta was evident (Figure 3.6 [3] and [4]). The remaining six periodograms showed either three or four peaks; these peaks were all still within the LIA and theta frequency ranges (see Figure 3.6 [5]). Most theta oscillations had a frequency of between 6 and 7 Hz. Examples of subicular EEG can be seen in Figures 3.7, 3.7, and 3.8.

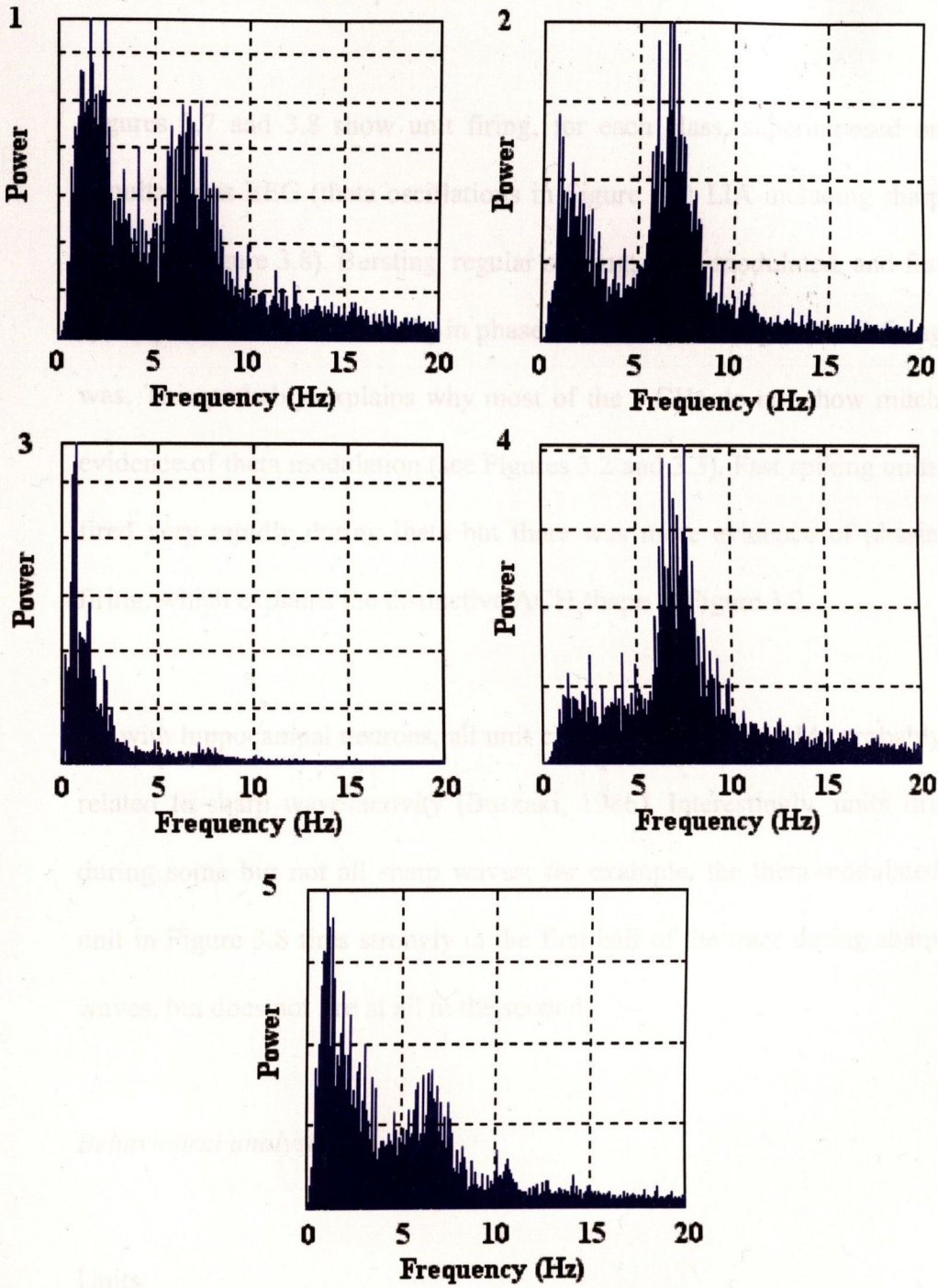


Figure 3.6. Periodograms, formed from fast Fourier transforms of each session's EEG trace, revealed a bimodal distribution of dominant frequencies in 20 / 26 sessions, as shown in [1] to [4]; the relative size of the two peaks varied (there is a slight hump at 7 Hz in [4]). One peak is in the LIA frequency range, while the other is in the theta frequency range. The remaining six sessions showed three or four peaks still within LIA and theta frequency ranges, as in [5]. See text for discussion.

EEG and unit relationship

Figures 3.7 and 3.8 show unit firing, for each class, superimposed on simultaneous EEG (theta oscillations in Figure 3.7; LIA including sharp waves in Figure 3.8). Bursting, regular spiking, theta-modulated, and fast spiking units all showed firing in phase with theta, although not all firing was. This probably explains why most of the ACHs do not show much evidence of theta modulation (see Figures 3.2 and 3.3). Fast spiking units fired very rapidly during theta but there was more evidence of phasic firing, which explains the distinctive ACH shape in Figure 3.2.

As with hippocampal neurons, all unit classes fired during LIA, probably related to sharp wave activity (Buszaki, 1986). Interestingly, units fire during some but not all sharp waves; for example, the theta-modulated unit in Figure 3.8 fires strongly in the first half of the trace during sharp waves, but does not fire at all in the second.

Behavioural analysis

Units

PETHs revealed few significant differences between pre- and post-flag firing rates (15 significant differences across 14 units). It may be that using PETHs is not the best way to look for firing rate differences between behavioural states because it is difficult to determine precisely

when those states shift. All of the significant differences ($p < 0.001$ to $p < 0.05$) appeared to be related to changes in arousal levels or related to

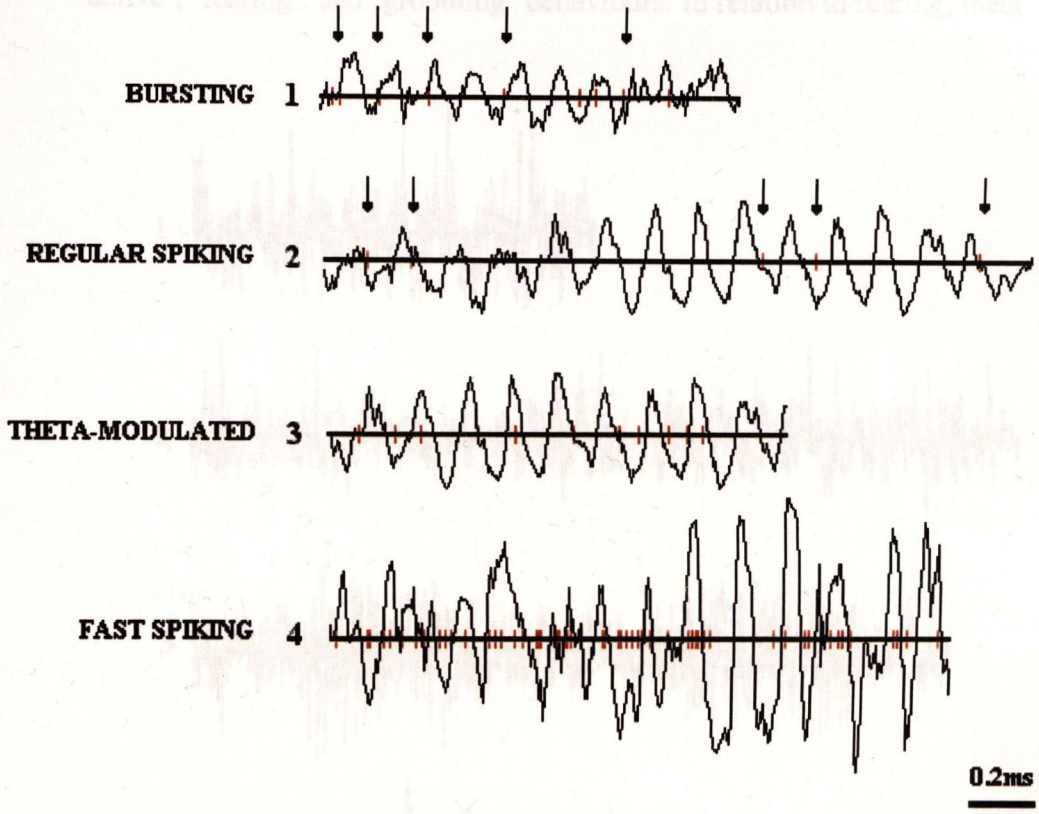


Figure 3.7. Theta oscillations (black continuous line) and simultaneous unit firing (small red lines) for each unit class (1 = bursting unit; 2 = regular spiking unit; 3 = theta-modulated unit; 4 = fast spiking unit). The bursting unit and regular spiking unit both fire during the same phase of the background theta oscillation (as indicated by arrows).

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movement since they were associated with either the 'alert, still', 'alert, moving', or 'rearing' flags, and they were not specific to a unit class.

EEG

Subicular EEG showed a similar relationship with behaviour to hippocampal EEG. Theta rhythms were invariably evident during 'alert, active', 'rearing', and 'grooming' behaviours. In relation to rearing, theta

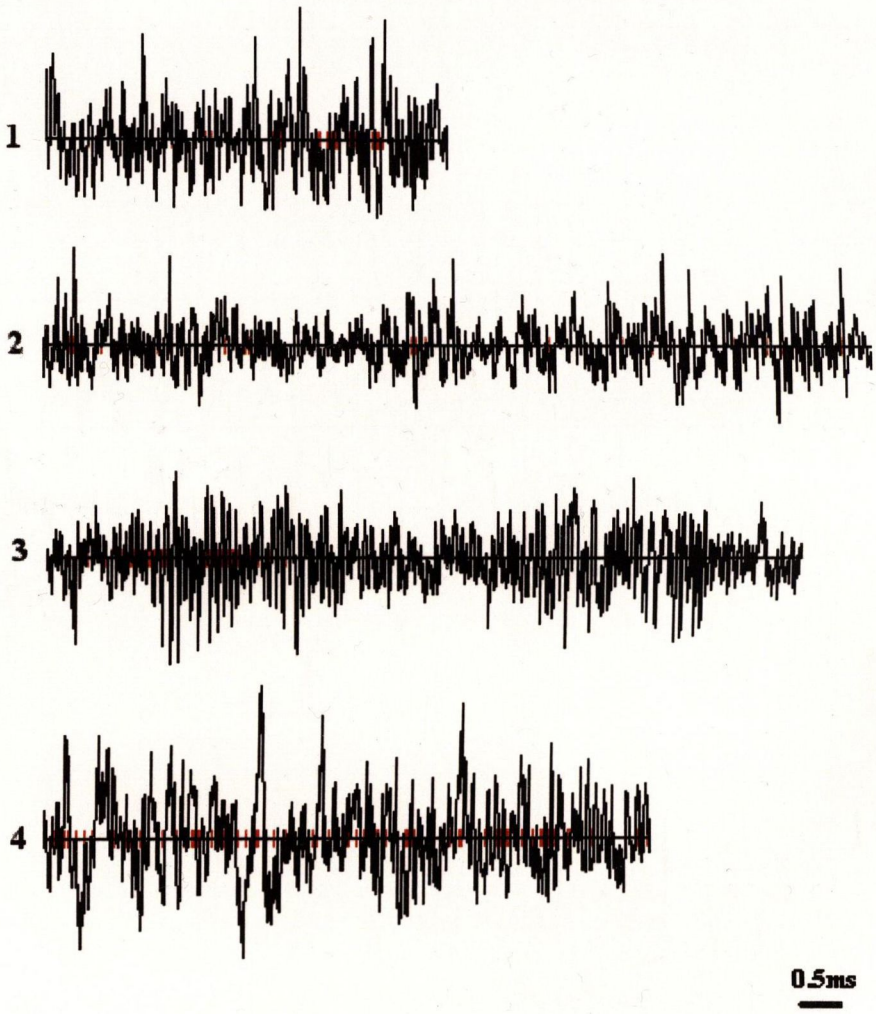


Figure 3.8. LIA including sharp waves (black continuous line) and simultaneous unit firing (small red lines) for each unit class (1 = bursting unit; 2 = regular spiking unit; 3 = theta-modulated unit; 4 = fast spiking unit). See text for discussion.

rhythms would often continue after the initial rearing movement, presumably related to sniffing the air. Both theta and irregular activity appeared intermittently during 'alert, still' and 'quiet' behaviours, though it was the irregular activity, and sharp waves, which dominated these periods (see Figure 3.8).

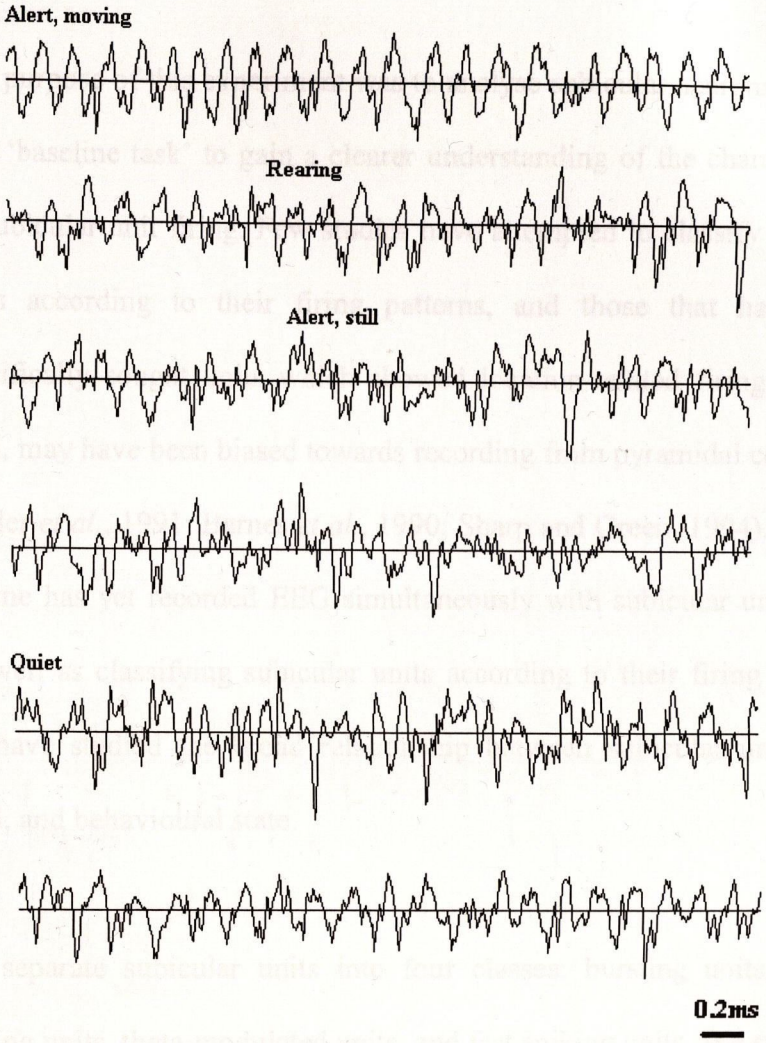


Figure 3.9. EEG samples with concurrent behaviours showing the predominance of theta oscillations during 'alert, movement' periods, giving way to LIA including sharp waves during 'alert, still' and 'quiet' periods. Note the re-occurrence of theta oscillations during the quiet period. See text for discussion.

3.5 Chapter discussion

The purpose of this experiment was to analyse subicular neuronal activity in a 'baseline task' to gain a clearer understanding of the characteristics of subicular unit firing. Few studies have attempted to classify subicular units according to their firing patterns, and those that have, have specifically sought units which showed location-related firing, and, as such, may have been biased towards recording from pyramidal cells (e.g., Muller *et al.*, 1991; Barnes *et al.*, 1990; Sharp and Green, 1994). Further, no one has yet recorded EEG simultaneously with subicular unit firing. As well as classifying subicular units according to their firing patterns, we have studied the broad relationship between subicular unit firing, EEG, and behavioural state.

We separate subicular units into four classes: bursting units, regular spiking units, theta-modulated units, and fast spiking units. We stress that this is a tentative classification. It is a somewhat different classification to a previous *in vivo* study (Sharp and Green, 1994) where units were classified as either bursters (presumably our bursting units), non-bursters (presumably our regular spiking units), depolarized bursters (see below), or theta units (possibly our fast spiking units). It is difficult to say whether or not we encountered what Sharp and Green call 'depolarized bursters': certainly some of the bursting units we recorded show many ISIs longer than the typical burst ISIs but whether this means that these units are shifting from a bursting to a regular spiking mode is another matter, especially so in light of a recent patch-clamping study which

failed to find any cells which showed such a shift (Staff *et al.*, 2000). Indeed, Sharp and Green themselves say that their classification is tentative, and only that it 'is possible that...[the depolarized bursters'] pattern [of firing] resulted from the oscillation of bursting cells between a bursting and non-bursting mode, as described by Stewart and Wong (1993)'.

The variation in firing characteristics of our bursting units is an interesting finding in itself, and may be explained by a recent patch clamping study of subicular principal cells (Staff *et al.*, 2000). Staff *et al.* conclude that subicular bursting units may be better conceived of as lying on a continuum of their propensity to burst, given that (1) they find a range of cells, from their 'weak bursting' cells to their 'strong bursting' cells, which exhibit different levels of bursting activity, and (2) that, despite the 'variety in firing modalities', these neurons do not differ in any other measured parameter, both resting and active. This may explain the variation in bursting unit ACH shape and large spread in the propensity to burst measure displayed in Figure 3.4. Bursting, it is hypothesised, increases the probability of synaptic vesicle release and as such may increase the reliability of synaptic transmission (Lisman, 1997; Commins *et al.*, 1998). This mechanistic advantage of bursting may also convey an informational advantage - place cells appear to have smaller place fields when single spikes are ignored and only bursts are taken into account (Otto *et al.*, 1991). If the subiculum does indeed mediate hippocampal-cortical interaction then reliability in transferring

information to downstream cortical circuits would be expected. While this suggests why the subiculum contains a large proportion of bursting units to non-bursting units, it does not explain why bursting neurons within the subiculum should vary according to their propensity to burst.

Fast spiking units are very probably interneurons. They fire at very high rates and have narrow spikes, both of which are known features of hippocampal interneurons (e.g., O'Keefe, 1979). We also find them in a similar proportion in the subiculum that previous studies have reported (e.g., Greene and Totterdell, 1997 [their 'fast spiking units']; Sharp and Green, 1994 [their 'theta units']).

It is possible that the theta-modulated unit class is only evident in the *in vivo* preparation with simultaneous EEG recordings (the latter because theta-modulated units were distinguished from regular spiking units only by their increased firing when a theta rhythm was present in the EEG). Of course, *in vitro* studies cannot help to explain this finding because many inputs to the subiculum are severed in the process of making a slice, including what is traditionally viewed as a source of at least one type of theta-rhythmic activity in the hippocampus, the septum (Butcher and Woolf, 1986). It is also the case that no other *in vivo* subicular study (Barnes *et al.,* 1990; Sharp and Green, 1994; Sharp, 1997; Sharp, 1999a,b,c) reports simultaneously recorded EEG, and hence no other study would be able to discriminate theta-modulated units from regular spiking units. It should be noted, however, that we do not discount the

possibility that theta-modulated units are in fact interneurons, possibly different from the interneurons we call fast spiking units. It is widely accepted that 'theta units', as classified by Ranck (e.g., Feder and Ranck, 1973), are in fact interneurons, and that theta units increase their firing rates when a theta rhythm appears in the EEG (O'Keefe, 1979). Our theta-modulated units also increase their firing rates when a theta rhythm appears in the EEG; however, the large spike width of these units, which was not significantly different from the bursting unit and regular spiking unit spike widths (presumed pyramidal cells) but highly significantly different from the fast spiking unit spike width, led us to assign these units to their own class. It will be interesting to see if other *in vivo* studies also report this unit class.

The analysis of unit firing against behavioural state revealed little. All of the few significant differences between pre- and post-event flag firing rates appeared to be related to arousal levels or movement.

A comprehensive theory of the functions of the hippocampal formation will be required to explain, among many things, why the intrinsic cell-types of the hippocampal formation show markedly different firing characteristics, and precisely how these differences affect the contribution these cell-types make to the hippocampal network. Of course, before this 'comprehensive' theory is realised, all intrinsic cell-types will have to be fully described. It is hoped that this study contributes to the description.

Chapter 4

Pellet chasing task

Spatial correlates of subicular unit firing

Chapter contents

4.1 Summary

4.2 Introduction

4.3 Chapter methods

4.4 Results

4.5 Chapter discussion

4.1 Summary

We have recorded single-unit activity in the subiculum of freely-moving rats during a pellet-chasing task in a black circular tub. A white card was attached to the side wall to act as a polarising cue. In a similar task, Sharp and Green (1994) showed that subicular units demonstrate place-related firing in a manner qualitatively different from hippocampal place cells. Here we extend their study by examining the effects of cue rotation, cue removal, and subsequent cue replacement on the activity of subicular unit firing, using both qualitative and quantitative measures. We confirm that subicular units demonstrate location-related firing; that the place fields of subicular units appear to be larger and more diffuse than the place fields of hippocampal units; and that subicular units typically display several peaks of firing within a more general firing field. We describe subicular spike trains using quantitative measures: subicular spike trains signal less spatial information than hippocampal spike trains.

4.2 Introduction

Single-unit recordings in freely-moving rats have shown that some hippocampal units ('place cells') fire strongly when the rat occupies discrete areas in its current environment ('place fields') (for reviews, see O'Keefe, 1979; O'Mara, 1995). Recordings in the subiculum have revealed that this structure also contains place cells, together with head direction cells, and cells which encode both place and head direction (O'Mara *et al.,* 2000; Anderson and O'Mara, 2000; Sharp and Green, 1994; Muller *et al.*, 1991; Barnes *at al.,* 1990). Subicular place cells, however, appear to encode places in the environment differently to hippocampal place cells: they usually have larger place fields than CA1 and CA3 place cells, typically with more than one peak of activity in the overall field (O'Mara *et al.,* 2000; Anderson and O'Mara, 2000; Sharp and Green, 1994; Barnes *at al.,* 1990). It is apparent, therefore, that there are important differences in spatial representation between the subiculum and other areas of the hippocampal formation. Exactly why there is a difference between the representations in these structures, and what the precise contribution each area makes to the spatial representation in the hippocampal formation is, however, unknown.

Here, in an attempt to address the questions surrounding the subicular spatial representation, we recorded subicular units during a pellet-chasing task (Muller *et al.*, 1987). This task, as well as examining the production of place fields in an environment, tests the effects on unit firing of

manipulating a salient visual cue. Sharp and Green (1994) examined the spatial representations of subicular units in a similar task, in which they showed that subicular place fields were able to follow the rotation of the cue; we extend that study by examining the effects of cue removal and subsequent cue replacement, as well as cue rotation, on subicular unit representations. We adopt a quantitative approach to the unit activity, quantifying the amount of spatial information carried by each spike (using an information content measure [Skaggs *et al.*, 1993]). These analyses will assist in our understanding of how the subiculum represents space, of how its representation differs from that of the hippocampus, and of what information the subiculum might send to its output structures.

4.3 Method

Subjects

Four adult male Wistar rats (Bio Resources Unit, University of Dublin), weighing approximately 300 – 500 g upon arrival in the laboratory, were used as subjects. Rats were housed singly in a temperature-controlled laminar airflow holding unit, and maintained on a 12:12 light-dark cycle (0800-2000hrs). All testing was carried out during the light phase. In preparation for the experiment all rats were well-handled and gradually reduced to approximately 85 % of their *ad libitum* feeding weight and were maintained at this level throughout the experimental period by allowing each rat 15g of rat chow per day as a supplement to the food used as reward during the task.

Apparatus

The apparatus was based upon a design used by Muller and co-workers (e.g., Muller *et al.*, 1987). It consisted of a plastic matt black circular tub (diameter 45 cm, height 39 cm) which rested on a table 80 cm above the ground. An A4 sheet of white paper was positioned on the wall of the tub; its position could be easily changed. The experimental room was illuminated by two 12 volt bulbs attached to the ceiling (for full details of the experimental room see Chapter 2). In order to examine the control of *both* distal cues (extramaze) and the proximal cue (cue card) over

subicular unit firing no attempt was made to hide distal cues in the surrounding environment.

Surgery

Electrodes were implanted using the standard method in this thesis (see Chapter 2). Electrodes were aimed at the following co-ordinates in dorsal subiculum: AP –6.8 mm (relative to bregma), L 4.0 mm, DV 2.4 mm (Paxinos and Watson, 1996).

Following the experiment, rats were deeply anaesthetized using sodium pentobarbitol, then perfused. Brains were removed and stored in 4 % formaldehyde for several days. They were subsequently sliced on a vibrotome and all electrode placements were verified as being in dorsal subiculum.

Recording techniques

One channel of neuronal activity was recorded during each session: it was filtered with bandpass 300 – 5000 Hz and amplified by a factor of 10,000. The channel was fed into the analogue-to-digital conversion system (DataWave Technologies, Colorado, USA) described in detail in Chapter 2. Output was also sent to a loudspeaker for auditory feedback.

An overhead video tracking system was used to follow the location of the rat in the maze. The system operated in one-spot (monochrome) tracking mode and detected a small LED attached to the headstage (see Chapter 2). The tracking system split the environment into 256 x 256 pixels and sent time-stamped positional data to the acquisition system.

After each session the electrodes were driven ventrally by approximately 50 μm in an effort to encounter new units.

Protocol

Each recording session consisted of four trials. During each trial, rats foraged for food pellets in the task arena; the pellets were thrown into the arena at irregular intervals and positions (average of one every 20 s).

In each trial the position of the card in the arena was changed as follows: **Trial 1:** card on the inside wall in the north position. **Trial 2:** card on the inside wall in the south position. **Trial 3:** card removed. **Trial 4:** card returned to the inside wall in the north position (see Figure 4.1).

Each trial lasted 10 min and the period between stages when the cue card was manipulated lasted approximately 4 min. Between trials, the rat was placed in a holding chamber close to the recording arena and could not see the changes being made to the environment.

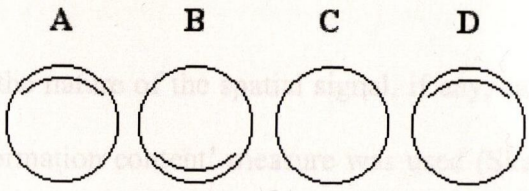


Figure 4.1. The task consisted of four conditions in which the position of the cue card was varied. **A** : card on the inside wall in the north position. **B** : card on the inside wall in the south position. **C** : card removed. **D** : card returned to the inside wall in the north position.

Data analysis

Output ASCII files were exported from the DataWave system into custom-written analysis software (M.Anderson using Matlab; see Chapter 2 for a full discussion of all analysis software and techniques). Spike sorting was conducted using a template-matching algorithm with conservative criteria for acceptance.

After spike sorting, individual unit data were further processed to display inter-spike interval histograms (ISIHs), autocorrelation histograms (ACHs), and raster plots (see Chapter 2). ISIHs, ACHs, and raster plots were used, together with the electrophysiological characteristics of the units (e.g., spike duration, rate, etc.) in an attempt to classify each unit according to known subicular cell types. The session rate (Hz) was calculated for each unit by dividing the total number of spikes by the session time.

To determine the nature of the spatial signal, if any, carried by subicular units, the 'information content' measure was used (Skaggs *et al.*, 1993), which calculates the amount of information, *I,* contained in the firing of a unit, using the formula

$$I = \Sigma_i (\lambda_i / \lambda) \log_2 (\lambda_i / \lambda) P_i ,$$

where λ_i is the mean firing rate in pixel i, λ is the overall mean firing rate, and P_i is the probability of the animal occupying pixel i (which is simply the time spent in pixel i divided by the total trial time). For a fuller description of this measure see Chapter 2. Spatial information content is measured in 'bits of information per spike' (bits / spike).

Firing rate maps were constructed in Matlab to enable easy visualisation of location-specific firing, using the system described in *Main Methods.* Briefly, the maps are constructed by dividing the open-field environment into pixels (square measuring 6 x 6 cm). The number of spikes that fired when the rat occupied a pixel is divided by the total time the rat spent in that pixel (s) to produce a firing rate for that pixel (Hz). All the pixel firing rates are mapped as a contour plot using Matlab's in-built contouring algorithm. The maps are colour-coded in increments of 20% of the peak firing rate for each individual map. The 'peak rate' of each map is equal to the rate of the pixel with the highest firing rate in each map. Other spatial measures were also calculated, based on the firing rate maps. 'Non-zero rate' represents the mean of all the pixels in a firing rate map displaying a rate above 0 Hz. This measure controls for the

possibility that units would not fire in all areas of the arena (i.e., in all pixels) but only in discrete locations, thus biasing the session rate; it is probably closer to the true firing rate of the cell. Also, the size of the firing field for each cell was calculated by dividing the number of pixels with a firing rate of greater than zero by the total number of pixels in the environment. Given that subicular place fields have multiple fields and cover a large proportion of the environment surface this measure was reasoned to be the most appropriate. It also allowed comparisons to be made with previous work (Sharp and Green [1994], from where the measure originates).

Statistical analysis

Where appropriate, differences between firing rates were analysed parametrically with one-way analyses of variance (ANOVA) and post-hoc tests. All statistics were calculated using the Statistical Package for the Social Sciences (SPSS) software.

4.4 Results

47 units were isolated from recordings from the four rats. The tracks of electrode bundles were reconstructed by visual inspection after histological processing (see Figure 4.2). The recording electrodes from all rats were positioned in dorsal subiculum.

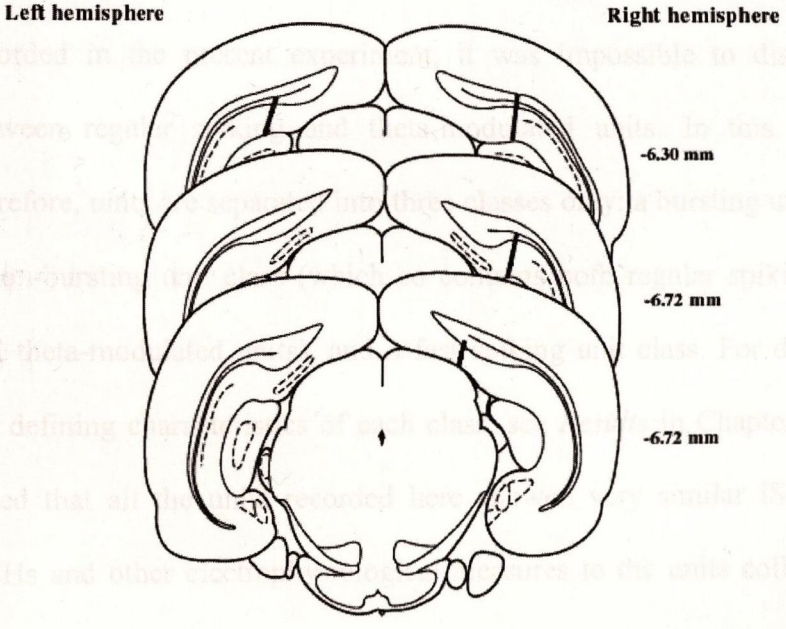


Figure 4.2. Results of the histological analysis showing the reconstructed electrode tracks from two of the three animals. The heavy black line in each section represents the electrode track. Slice measurements are posterior to bregma (Paxinos and Watson, 1996).

Unit classification

In the baseline recordings of subicular units in this thesis (see Chapter 3), each unit was assigned to one of four classes: a bursting unit class, a regular spiking unit class, a theta-modulated unit class, and a fast spiking unit class. In order to distinguish regular spiking units from theta-modulated units, it was necessary to use the simultaneous EEG records in a method detailed in Chapter 3. Unfortunately, because EEG was not recorded in the present experiment, it was impossible to distinguish between regular spiking and theta-modulated units. In this chapter, therefore, units are separated into three classes only: a bursting unit class, a non-bursting unit class (which so contains both regular spiking units and theta-modulated units), and a fast spiking unit class. For details of the defining characteristics of each class, see *Results* in Chapter 3. It is noted that all the units recorded here showed very similar ISIHs and ACHs and other electrophysiological measures to the units collected in the baseline experiment.

Unit electrophysiological characteristics

Table 1 displays the electrophysiological characteristics of units recorded in the present experiment, separated by unit class. One-way ANOVAs with Tukey's Honestly Significant Difference (Tukey's HSD) post-hoc tests were conducted to look for any significant differences between unit

classes in these firing characteristics. As expected, fast spiking units had significantly higher session rates than both bursting and non-bursting units (both comparisons, $p < 0.0001$), and had significantly narrower spike widths (both comparisons, $p < 0.05$). Bursting and non-bursting units did not differ on either of these measures.

TABLE 1. Electrophysiological characteristics of subicular units separated by unit class

Unit class	Bursting	Non-bursting	Fast spiking
N	11	20	16
Session rate (Hz)	0.56 ± 0.15	1.45 ± 0.19	5.50 ± 0.38
Height (μV)	256 ± 34	197 ± 19	238 ± 12
Width (ms)	0.72 ± 0.07	0.67 ± 0.05	0.50 ± 0.03

Spatially-related firing characteristics

Introduction

The firing rate maps revealed that all units showed firing rate gradients across the surface of the environment, whether that gradient was gentle across most of the surface as in the case of the majority of the fast spiking units, or whether it consisted of sharper, localized gradients as in the case of the majority of the bursting units. How exactly can we tell whether or not the areas on the arena surface of high firing rate are actually place fields? It is possible, though often not considered, that such differences are in fact related to variables other than the location of the

animal, such as the animal's engagement in specific behaviours or the effects of ongoing internal processes. Given that this task is specifically designed to homogenize all behavioural and experimental variables across the arena surface *except the location of the animal*, it was presumed that, at least, a proportion of the variation in firing rates between areas on the arena surface was due to location-related firing of the unit, and that these areas could be called 'place fields'. Further evidence that these areas of high firing rate are indeed place fields could also be obtained by finding similar firing rate maps across at least two of the conditions in the task (although the task was designed specifically to examine the plasticity of the spatial representation in the subiculum, the cue card did not exert much control over the position of a unit's place field on the arena surface, and so place fields would often remain in the same locations from one condition to the next). Pixel-to-pixel correlations (Pearson correlation coefficient - r) conducted between all four firing rate maps for each unit often showed significant high values (> 0.7; $p < 0.01$) between two firing rate maps, indicating similarity in the unit's spatially-related firing characteristics between conditions. The largest value of r for any unit was 0.84 ($p < 0.01$) which gives a coefficient of determination (r^2) of 0.71, indicating that 71 % of the variance in one firing rate map is explained by the other firing rate map. Some units showed very little, if any, evidence of consistent spatially-related firing, despite their firing rate maps showing firing rate gradients across the surface of the environment: these firing rate gradients did not behave consistently across conditions (as shown by r values close to zero

for correlations between firing rate maps across conditions [mean of 0.10 ± 0.02]), and, on the other hand, they did not respond coherently to movements of the cue card (these units are considered in more detail below). The rest of the units showed higher and significant ($p < 0.01$ or 0.05) *r* values between firing rate maps (mean of 0.42 ± 0.02), and hence at least some evidence of consistent spatially-related firing.

Data analysis

Examples of firing rate maps for each unit class are shown in Figures 4.3, 4.4, 4.6, and 4.7. Most of the firing rate maps have a rather broken appearance, with peaks of firing evident within more general areas of medium to low rate firing. Bursting units typically had the smallest place fields, with one peak or, more often, multiple peaks (two upwards) of firing. Fast spiking units typically displayed very large place fields, with extended peaks of firing within one overall firing field. Non-bursting units showed place fields of either variety (those more like bursting unit place fields or those more like fast spiking unit place fields), which is not unexpected given that the non-bursting class here probably contains both regular spiking units and theta-modulated units (of which the latter may well be interneurons [see chapter 3]).

Table 2 displays the spatially-related firing characteristics of units recorded in the present experiment. One-way ANOVAs with Tukey's HSD post-hoc tests were conducted to look for any significant

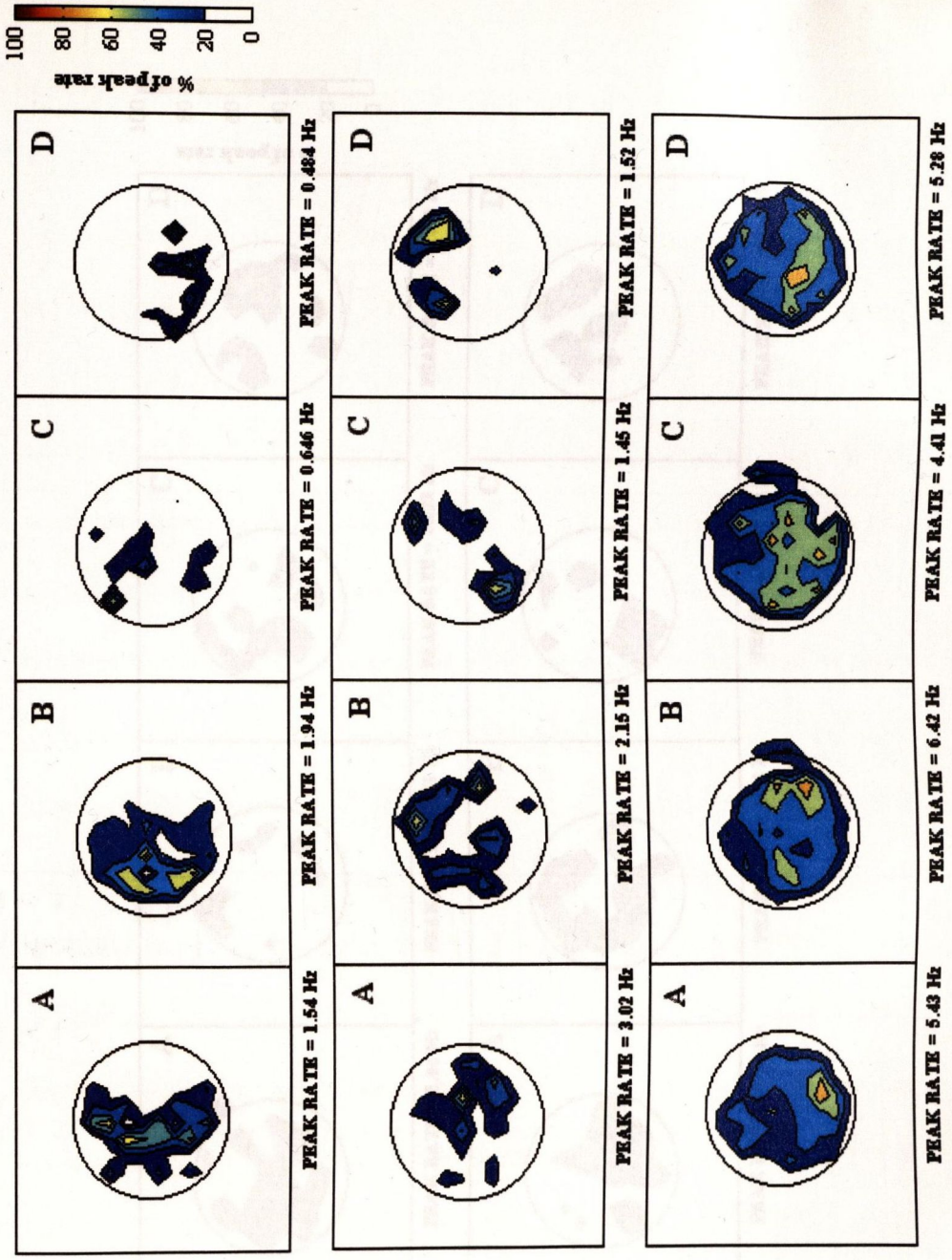


Figure 4.3. Firing rate maps for each unit class across the four conditions (**A, B, C, D**) of the task (upper - bursting unit; middle - non-bursting unit; lower - fast spiking unit). The peak rate beneath each map shows the rate of the pixel peak rate for that particular map. The colourbar displays the contour colouring, as a percentage of each map's peak rate. See text for discussion.

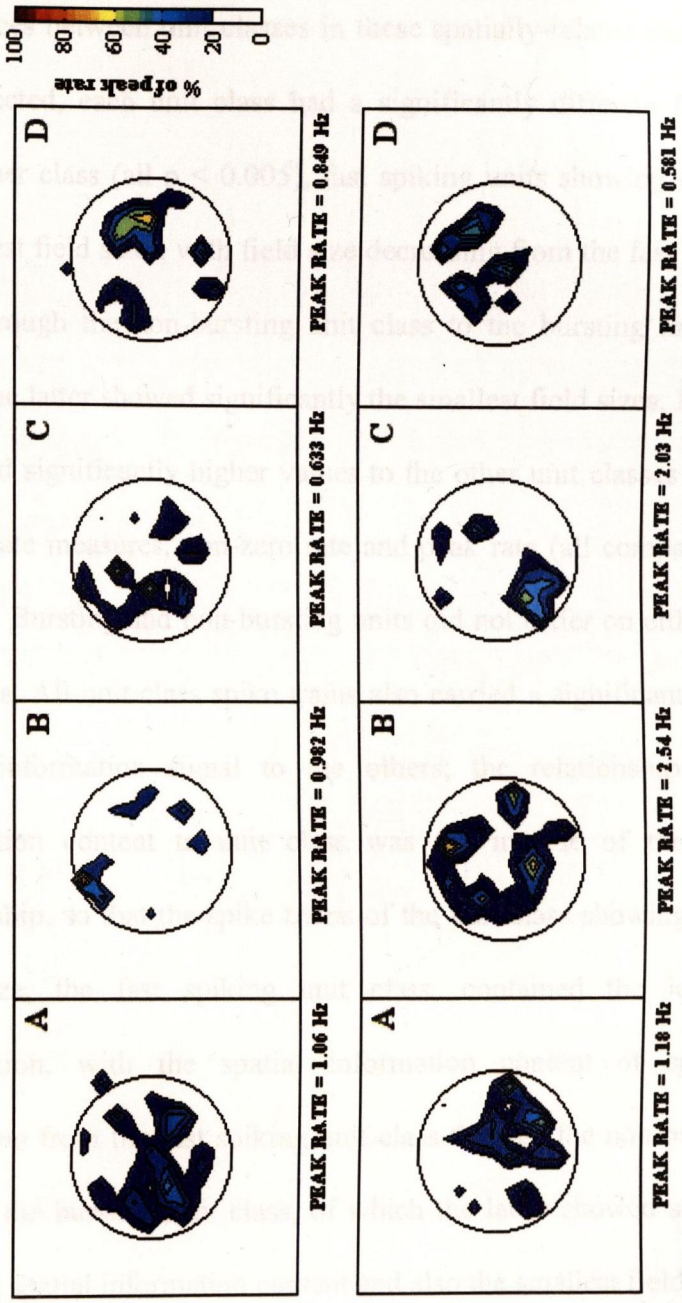


Figure 4.4. Examples of units that did not appear to encode place (upper - bursting unit; lower - non-bursting unit). There is no stability, or consistent changes, in the firing fields across the four task conditions (**A, B, C,** and **D**). See text for discussion.

differences between unit classes in these spatially-related characteristics. As expected, each unit class had a significantly different field size to each other class (all $p < 0.005$), fast spiking units showing significantly the largest field sizes, with field size decreasing from the fast spiking unit class through the non-bursting unit class to the bursting unit class, of which the latter showed significantly the smallest field sizes. Fast spiking units had significantly higher values to the other unit classes on the two spatial rate measures, non-zero rate and peak rate (all comparisons, $p < 0.0001$). Bursting and non-bursting units did not differ on either of these measures. All unit class spike trains also carried a significantly different spatial information signal to the others; the relationship of spatial information content to unit class was the inverse of the field size relationship, so that the spike trains of the unit class showing the largest field size, the fast spiking unit class, contained the least spatial information, with the spatial information content of spike trains *increasing* from the fast spiking unit class through the non-bursting unit class to the bursting unit class, of which the latter showed significantly the most spatial information content and also the smallest field sizes.

To test the relationship of the spatial information content of each unit class's spike train with the other spatially-related measures, plots were constructed of the bivariate relationships, as in Figure 4.5. As can be seen in the curvilinear trends evident in all three plots (though most strongly in Figure 4.5 B and C), there exist non-linear negative relationships between spatial information content and the other spatially-related

TABLE 2. Spatially-related firing characteristics of subicular units separated by unit class

Unit class	Bursting	Non-bursting	Fast spiking
Field size	0.53 ± 0.05	0.71 ± 0.04	0.93 ± 0.007
Peak rate (Hz)	1.28 ± 0.24	2.15 ± 0.18	5.78 ± 0.33
Non-zero rate (Hz)	0.68 ± 0.18	1.58 ± 0.19	5.57 ± 0.39
Spatial info. c.* (bits/spike)	0.82 ± 0.11	0.41 ± 0.08	0.14 ± 0.008

*Spatial information content

measures. To test these hypotheses, a non-parametric correlation coefficient (Spearman's ρ) was calculated for each bivariate correlation (a non-parametric correlation coefficient was chosen because of the strong evidence of the non-linear relationships between variables). As expected, very high significant negative correlation coefficients were produced: Spearman's ρ for spatial information content and field size was -0.92 ($p < 0.01$); Spearman's ρ for spatial information content and non-zero rate was -0.88 ($p < 0.01$); Spearman's ρ for spatial information content and peak rate was -0.87 ($p < 0.01$).

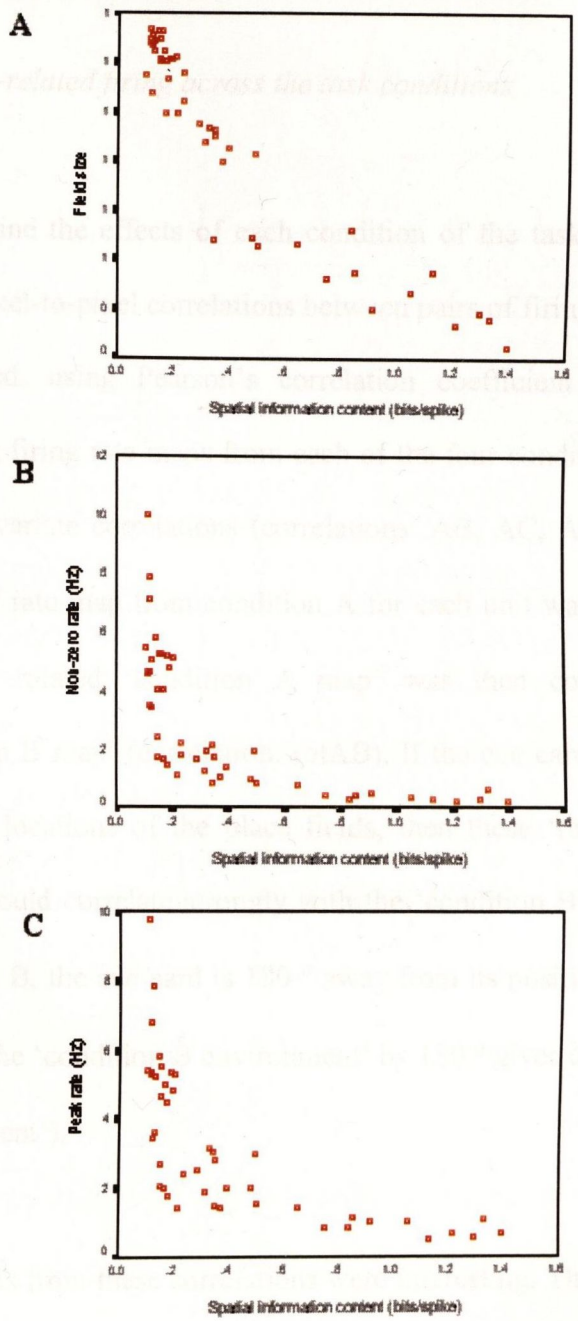


Figure 4.5. Spatial information content plotted against the three other spatially-related measures, field size (**A**), non-zero rate (**B**), and peak rate (**C**). There is evidence of a strong, non-linear, negative relationship in all three plots.

Spatially-related firing across the task conditions

To examine the effects of each condition of the task on the unit place fields, pixel-to-pixel correlations between pairs of firing rate maps were conducted, using Pearson's correlation coefficient (*r*). As well as including firing rate maps from each of the four conditions for each unit in the bivariate correlations (correlations: AB, AC, AD, BC, BD, CD), the firing rate map from condition A for each unit was rotated by 180 ° and this rotated 'condition A map' was then correlated with the 'condition B map' (correlation: rotAB). If the cue card did exert control over the locations of the place fields, then these 'rotated condition A maps' should correlate strongly with the 'condition B map' (because, in condition B, the cue card is 180 ° away from its position in condition A; rotating the 'condition B environment' by 180 ° gives us the 'condition A environment').

The results from these correlations were interesting. The cue card did not exert much control over place fields: one unit showed a higher *r* for the rotAB correlation than for the AB correlation, which suggests that the rotation of the cue card led to rotation of the place fields (most rotAB *r* values for other units were close to zero) though this finding may have occurred by chance alone.

Correlations could be grouped into different correlational 'types':

- **CORRELATIONAL TYPE 1.** 28 units (1 bursting unit [9% of bursting units], 11 non-bursting units [55 % of non-bursting units], and 16 fast spiking units [100 % of fast spiking units]; total = 60 % of all the units) showed similar *r* values in the AB, AC, and AD correlations, indicating stability in the location of place fields across conditions.
- **CORRELATIONAL TYPE 2.** 8 units (4 bursting units [36 % of bursting units], 4 non-bursting units [20 % of non-bursting units]; total = 17 % of all the units) showed *r* values that decreased from AB to AC to AD (and BC > AC, BD > AD, CD > BD > AD), indicating that the place fields were either altering gradually or disintegrating from one condition to the next.
- **CORRELATIONAL TYPE 3.** 1 unit (1 bursting unit [9% of bursting units]; total = 2 % of all the units) showed very small *r* values in the AB, AC, and AD correlations, but much larger *r* values in the BC, BD, and CD correlations, indicating that the place fields in this unit underwent an abrupt change in condition B but then remained stable.
- **CORRELATIONAL TYPE 4.** 1 unit (1 non-bursting unit [5 % of non-bursting units]; total = 2 % of all the units) showed a higher *r* for the rotAB correlation than for the AB correlation, as described above, suggesting that this unit's place fields may have responded to the movements of the cue card.

- 9 units (5 bursting units [46 % of bursting units], 4 non-bursting units [20 % of non-bursting units]; total = 19 % of all the units) did not behave in a manner consistent with a spatially-related interpretation of their firing: there was no correlation between any maps, rotated or otherwise (examples of these units were shown in Figure 4.3)

Examples of the different correlational types are shown in Figures 4.6 and 4.7.

To investigate further the spatially-related firing across the four task conditions, a two-way ANOVA of unit class by condition was conducted on the spatial measures given in table 2. The aim was to see whether the spatially related measures altered over the four conditions of the task, and if they did, whether these changes were specific to unit classes. As would be expected from the one-way ANOVA findings reported above, the main effect of unit class was significant for all measures, and as the pairwise comparisons have already been reported, they will not be dealt with further here. The main effect of condition was significant only for the place field size, peak rate, and spatial information content measures. Figure 4.7 shows a plot of the grand mean of each spatial measure across the four conditions of the task. While session rate and non-zero rate remain relatively equal across the conditions (no significant differences between conditions), an impressive increase in spatial information content can be seen, with spike trains in condition D containing the most

spatial information, and condition B the least. Interestingly, the spatial information content decreases in condition B compared with condition A, before increasing in condition C and condition D. The two other measures which showed significant results in the ANOVA, peak rate and field size, show an inverse relationship with condition to the one described for the spatial information content measure. Here, peak rate and field sizes *decrease* from condition A to D and, mirroring the spatial information content trend, condition B shows higher peak rates and larger field sizes than condition A.

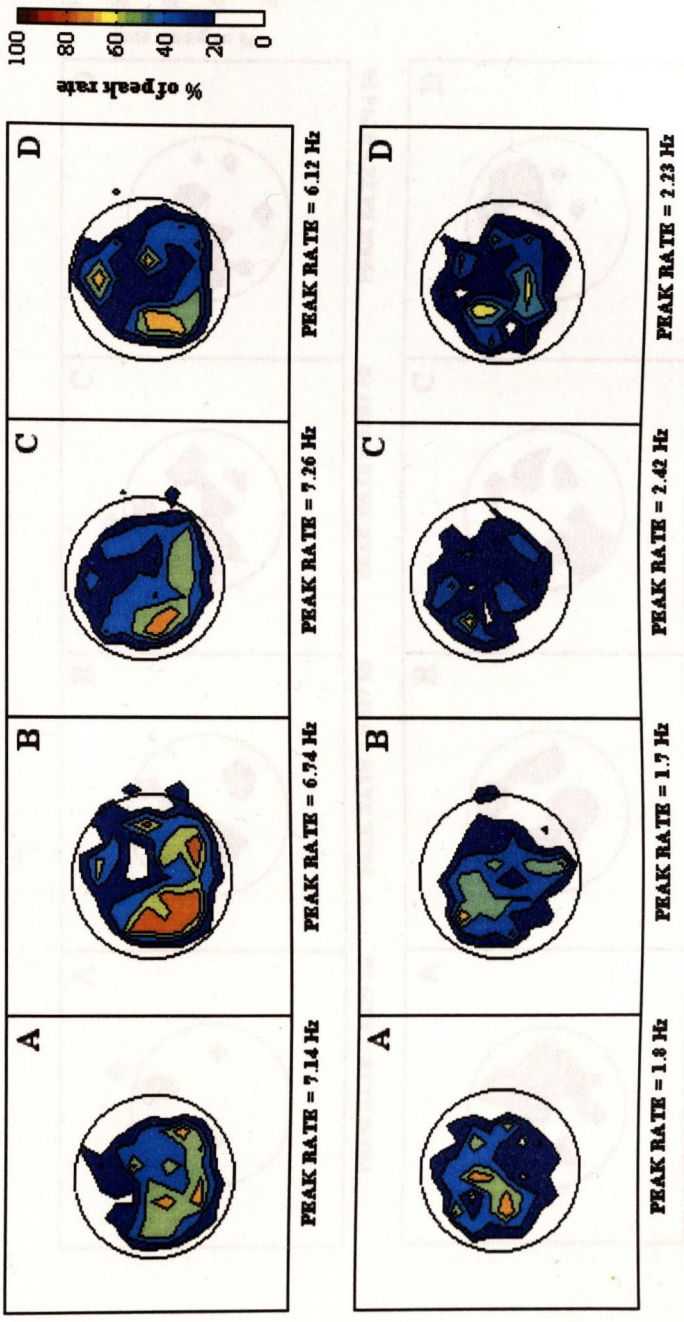


Figure 4.6. Correlational types 1 (upper - fast spiking unit) and 2 (lower - non-bursting unit). Correlational type 1 units showed stability in the location of their place fields across conditions. Correlational type 2 units showed gradual changes in the location and/or shape of their place fields over the conditions of the task. See text for discussion.

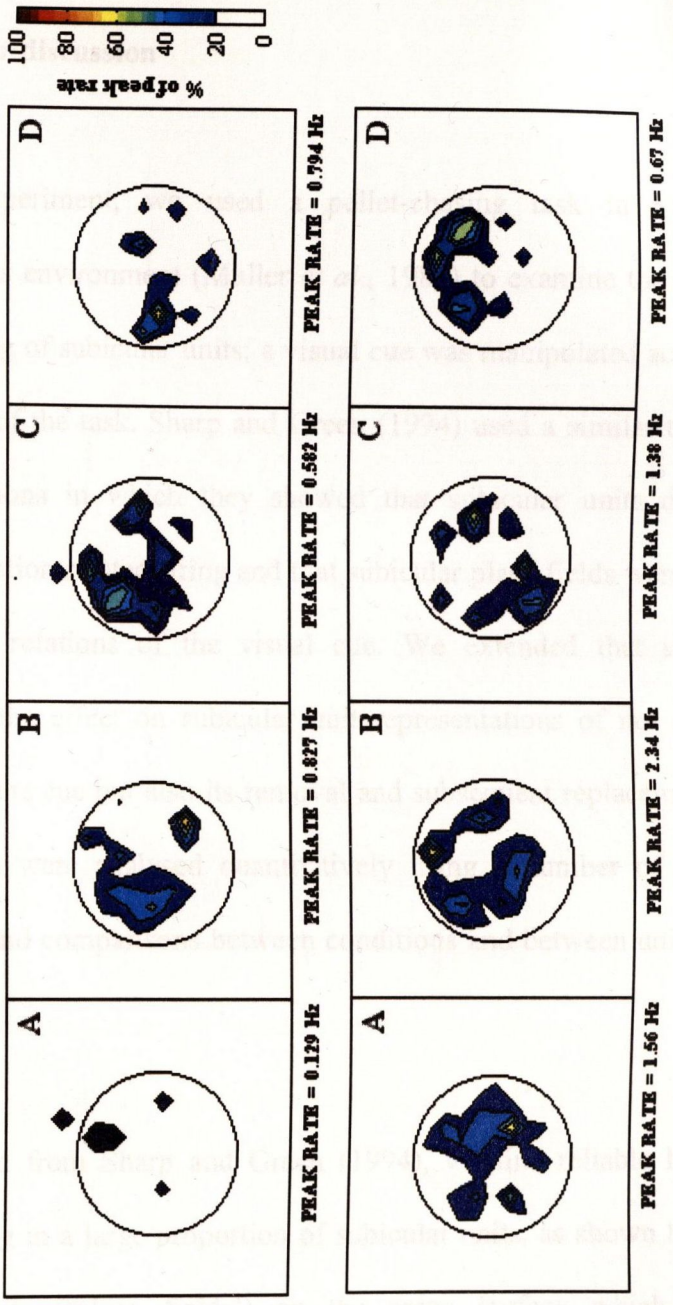


Figure 4.7. Correlational type 3 (upper - bursting unit) and 4 (lower - non-bursting unit). The correlational type 3 unit's place fields underwent an abrupt change in condition B, possibly as a result of 'remapping', but then remained relatively stable. The correlational type 4 unit's place fields may have responded to the first movement of the cue card: the peak towards the bottom of the condition A map appears at the top of the condition B map; it then remains in approximately the same place in the final two conditions. See text for discussion.

4.5 Chapter discussion

In this experiment, we used a pellet-chasing task in a circular homogenous environment (Muller *et al.*, 1987) to examine the location-related firing of subicular units; a visual cue was manipulated across four conditions of the task. Sharp and Green (1994) used a similar task with two conditions in which they showed that subicular units displayed reliable location-related firing and that subicular place fields were able to follow the rotations of the visual cue. We extended that study by examining the effect on subicular unit representations of not only the rotation of the cue but also its removal and subsequent replacement. The place fields were analysed quantitatively using a number of standard measures, and comparisons between conditions and between unit classes were made.

As expected from Sharp and Green (1994), we find reliable location-related firing in a large proportion of subicular units, as shown by firing rate gradients ('place fields') on the arena surface which behave spatially-consistently across conditions. We also find that bursting units have smaller place fields than the other unit classes, while fast spiking units (putative interneurons) have the largest. Fast spiking units here are most probably interneurons; hippocampal interneurons, or 'theta cells' (Ranck, 1973), are known to fire over virtually the whole arena surface, with some location-related firing rate gradients (e.g., Muller *et al.*, 1991; Barnes *et al.*, 1990).

The spatial information content measure (Skaggs *et al.*, 1993) revealed low estimates, relative to findings from hippocampal recordings, of the amount of information, in bits per spike, carried by subicular unit spike trains (e.g., subicular bursting units here carry 0.82 ± 0.11 bits / spike, while hippocampal place cells carried 1.76 ± 0.11 bits / spike in Save *et al.* [1998]; and a 'typical' hippocampal place cell carried 1.82 bits / spike in Skaggs *et al.* [1993]) indicating that the subicular spatial representation is more distributed than the hippocampal representation (Skaggs *et al.*, 1993); however, the trend was in the expected direction: bursting units signalled the most spatial information (0.82 bits / spike), while fast spiking units signalled the least (0.14 bits / spike). Non-bursting units signalled an intermediate amount of spatial information (0.41 bits / spike); it may be that bursting neurons do indeed signal more spatial information than non-bursting units (cf. Lisman, 1997; Commins *et al.*, 1998), but it seems more plausible that this lower spatial information content for bursting units compared with non-bursting units is a result of combining what we call 'theta-modulated units' (see Chapter 3), which may be interneurons themselves, with other non-bursting units into the general non-bursting class adopted here.

If the spike trains of subicular units carry much less spatial information than their hippocampal afferents, what happens to the spatial signal received from the hippocampus? As well as the massive projection it receives from CA1, the subiculum is known to receive inputs from the presubiculum (Kohler, 1985) and parasubiculum (Kohler, 1985) (from

where head direction information may derive), and a variety of cortical and subcortical areas, including entorhinal cortex (Stewart, 1976; Witter *et al.,* 1989), perirhinal cortex (Kosel *et al.*, 1983; Naber *et al.*, 1999, 2000), and the thalamus (nucleus reuniens [Zhang, 1994], anteromedial and anteroventral nuclei [Shibata, 1993]) (from which structures highly-processed sensory information may derive). It is reasonable to suppose, therefore, that subicular unit spike trains carry, as well as spatial information from the CA1 input, information particular to each of these other inputs. Evidence of this comes from Martin and Ono (2000) who find that subicular units convey more information about reward, head direction, and running speed, when these information content values are summed, than about location (0.64 bits / s compared with 0.45 bits / s). The head direction input has been examined before: Muller *et al.* (1991) find a class of subicular units whose firing is modulated by head direction, but do not say to what degree; Sharp and Green (1994) quantified the head direction input, finding that only mean of 1 % of the variance in subiculum unit firing could be explained by head direction, an amount which they considered negligible. Future experiments should examine the influence on subicular unit firing of the other known inputs to the subiculum (and see Chapters 6 and 7).

The pixel-to-pixel correlations revealed two major correlational 'types': the first type showed similar *r* values in the AB, AC, and AD correlations, indicating stability in the location of place fields across conditions; the second type showed *r* values that decreased from AB to

AC to AD (and BC > AC, BD > AD, CD > BD > AD), indicating that the place fields were either altering gradually or disintegrating from one condition to the next. Interestingly, only one bursting unit was of the first type, whereas *all* of the fast spiking units were this type, indicating that fast spiking units signal more reliable trial-by-trial spatial information, despite not signalling much spatial information per spike. In fact, bursting units were much more likely to show either the second correlational type (changing place fields) or no place correlate at all, further evidence that their firing may indeed be correlated with other factors (non-bursting units did not cluster according to correlational type).

The two-way ANOVA of unit class by condition conducted on the spatial measures revealed an interesting trend: while session rate and non-zero rate remain relatively equal across the conditions (no significant differences between conditions), an impressive increase in spatial information content can be seen, with spike trains in condition D containing the most spatial information, and condition B the least; interestingly, the spatial information content decreases in condition B compared with condition A, before increasing in condition C and condition D. The two other measures which showed significant results in the ANOVA, peak rate and field size, show an inverse relationship with condition to the one described for the spatial information content measure. Here, peak rate and field sizes *decrease* from condition A to D and, mirroring the spatial information content trend, condition B shows

higher peak rates and larger field sizes than condition A. This 'mirrored' trend of spatial information content vs. peak rate and field size is interesting: first, units signal *more* spatial information by condition D using *smaller* place fields and a *lower* peak rate; and second, 'condition B spike trains' contain the least spatial information. We can provide no explanation for these two findings.

Using pixel-to-pixel correlations we find only one unit which appeared to be under the control of the visual cue. It may be that the conditions under which this task were conducted, where no attempts to cue-control were made, led to this lack of place field control by the cue card (Sharp and Green [1994] *did* use a cue-controlled environment and found that the large majority of cells responded to the movements of the cue).

Units of all classes showed multiple place fields, an observation consistent with previous studies (Sharp and Green, 1994; Phillips and Eichenbaum, 1998; Barnes *et al.*, 1990). The prevailing explanation of single subicular units showing multiple place fields is that multiple CA1 principal cells converge onto single subicular principal cells; hence, if the convergent CA1 cells have place fields in the current environment themselves, then their target subicular units may show multiple place fields (e.g., Sharp and Green, 1994); anatomical evidence of this convergence exists (Tamamaki *et al.*, 1987). What purpose the qualitatively different hippocampal and subicular representations serve is at present unknown. Perhaps subicular processing connects together

different areas of the environment, areas represented singly by CA1 place cells, via Hebbian modification of these hippocampal afferent connections. Given, however, that the multiple place fields do not appear to necessarily occupy adjacent locations in the environment, one may ask what is the purpose of connecting together disparate places in an environment. Morris *et al.* (1990), in their study of the effects of selective lesions in the hippocampal formation on behaviour in the water maze, may provide a clue. They showed that rats with subicular lesions were impaired at performing the water maze task; interestingly, their deficits were qualitatively different from the deficits exhibited by rats with hippocampal lesions: while hippocampal lesioned rats showed stereotyped circling patterns of swimming in their attempts to find the hidden platform, subicular lesioned rats showed patterns of searching which 'closely resembled those of normal rats who do not know where the platform is located.' (Morris *et al.*, 1985 : 1020). This qualitatively different subicular-lesioned deficit suggests a further hypothesis: if the subiculum represents disparate areas of the environment, then subicular lesioned animals may lack the neural mechanisms to navigate efficiently between these areas, showing swimming patterns reminiscent of naïve rats. It will be interesting to design experiments which directly test the different contributions the subiculum and hippocampus make to spatial processing and behaviour.

Chapter 5

Gradient maze task

Chapter contents

5.1 Summary

5.2 Introduction

5.3 Chapter methods

5.4 Results

5.5 Chapter discussion

5.1 Summary

Animals can use idiothetic information to maintain and update the spatial representations that guide navigation. Cells in the hippocampus, itself strongly implicated in spatial behaviour, are modulated by vestibular-derived inputs.

Subicular units are influenced by both place-related activity from the hippocampus and head-direction information from the presubiculum. We hypothesised that the subiculum may be an important site of convergence for information about location (coming, presumably, from the hippocampus) and information about orientation and changes in orientation (coming, presumably, as idiothetic inputs from structures such as the vestibular system [via the presubiculum and thalamic nuclei]). To test this hypothesis we recorded subicular unit activity while rats ran in search of reward on an adapted plus maze called a 'gradient maze'. Arms were set at angles of elevation of either 0 °, 10 °, or 20 ° relative to the ground. We found only two units (of 41) correlated with gradient. More than 60 % of units, however, were correlated with movement-related features. It appears that, while the subiculum does not participate in encoding gradient information, it is strongly activated by movement.

5.2 Introduction

Activation of the vestibular system can influence unit activity in the hippocampal formation (HF), as has been shown in the rodent (Wiener *et al.*, 1995; Sharp *et al.*, 1994) and the primate (O'Mara *et al.*, 1994). Wiener *et al.* (1995) showed that hippocampal place cells in rats could rotate or remap following rotation of the animal and its environment in darkness; O'Mara *et al.* (1994) reported that whole-body motion in the absence of visual information can modulate the firing rate of hippocampal neurons in the alert monkey. Further evidence from rats passively displaced on a robot suggests that this remapping of place cells is related to cues derived from the vestibular system, rather than to cues derived from postural reflexes or locomotor reactions (Gavrilov *et al.*, 1998). Direct electrophysiological evidence for vestibular influences on the HF also exists: stimulation of vestibular regions induces field potentials in the HF of anaesthetized guinea pigs (Cuthbert *et al.*, 2000).

The vestibular influence on the HF has been implicated in different processes, including the updating of hippocampal maps during self-motion (Wiener *et al.*, 1995; Taube *et al.*, 1996), and plays a crucial role in specific models of path integration (e.g., McNaughton *et al.*, 1996). Interestingly, the HF itself may be needed to interpret vestibular information since humans with unilateral hippocampal lesions exhibit errors in 'vestibular memory' for whole-body rotations (Wiest *et al.*, 1996).

Single unit recordings have revealed cell populations in the anterodorsal thalamic nuclei (Taube, 1995) and the presubiculum (Taube *et al.*, 1990a,b) which discharge as a function of head direction (HD); this HD information is believed to derive from, or at least to be updated by, the vestibular system (Stackman and Taube, 1997). Given that the subiculum receives input from both the presubiculum (Kohler, 1985) and the anterodorsal thalamic nucleus (Van Groen and Wyss, 1995) (though both inputs are modest at best), it is unsurprising that subicular unit firing is also modulated by HD (Martin and Ono, 2000; Sharp and Green, 1994; Muller *et al.*, 1991), albeit rather weakly (Sharp and Green, 1994).

In light of these anatomical and electrophysiological findings, we hypothesised that the subiculum may be an important site of convergence for information about location (coming, presumably, from the hippocampus) and information about orientation and changes in orientation (coming, presumably, as idiothetic inputs from structures such as the vestibular system [via the presubiculum and thalamic nuclei]). To test this hypothesis, we developed a task, based on a design by Grobéty and Schenk (1992), that would activate parts of the vestibular apparatus, making use of an adapted plus maze, in which each arm could be elevated independently to an angle of 10 ° or 20 ° above level (a 'gradient maze'). Using the gradient maze, we recorded subicular unit activity while rats performed a simple task in which they were required to run to a food well at the end of each arm in turn to receive a reward. A simple task was developed so as to homogenize the animal's behaviour

over the entire maze, in the hope that any effects of the gradient on subicular unit firing would be revealed. As well as testing for the effects of gradient on subicular unit firing, we took the opportunity to examine other possible firing correlates, such as location-related firing, and also firing correlates around the time of arrival at the reward; a previous study suggested that subicular unit firing was differentially correlated with random and predicted reward (delivered via stimulation of the medial forebrain bundle) (Martin and Ono, 2000). It was of interest to examine whether such firing rate changes would follow the delivery of more 'ethological' reward (food).

5.3 Method

Subjects

Three adult male Wistar rats (Bio Resources Unit, University of Dublin), weighing approximately 350 g on arrival in the laboratory were used as subjects. Rats were housed singly in a temperature controlled laminar airflow cupboard, and maintained on a 12:12 hour light-dark cycle (light: 0800 – 2000 hours). All testing was carried out during the light phase. In preparation for the experiment several days after surgery, all rats were regularly handled and gradually reduced to approximately 80 % of their *ad libitum* feeding weight and were maintained at this level throughout the experimental period by providing each rat 15 g of rat chow per day as a supplement to the food used as reward during the task.

Apparatus

Rats were tested on a 'gradient maze' which consisted of an adapted four-arm 'plus' maze based on a design by Grobéty and Schenk (1992) (see Figure 5.1). Each arm (60 x 10 x 15 cm) extended from a central platform (40 x 40 cm) in the direction of a different cardinal point on the compass, and had a concealed food well at the end. Each arm pivoted on a screw close to the central platform which allowed arms to be raised at an angle relative to the floor. During each trial, two arms remained level on the floor (0 °), one arm was sloped at 10 °, and one arm was sloped at

20 °. The maze rested on the floor of the experimental room. The experimental room was illuminated as usual by two 12 volt bulbs attached to the ceiling (for full details of the experimental room see Chapter 2). The maze was surrounded by numerous potential cues.

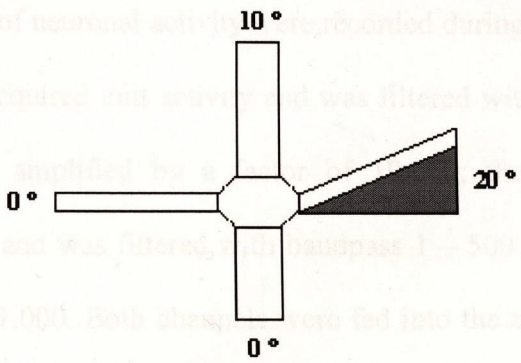


Figure 5.1. The 'gradient maze'. A plus-maze was adapted so that each arm could be independently raised to angles of 10 ° or 20 °. In each trial, one arm was raised to 10 °, one arm was raised to 20 °, and two arms remained level on the floor. This figure is for illustrative purposes only: it is not to scale and does not show the maze walls.

Surgery

Electrodes were implanted using the standard method (see Chapter 2). Electrodes were aimed at the following coordinates in dorsal subiculum: AP –6.8 mm (relative to bregma), L 4.0 mm, DV 2.4 mm (Paxinos and Watson, 1996).

Following the experiment, rats were deeply anaesthetized using sodium pentobarbitol then perfused. Brains were removed and stored in 4 %

formaldehyde for several days. Brains were subsequently sliced on a vibrotome and all electrode placements were verified as being in dorsal subiculum.

Recording techniques

Two channels of neuronal activity were recorded during each session: the first channel acquired unit activity and was filtered with bandpass 300 – 5000 Hz and amplified by a factor of 10,000; the second channel acquired EEG and was filtered with bandpass 1 – 500 Hz and amplified by a factor of 1,000. Both channels were fed into the analogue-to-digital conversion system (DataWave Technologies, Colorado, USA) described in detail in Chapter 2. Unit activity output was also sent to a loudspeaker.

An overhead video tracking system was used to follow the location of the rat in the maze. The system operated in one-spot (monochrome) tracking mode and detected a small LED attached to the headstage (see Chapter 2). The tracking system split the environment into 256 x 256 pixels and sent time-stamped positional data to the acquisition system which gave the moment-to-moment location of the rat and also permitted calculation of its velocity.

After each session the electrodes were driven ventrally by approximately 50 μm with the hope of encountering new units.

Protocol

The task was very simple: rats were required to retrieve a chocolate milk reward (~ 1 ml) from the food well at the end of each arm. Before each trial all arms were baited and two of the arms were sloped (one at 10 °; one at 20 °) according to a pseudorandom list compiled prior to the experiment (see Appendix 3). Thus, in every trial, rats traversed each maze arm at least once. If the rat re-entered a previously-entered maze arm, the mistake was noted and the data from the re-entry was discarded. Data from mistake trials was not analysed because rats made few mistakes (6 % of all arm entries) and, when they did, they usually corrected the mistake before reaching the food well.

Rats were trained on the maze approximately seven days after surgery. All rats were accustomed to the maze environment over the period of a few days prior to testing by placing small drops of the reward in all areas of the maze and allowing them to retrieve it until they learned to find the reward only in the food wells at the end of the arms. Once each rat traversed the maze quickly, with minimal errors, testing began.

Each recording session typically lasted from 30 - 50 mins. At the start of the session, rats were taken from a holding chamber close to the maze and placed into the centre of the maze at a random orientation. Each session consisted of eight trials in which the orientation of each arm was pseudorandomly varied in each trial (see above). At the end of each trial

the rat was returned to the holding chamber and remained there until the start of the next trial.

As well as acquiring neuronal activity and positional data during each trial, the experimenter also recorded the ongoing behaviour of the rat by pressing a key on the keyboard at the time of the behaviour; key-presses were saved to file as time-stamped 'event-flags'. Event flags used in this experiment were: F = 'arrival at food reward'; T = 'turn away from food reward facing towards the centre of maze'; M = 'arm mistakenly re-entered'.

Data analysis

Output ASCII files were exported from the DataWave system into custom-written analysis software (M.Anderson using Matlab; see Chapter 2 for a full discussion of all analysis software and techniques). Spike sorting was conducted using a template-matching algorithm with conservative criteria for acceptance. EEG was visualized using a data plotter which also allowed the simultaneous viewing of spike times and event flags; as such, portions of the acquired data could be cropped for further analysis.

After spike sorting, individual unit data were further processed to display inter-spike interval histograms (ISIHs), autocorrelation histograms (ACHs), and peri-event time histograms (PETHs) and raster plots based

on the event flags (see Chapter 2). The ISIHs and ACHs were used, together with the electrophysiological characteristics of the units (spike duration and spike amplitude) to classify each unit according to previously determined subicular cell types (see Chapter 3). Simple mean rate (Hz) was also calculated for each unit by dividing the total number of spikes by the session time.

To determine the nature of the spatial signal, if any, carried by subicular units, the 'information content' measure was used (Skaggs *et al.*, 1993), which calculates the amount of information, *I,* contained in the firing of a unit, using the formula

$$I = \sum_i (\lambda_i / \lambda) \log_2 (\lambda_i / \lambda) P_i ,$$

where λ_i is the mean firing rate in pixel i, λ is the overall mean firing rate, and P_i is the probability of the animal occupying pixel i (which is simply the time spent in pixel i divided by the total trial time). For a fuller description of this measure see Chapter 2. Spatial information content is measured in units of 'bits of information per spike' (bits / spike).

Firing rate maps were constructed in Matlab to enable easy visualisation of location-specific firing, using the system described in *Main Methods*. Briefly, the maps are constructed by dividing the open-field environment into pixels (square measuring 16 x 16 cm). The number of spikes that fired when the rat occupied a pixel is divided by the total time the rat spent in that pixel (in seconds) to produce a firing rate for that pixel (in

Hz). All the pixel firing rates are mapped as a contour plot using Matlab's in-built contouring algorithm. The maps are colour-coded in increments of 20% of the peak firing rate for each individual map. The 'peak rate' of each map is equal to the rate of the pixel with the highest firing rate in each map. Other spatial measures were also calculated, based on the firing rate maps. 'Non-zero rate' represents the mean of all the pixels in a firing rate map displaying a rate above 0 Hz. This measure controls for the probability that units would not fire in all areas of the arena (i.e., in all pixels) but only in discrete locations. Also, an estimate of the size of the firing field for each cell was calculated by dividing the number of pixels with a firing rate of greater than zero by the total number of pixels visited in the environment. Given that subicular place fields have multiple fields and cover a large proportion of the environment surface this measure was reasoned to be the most appropriate. It also allowed comparisons to be made with previous work (Sharp and Green [1994], from where the measure originates).

It was considered that a number of factors in this experimental design were capable of influencing unit firing rates. To this end, a three-way fixed factor ANOVA with a covarying factor was conducted for each unit: the three fixed factors were arm gradient, arm location, and trial, while the covarying factor was mean velocity on each arm. For this analysis, firing on the central platform was ignored. Note that the gradient factor had five levels: level, 10 °, and 20 ° as one would expect, but also –20 ° and –10 °, given that animals heading along an angled arm

towards the central platform were moving *down* a gradient. The arm location factor had four levels: north, south, east, and west (with the consideration borne in mind that subicular units might not necessarily represent the north arm angled at 20 ° in the same way as a level north arm) and was included because, while we were most interested in the possible effects of gradient on unit firing, clearly subicular cells would also be influenced by spatial location. The covarying factor of mean velocity on each arm (two speeds on each arm, one for each direction [outward and inward]) was included because it was considered that the greater effort expended by the rats in climbing an arm angled at 20 ° than in moving along a level arm might influence firing rates: to this end, the speed of the animal on the maze arms, as an index of 'effort', was calculated and used in the analysis (also, velocity has been shown to influence the firing rate of hippocampal units [McNaughton *et al.*, 1983; Wiener *et al.*, 1989; Zhang *et al.*, 1998]). As well as examining the main effects of arm gradient, arm location, trial, and mean arm velocity, the interaction effect of arm and angle was considered of potential interest and was also included in the ANOVA model.

The independent variable used in the three-way ANOVA was the transformed (see below) mean firing rate of each unit on each arm in each direction (outward or inward) and was calculated using both the known locations of the start of each arm and the event-flag data. Data for the outward direction was taken from the time between entry into the arm and the arrival at the food reward; data for the inward direction was taken

from the time between turning away from the food reward and exit of the arm. Sixty-four mean firing rates were produced for each unit (8 trials x 4 arms x 2 directions).

Prior to analysis, firing rates were transformed using the following formula,

$$X' = (\sqrt{X} + \sqrt{X+1}) ,$$

since, as found in previous studies (e.g., Wiener *et al.*, 1995; O'Keefe and Speakman, 1987), firing rates showed skewed distributions. An assumption behind ANOVA is the normality of the data, and in agreement with previous studies (Wiener *et al.*, 1995; O'Keefe and Speakman, 1987; and see Winer, 1971) we found that this was the most effective transform.

Statistical analysis

Where appropriate, differences between firing rates were analysed parametrically with analyses of variance (ANOVA) and with post-hoc tests (Tukey's Honestly Significant Difference [HSD] tests). For analyses of firing rates before and after the onset of a particular behaviour (as recorded with the event flags and displayed in the PETHs), paired t-tests were used (since a plot of the differences between pre-and post-flag spike counts showed them to be normally distributed). All statistics were calculated using either the Statistical Package for the Social Sciences (SPSS) software, or MINITAB.

5.4 Results

Histological findings

The tracks of electrode bundles were reconstructed by visual inspection after histological processing (see Figure 5.2). The recording electrodes from all rats were positioned in dorsal subiculum (one bordered hippocampal area CA1 and was included because it recorded units with similar electrophysiological characteristics to the other recorded units).

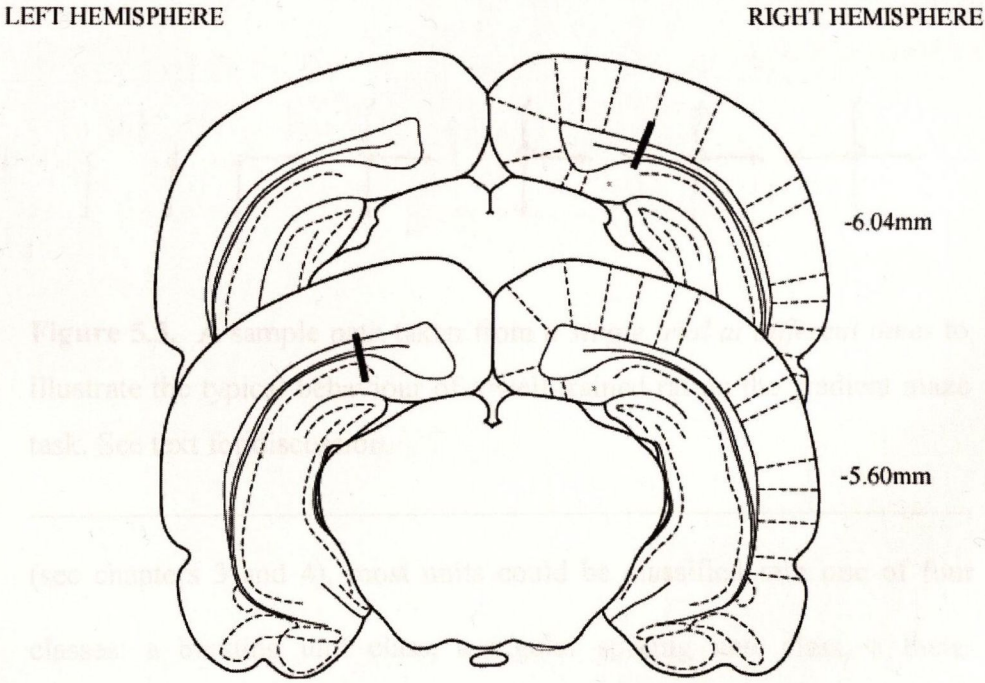


Figure 5.2. Results of the histological analysis showing the reconstructed electrode tracks from two of the three animals. The electrode track from the remaining animal could not be located but this data was included because the units had very similar electrophysiological characteristics to units recorded from other animals. The heavy black line in each section represents the electrode track. Slice measurements are posterior to bregma (Paxinos and Watson, 1996).

The electrode track from one animal could not be located but was included because the units had very similar electrophysiological characteristics to units recorded from other animals.

Unit electrophysiological characteristics and classification

All rats learned the task quickly (a sample path is shown in Figure 5.3). In total, 41 subicular units were isolated from the three rats. Examination of the ISIH and ACH, and unit waveform characteristics (amplitude and width), for each unit showed that, as expected from previous chapters

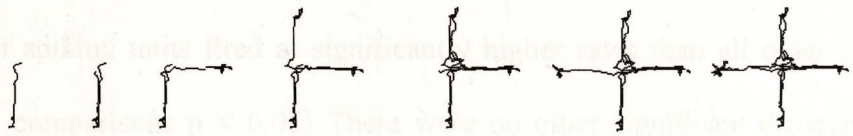


Figure 5.3. A sample path taken from *a single trial at different times* to illustrate the typical behaviour of a well-trained rat on the gradient maze task. See text for discussion.

(see chapters 3 and 4), most units could be classified into one of four classes: a bursting unit class, a regular spiking unit class, a theta-modulated unit class, and a fast spiking unit class. For details of the defining characteristics of each class, see *Results* in Chapter 3. It is noted that all the units recorded here showed very similar ISIHs and ACHs and other electrophysiological measures to the units collected in the baseline experiment. Table 1 displays the firing characteristics of the classified units, separated by unit class.

TABLE 1. Electrophysiological measures of subicular units separated by class

Unit class	Bursting	Regular	θ-mod	Fast spike
N	15	22	2	2
Rate (Hz)	0.82 ± 0.15	1.25 ± 0.14	0.59 ± 0.12	3.59 ± 0.34
Height (μV)	269 ± 16	257 ± 19	188 ± 9	181 ± 21
Width (ms)	0.80 ± 0.05	0.72 ± 0.04	0.78 ± 0.06	0.48 ± 0.08

A one-way ANOVA with Tukey HSD post-hoc tests revealed differences between unit classes in firing rate only ($F = 12.42$, d.f.$= 3, 37$, $p < 0.001$). Fast spiking units fired at significantly higher rates than all other units (all comparisons $p < 0.05$) There were no other significant differences between unit classes on the electrophysiological measures displayed in Table 1.

EEG

EEG recorded during each session was analysed using fast Fourier transforms. Nearly all of the EEG was 6 – 8 Hz theta, as would be expected from this task in which animals ran for most of the trial period. A sample periodogram is displayed in Figure 5.4, together with a section of EEG taken from one of the trials.

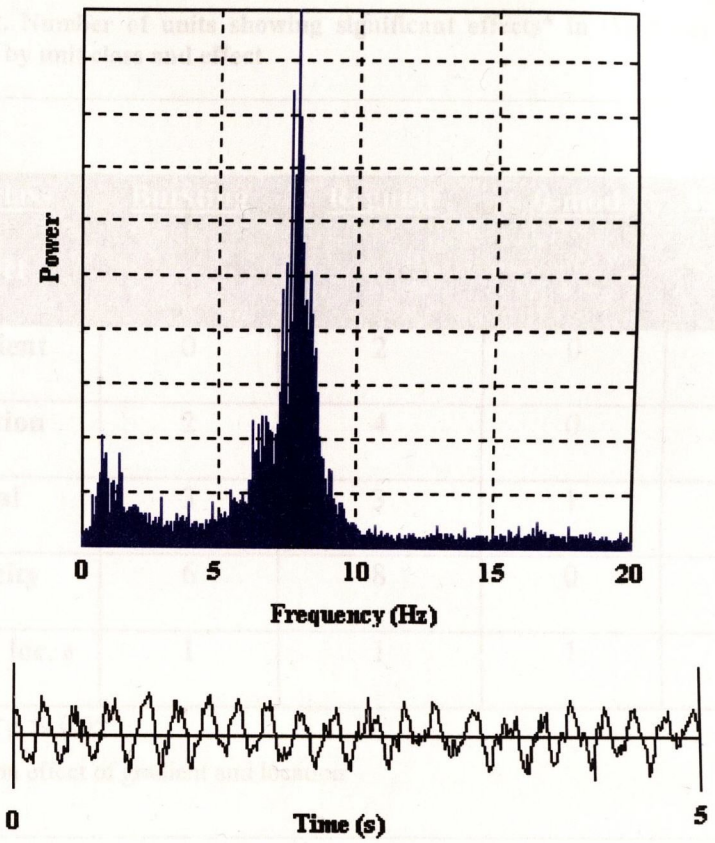


Figure 5.4. A periodogram and a section of EEG taken from a typical experiment and shown to illustrate that theta rhythms in the 6 – 8 Hz were the dominant EEG pattern recorded during this task.

Gradient analysis and other analyses

Table 2 displays the number of units showing significant main or interaction effects in the three-way ANOVA.

Some units displayed multiple correlates:

- Of the bursting units, four units had single correlates (one of location and three of velocity), one unit had two correlates (trial and velocity), and two units had three correlates (unit 1: trial, velocity, and the interaction effect of gradient and location; unit 2: location, trial, and velocity).

TABLE 2. Number of units showing significant effects* in the 3-way ANOVA, separated by unit class and effect

Unit class / Effect	Bursting	Regular	θ-mod	Fast spike
Gradient	0	2	0	0
Location	2	4	0	0
Trial	3	3	1	2
Velocity	6	8	0	1
Grad. x loc. §	1	1	1	0

* $0.0001 < p \leq 0.05$

§ Interaction effect of gradient and location

- Of the regular spiking units, five units had single correlates (one of trial and four of velocity), five units had two correlates (unit 1: location and trial; unit 2: angle and velocity; unit 3: velocity and the interaction effect of gradient and location; unit 4: angle and location; unit 5: location and velocity), and one unit had three correlates (location, trial, and velocity).
- Of the theta-modulated units, both units had one correlate (unit 1: trial; unit 2: the interaction effect of gradient and location).
- And of the fast spiking units, one unit had one correlate (trial) and one unit had two correlates (trial and velocity).

Main effect of gradient

Gradient had little effect on unit firing: only two units (5 %) showed a significant main effect of gradient on firing rate (Unit 1: $F = 2.81$, d.f. = 4, 36, $p < 0.05$; Unit 2: $F = 2.63$, d.f. = 4, 36, $p < 0.05$). Post-hoc tests failed to pinpoint a significant difference for the first unit; the second unit revealed a difference in firing rate restricted to one comparison, between $-10°$ and $-20°$ ($p < 0.05$). Both units were regular spiking units.

Main effect of location

Interestingly, only six units (15 %) showed significant main effects of arm location (N, S, E, or W) on their firing rates (for statistical output see Appendix 4). This rather low number of cells showing location-related firing owes, we believe, to the concern raised above that subicular units might not necessarily represent the north arm angled at 20 ° in the same way as a level north arm. These six units consisted of two bursting units, and four regular spiking units.

Main effect of trial

Nine units (22 %) showed a significant main effect of trial (for statistical output see Appendix 5). These nine units consisted of two fast spiking units, three bursting units, three regular spiking units, and one theta-modulated unit, indicating that trial differences in firing rate were not specific to a unit class. The effects for the majority of units only just reached significance, however, and post-hoc tests were able to find a

significantly different comparison for only three units. The differences in firing rate between trials did not appear to be related to differences in velocity.

Covariation of arm velocity with unit firing

15 units (37 %) showed a main effect of mean arm velocity on their firing rates (for statistical output see Appendix 6). Many of these effects were highly significant ($p < 0.0001$). Plots of mean arm velocity

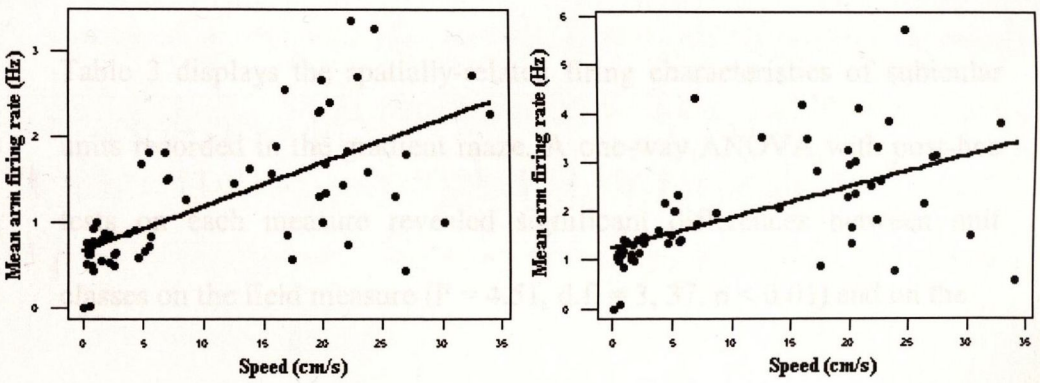


Figure 5.5. Plots of mean arm firing rate vs. speed for two units showing significant covarying effects of speed. See text for discussion.

against firing rate showed a positive relationship between these two variables: higher firing rates accompanied higher velocities (see Figure 5.4).

Interaction effect of location and gradient

Three units (7 %) showed significant interaction effects between arm gradient and arm location (Unit 1: $F = 3.30$, d.f. = 12, 36, $p < 0.005$; Unit

2: $F = 2.07$, d.f. = 12, 36, $p < 0.05$; Unit 3: $F = 3.11$, d.f. = 12, 36, $p < 0.005$).

Spatial analysis

Despite designing this experiment specifically to examine gradient-related firing, it was considered appropriate, in light of the location-related firing described in Chapter 4, to analyse all units in terms of their location-related firing. The difficulty in assigning a purely spatial interpretation to subicular unit firing is noted in the *Discussion.*

Table 3 displays the spatially-related firing characteristics of subicular units recorded in the gradient maze. A one-way ANOVA with post-hoc tests on each measure revealed significant differences between unit classes on the field measure ($F = 4.51$, d.f. = 3, 37, $p < 0.01$) and on the

TABLE 3. Spatially-related measures of subicular units separated by class

Unit class	Bursting	Regular	θ-mod	Fast spike
N	15	22	2	2
Field size	0.85 ± 0.02	0.91 ± 0.01	0.79 ± 0.04	0.92 ± 0.03
Peak rate (Hz)	1.85 ± 0.16	2.32 ± 0.24	1.50 ± 0.43	2.89 ± 0.32
Non-zero rate (Hz)	1.07 ± 0.16	1.90 ± 0.25	1.01 ± 0.19	4.17 ± 0.36
Spatial info. c.* **(bits / spike)**	0.26 ± 0.03	0.22 ± 0.02	0.19 ± 0.04	0.12 ± 0.01

*Spatial information content

non-zero rate measure ($F = 6.89$, d.f. = 3, 37, $p < 0.001$). Fast spiking units had significantly higher non-zero rates than all other unit classes (fast spiking vs. bursting, $p < 0.001$; fast spiking vs. regular spiking and theta-modulated, both $p < 0.05$), and bursting units had significantly smaller place fields than regular spiking units ($p < 0.05$). There were no other significant differences.

Firing rate maps were constructed to visualize location-related firing. Since only six units showed main effects of arm location on their firing rates, this analysis was restricted to these units. Firing rate maps were collapsed across all eight trials of each experimental session since individual trials did not last long enough to allow a good sampling of the maze surface. Maps were also separated as to whether the rat was making outward or inward journeys along the maze arms because in restrictive environments hippocampal place cells and subicular place cells can show directional place fields (i.e., present when the rat runs in one direction only) (McNaughton *et al.*, 1983; Barnes *et al.*, 1990). Four of the six units did indeed show directional effects: these units are displayed in Figure 5.6; the other two units did not show strong directional effects: these units are displayed in Figure 5.7.

PETH and raster analysis

38 of the 41 units were analysed using PETHs (the remaining three units were collected in experiments when event-flags were not used).

Paired t-tests on pre- and post-event flag firing in the PETHs revealed that 24 units (63 %) were significantly correlated in one of two ways with arrival at the food reward at the end of each arm ('F-correlate' units; $p < 0.05$). The first unit response type to arrival at food reward (*F-correlate increase/decrease* response) was a pre-arrival increase in firing followed immediately on arrival by a sharp decrease in firing relative to the baseline; 20 of the 24 (83 %) units correlated with arrival at food reward showed this response. The second unit type response to arrival at food reward (*F-correlate increase* response) was an increase in overall firing rate at the time of arrival at the reward; 4 of the 24 units (17 %) correlated with arrival at the food reward showed this response. Both correlates were not selective as to which arm the rat occupied. Figure 5.8 illustrates these two responses with PETHs, raster displays, and a plot of concurrent velocity.

Paired t-tests were also conducted on PETHs constructed around the turn the rat made at the end of each arm before its return to the centre. Not as many units showed significant differences pre- and post-event flag here ('T-correlate') as did for the F-correlate (here, 11 units [29 % of all units]). Notably, though, of the units showing *both* significant F- and T-correlates, all of the units which had displayed an *F-correlate increase/decrease* response to the arrival at reward now increased their firing (*T-correlate increase* response; 3 units; $p < 0.05$); and units which had displayed an *F-correlate increase* response to arrival at reward now

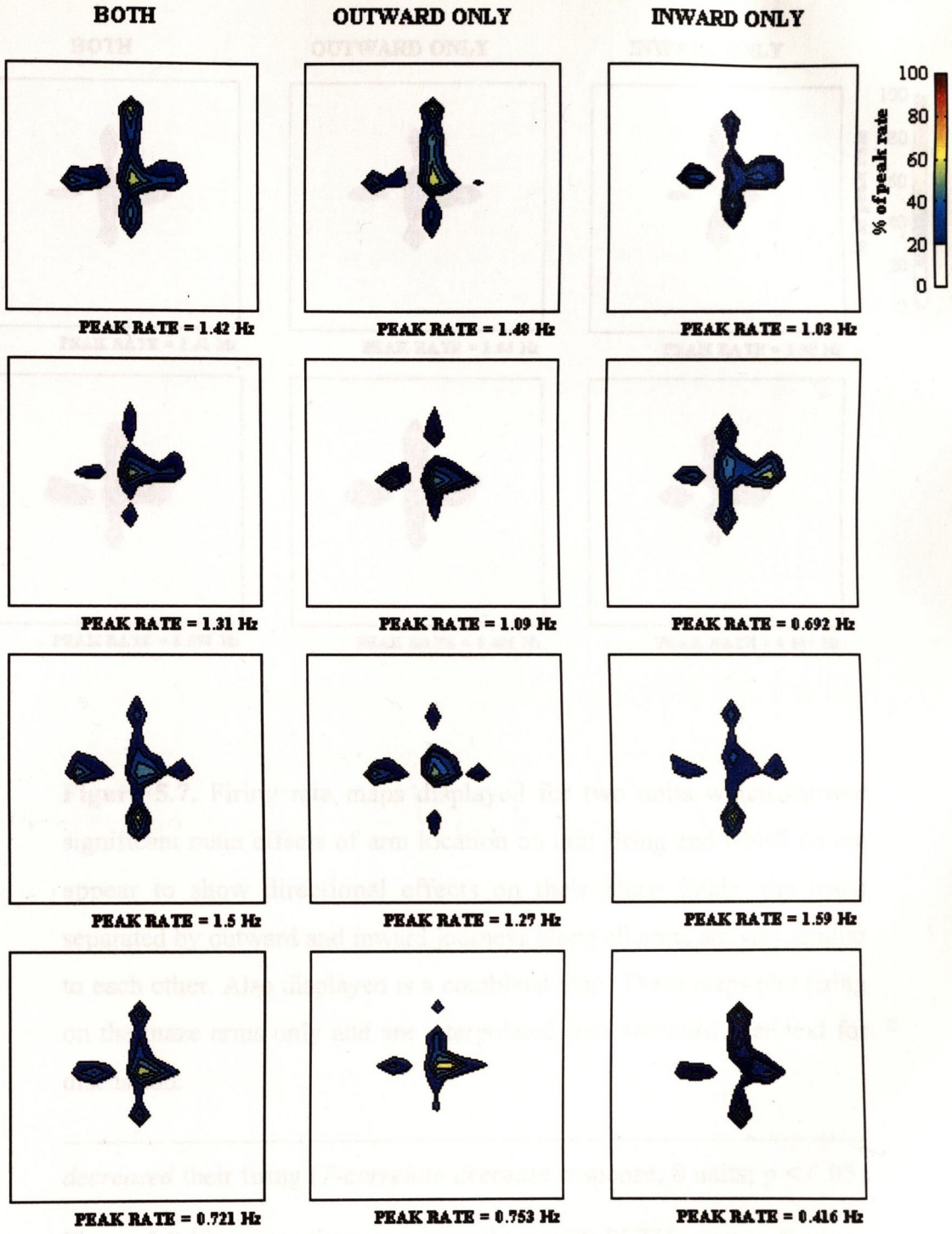


Figure 5.6. Firing rate maps displayed for four units which showed significant main effects of arm location on unit firing and which appear to show directional effects on their place fields: the maps separated by outward and inward journeys along all arms are substantially different from each other. Also displayed is a combined map. These maps plot firing on the maze arms only and are interpolated (see *Methods)*. See text for discussion.

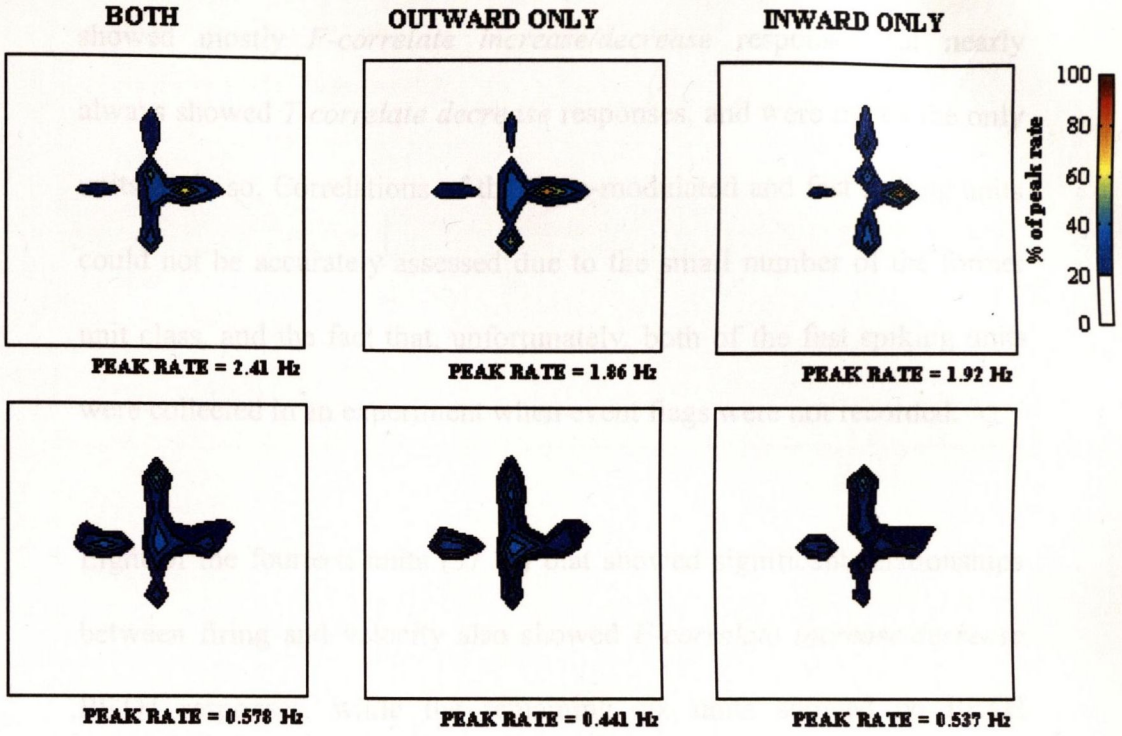


Figure 5.7. Firing rate maps displayed for two units which showed significant main effects of arm location on unit firing and which do not appear to show directional effects on their place fields: the maps separated by outward and inward journeys along all arms are very similar to each other. Also displayed is a combined map. These maps plot firing on the maze arms only and are interpolated (see *Methods)*. See text for discussion.

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decreased their firing (*T-correlate decrease* response; 8 units; $p < 0.05$). Figure 5.9 illustrates these two responses with PETHs, raster displays, and a plot of concurrent velocity.

Table 4 separates units which showed significant PETH effects by unit class. Bursting units that had significant F-correlate PETHs nearly always displayed an *F-correlate increase/decrease* response; and always here displayed *T-correlate increase* responses. Regular spiking units

showed mostly *F-correlate increase/decrease* responses but nearly always showed *T-correlate decrease* responses, and were in fact the only units to do so. Correlations of the theta-modulated and fast spiking units could not be accurately assessed due to the small number of the former unit class, and the fact that, unfortunately, both of the fast spiking units were collected in an experiment when event flags were not recorded.

Eight of the fourteen units (57 %) that showed significant relationships between firing and velocity also showed *F-correlate increase/decrease* PETH responses, while the remaining six units showed no PETH responses, indicating that this PETH pattern is most likely related to the velocity effects. The units showing *F-correlate increase* responses show an inverse relationship to velocity: these units increase their firing when velocity is low, or zero as happened when the animals reached the food reward. The *T-correlate* responses, we believe, are attributable to the velocity effects also: *T-correlate increase* response units increase their firing after the turn (when velocity increases) while the *T-correlate decrease* response units decrease their firing after the turn, mirroring the *F-correlate* response behaviour. To summarize this analysis, and to support this interpretation, units showed only one response to increasing velocity: either increased firing or decreased firing.

TABLE 4. Number of units showing significant PETH effects separated by unit class and by PETH response type

Unit class	Burster	Regular	θ-mod	Fast*
F-correlate *inc./decrease*	11^{6*}	8^{2}	1^{0}	-
F-correlate *decrease*	1^{0}	3^{0}	0^{-}	-
T-correlate *increase*	2^{1}	1^{0}	0^{-}	-
T-correlate *decrease*	0^{-}	8^{4}	0^{-}	-

* Superscript numbers indicate the number of the respective units which showed significant covarying effects of velocity on their firing.

NB: Unfortunately, the fast spiking units were collected in an experiment when event flags were not recorded; it does not indicate that fast spiking units were not correlated with arrival at the reward or turning away from the reward.

An alternative explanation, and one that Martin and Ono (2000) support (see *Discussion*), is that the increases and decreases in firing are related to reward rather than to velocity. It may seem that the *F-correlate increase* response in particular is indicative of reward-related firing. However, given that units showing *F-correlate increase* responses also showed *T-correlate* decrease responses (if they indeed showed a significant *T-correlate* response), and that all units had coherent relationships with velocity, we favour the velocity-related interpretation.

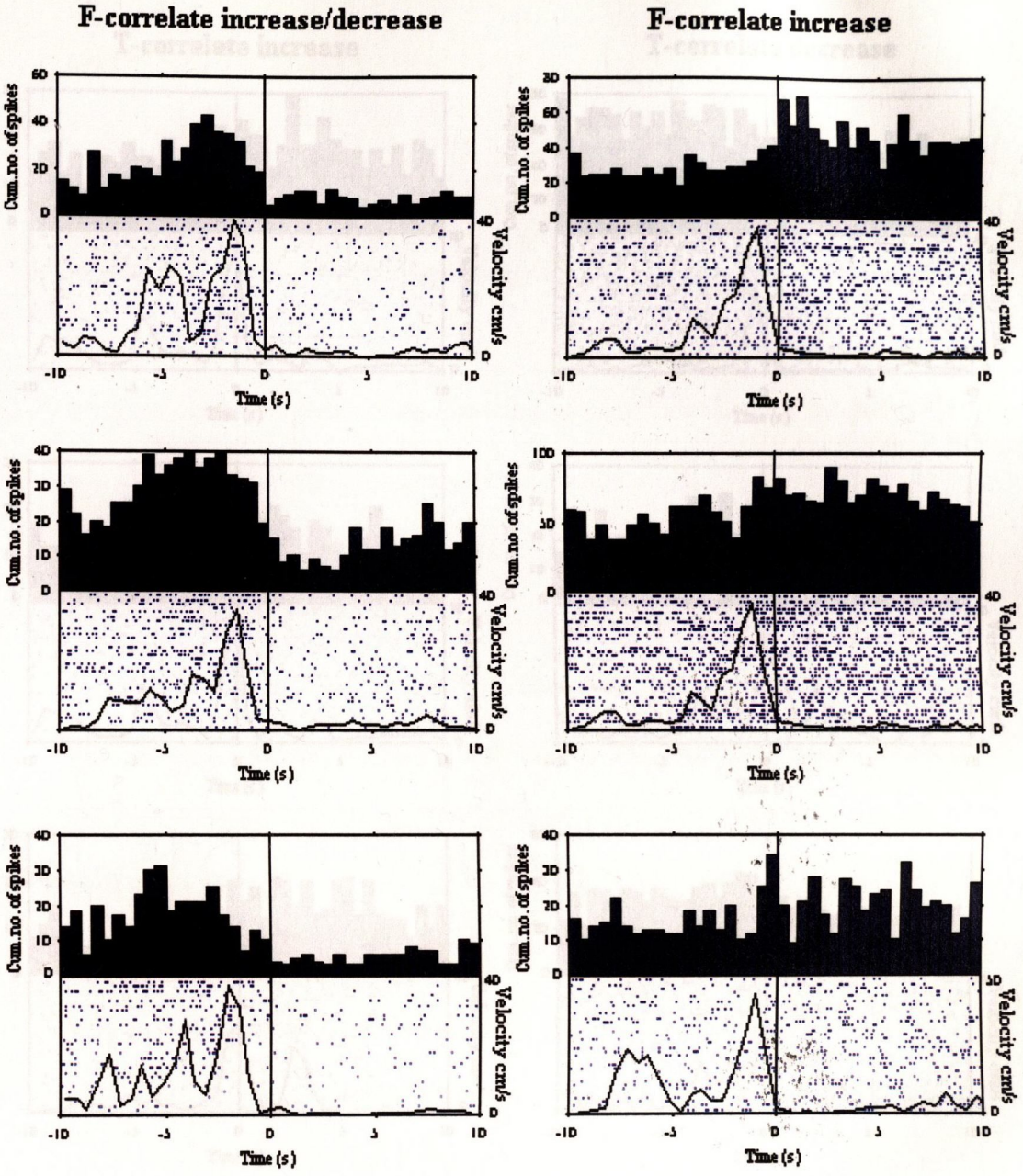


Figure 5.8. PETH and corresponding raster displays for unit responses to arrival at the food reward ('F-correlate' responses). The first column shows three different units responding with the *F-correlate increase/decrease* response; the second column shows three different units responding with the *F-correlate increase* response. Also displayed, overlaid on the rasters, is a plot of the averaged velocity of the animal during the displayed time period. See text for discussion.

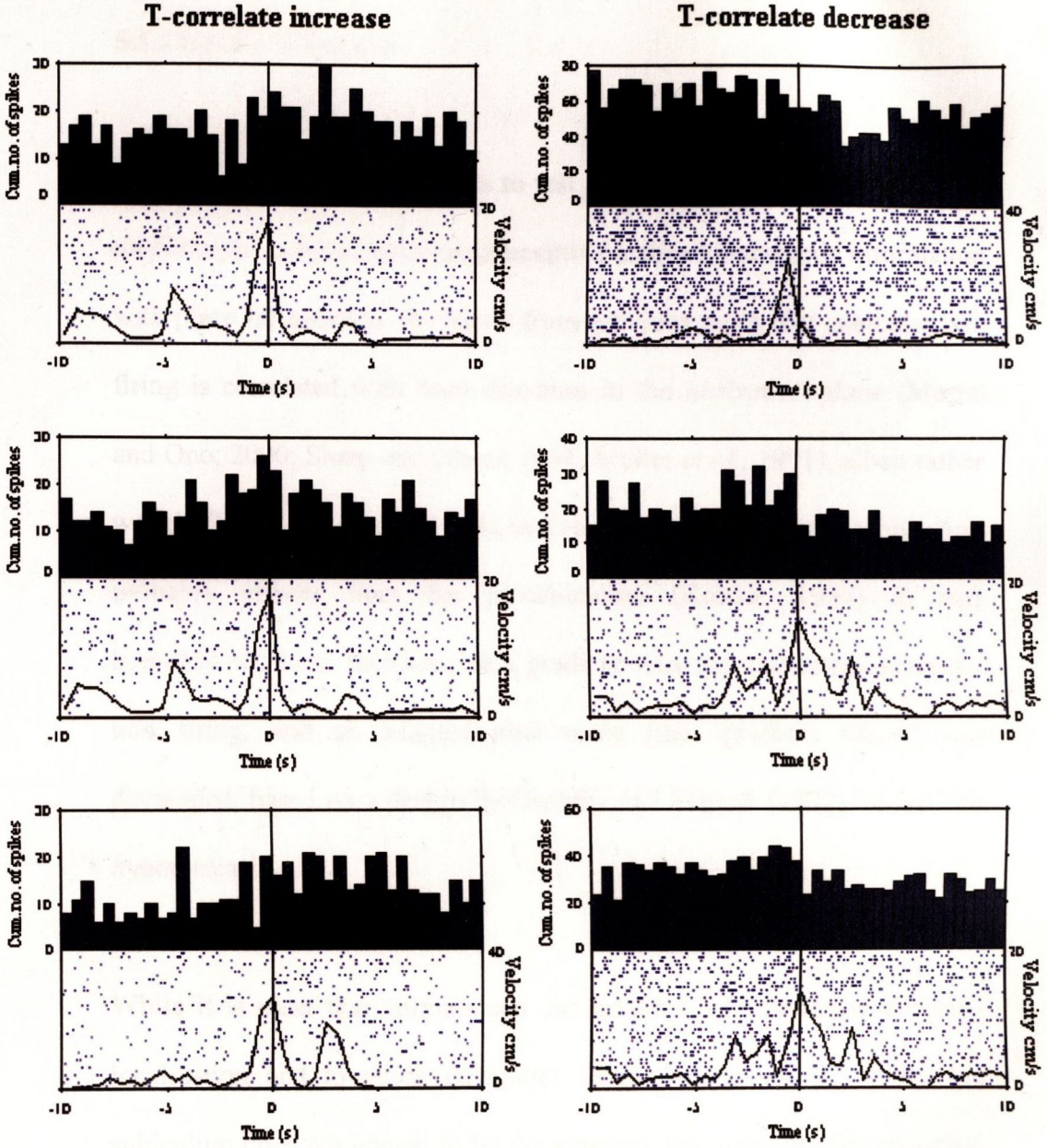


Figure 5.9. PETH and corresponding raster displays for unit responses to turn away from the end of each arm before the return to the centre ('T-correlate' responses). The first column shows three different units responding with the *T-correlate increase* response; the second column shows three different units responding with the *T-correlate decrease* response. Also displayed, overlayed on the rasters, is a plot of the averaged velocity of the animal during the displayed time period (some rats moved slowly back to the centre). See text for discussion.

5.5 Chapter discussion

The aim of this experiment was to test the hypothesis that the subiculum mediates the conjoining of proprioceptive and/or kinaesthetic information with place information. We know from previous work that subicular unit firing is correlated with head direction in the horizontal plane (Martin and Ono, 2000; Sharp and Green, 1994; Muller *et al.*, 1991), albeit rather weakly (Sharp and Green, 1994), and that this head direction information probably arrives from the presubiculum (Kohler, 1985). It was hypothesized here, therefore, that gradient may also influence subicular unit firing, and an adapted plus maze (the 'gradient maze') was developed, based on a design by Grobéty and Schenk (1992), to test this hypothesis.

While it is clear that animals can use gradient information to aid self-localization and to guide navigation (e.g., Miniaci *et al.*, 1999), the subiculum does not appear to be the structure that mediates the encoding of gradient information. Only two units (5 %) showed a significant main effect of gradient on their firing rates, a finding which might be expected by chance alone. Three more units (7 %) showed an interaction effect between arm location and gradient but it is difficult to interpret what these units might be encoding: none of these interactions was related to a directional effect on a single arm; rather, they were between disparate arms and gradients, such as significant differences in firing between the east arm at 10° and the west arm at 20 °. It is likely that gradient

information is encoded at a 'lower' level than the hippocampal formation, possibly in the thalamus, where units showing strong proprioceptive responses have already been found (HD cells – Ranck, 1984; Taube *et al.*, 1990a), or in vestibular circuits.

The other results from the three-way ANOVA were significant main effects of location (6 units; 15 %) and trial (9 units; 22 %) and a significant covarying effect of mean arm velocity (15 units; 37 %). The small number of units showing a main effect of location owes, we believe, to the fact that the experiment was designed to look for gradient effects, not locational effects, and hence the gradient changes made to the maze arms may have weakened the locational influence on unit firing. However, of the units showing a main effect of arm location on firing, firing rate maps revealed the typical diffuse multi-peaked place fields that were witnessed in Chapter 4. The majority of these units had place fields which showed strong directional effects, in that they would fire more strongly when the animal travelled in one direction along a maze arm (outward vs. inward); this is a characteristic of place fields which has been observed in restrictive environments such as the radial arm maze in both subicular place cells (Barnes *at al.*, 1990) and hippocampal place cells (McNaughton *et al.*, 1983). Some units interestingly did not appear to show this directional effect.

The trial effect is harder to explain: as stated in *Methods*, it was included in the 3-way ANOVA because of a suspicion that unit firing varied

between trials. The effect did not appear to be related to velocity; for example, the unit displayed in Figure 5.5 was not significantly affected by velocity. It may reflect reorganisation of subicular representations between trials, some trials requiring greater reorganisation, or it may be related to an unconsidered variable.

The most interesting characteristics of subicular unit firing in this experiment came from the PETH analyses, and they bear directly on the covarying effect of velocity. The *F-correlate* response characteristics are virtually identical to those recorded by Martin and Ono (2000) in a task which required rats to alternate between foraging for randomly delivered rewards (medial forebrain bundle [MFB] stimulation) and running to predicted rewards (MFB stimulation in the centre of the arena after the sounding of a tone). Martin and Ono (2000) state that the increases and decreases in firing are related to the effects of reward or anticipation of reward (in the case of the predicted reward delivery) rather than to velocity. Here, however, we attribute these effects to movement-related features: either to changes in velocity, or to our suspicion that subicular units may respond to changes in acceleration. How do we decide which explanation is the correct one? The answer will only arrive after further experiments or analysis. A further experiment would be to use a regular (i.e., no gradients) four or eight arm radial maze, and to bait with reward only half of the maze arms consistently between trials. One could then observe subicular unit firing on arrival at baited and unbaited arms: if rats learn which arms are consistently not baited, the anticipatory responses

of Martin and Ono (2000) would presumably change. Our prediction would be that unit firing would not differ between baited and unbaited arms if the rats ran along the baited and unbaited arms in a similar fashion. Alternatively, one could randomly bait half of the maze arms between trials and observe subicular unit responses to arrival at the baited and unbaited food wells under these conditions; again, we would predict no differences in subicular unit responses between baited and unbaited arms, whereas if subicular units were indeed responding to aspects of reward one might expect the same responses under these conditions that Martin and Ono (2000) find following the random delivery of rewards. On the other hand, further analysis would be to show that, while the firing changes are not perfectly predicted by velocity changes (this is one of Martin and Ono's major factors in their reward-related interpretation), they may be well-explained by acceleration changes, as we suspect, or other movement-related features.

It should be noted that Martin and Ono (2000) were primarily interested in firing time-locked with reward; here, however, we also investigated firing time-locked with the turn away from the end of the arm: these *T-correlate* responses provide further evidence of a movement-related explanation because (a) they show a similar relationship with velocity to the *F-correlate* response units, and (b) they are not directly related to reward.

These results raise an interesting caveat with regards to the inspection of firing rate maps constructed to show location-related firing. If subicular units are activated by locational *and* movement-related factors, among possible others, it will be of interest to ascertain which spikes are directly related to the spatially-related factor and which are related to the movement-related factors. The firing rate maps here are collapsed across eight trials in the belief that any differences in the movement-related factors are 'averaged-out' over the surface of the arena. However, it will be of interest to develop statistical means of removing the influence of the movement-related factor and visualizing only those spikes which are believed to encode location.

Chapter 6

Object exploration task

Chapter contents

6.1 Summary

6.2 Introduction

6.3 Chapter methods

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6.5 Chapter discussion

6.1 Summary

It has been known for some time that the subiculum receives a direct projection from the perirhinal cortex (Kosel et al., 1983); recent anatomical and electrophysiological studies have confirmed this finding (Naber et al, 1999). The perirhinal cortex is believed to play a part in object recognition processes, and the discovery that neurons in the perirhinal cortex respond more strongly when a rat encounters individual novel *objects compared with their responses to individual* familiar *objects in the environment (Zhu et al., 1995) supports this notion.*

In this experiment we hypothesised that, as a result of this projection, single units in the subiculum may be also differentially activated when novel and familiar objects are encountered in the environment. A recent lesion study supports this hypothesis (Galani et al., 1998). Using an object exploration task similar to the one used in this study, Galani et al. (1998) showed that rats with subicular lesions spent significantly less time than normal controls exploring a novel object placed in the task environment in the same position a familiar object had occupied (a non-spatial manipulation). Interestingly, in the same study rats with hippocampal lesions do not behave differently to normal controls.

Our results are compromised by the finding that, while rats did make more contacts with the changed objects, this difference was not

statistically significant. Regardless, on contact with novel objects in the environment, subicular units did not increase or decrease their firing rates, suggesting that the perirhinal cortex input to the subiculum may be too weak to influence subicular unit firing at a level detectable in the current analysis.

Unit firing did show movement-related correlates that were almost identical to movement-related correlates observed in previous experiments. It appears that an important role of the subiculum is to encode movement-related features.

6.2 Introduction

It has been known for some time that the subiculum receives a direct projection from the perirhinal cortex (Kosel *et al.*, 1983); recent anatomical and electrophysiological studies have confirmed this finding (Naber *et al.*, 1997; Naber *et al.*, 1999).

The perirhinal cortex is believed to play a part in object recognition processes (e.g., Wan *et al.*, 1999; Zhu *et al.*, 1995). Evidence of this centres on the discovery that neurons in the perirhinal cortex respond more strongly when a rat encounters individual novel objects compared with their responses to individual familiar objects in the environment (Wan *et al.*, 1999; Zhu *et al.*, 1995). Zhu *et al.*, (1995) used c-fos expression to analyse the activation of both hippocampus and perirhinal cortex following exposure to novel objects in either a familiar or a novel environment. They showed that whereas perirhinal neurons are activated by novel rather than familiar objects, hippocampal neurons are preferentially activated by novel rather than familiar environments. Wan *et al.* (1999) again used c-fos expression to analyse the activation of both hippocampus and perirhinal cortex following exposure to objects though here a paired viewing procedure was used. This allowed presentation of novel and familiar pictures simultaneously, one to each eye. They showed that perirhinal cortex is activated significantly more by pictures of novel than of familiar individual objects, and that the hippocampus is not differentially activated by this task. In contrast, pictures of novel

arrangements of familiar items produce significantly greater activation than familiar arrangements of these items in hippocampal area CA1 but, interestingly, significantly less activation in the dentate gyrus and subiculum. Perirhinal cortex was not differentially activated. They conclude that the hippocampus is importantly involved in processing information essential to recognition memory concerning the relative familiarity of arrangements of items, as needed for episodic memory of scenes, whereas the perirhinal cortex processes such information for individual items.

We hypothesised that as a result of this direct projection from perirhinal cortex, the subiculum may also be differentially activated by novel and familiar objects. To test this hypothesis, we used an object exploration task as developed by Poucet (1989) and similar to the one used by Galani *et al.* (1998 – see below). This task was chosen for its simplicity and its ecological validity. It also, at least in previous work, has a clear behavioural effect of an increase in object exploration following changes to the object task environment which can be used to verify that each rat is indeed responding to the objects.

A lesion study adds weight to this hypothesis. Galani *et al.* (1998) showed that rats with lesions restricted to the subiculum failed to respond to non-spatial changes in an object exploration task whereas they did respond to spatial changes (a non-spatial change consisted of replacing one of the familiar objects in the environment with a novel object

positioned in the same place; a spatial change consisted of moving one of the familiar objects to a new position in the environment). Conversely, rats with lesions restricted to the hippocampus failed to respond to spatial changes but were unimpaired in their responses to non-spatial changes.

6.3 Method

Subjects

Four adult male Wistar rats (Bio Resources Unit, University of Dublin), weighing approximately 400 g on arrival in the laboratory were used as subjects. Rats were housed singly in a temperature controlled laminar airflow cupboard, and maintained on a 12:12 hour light-dark cycle (light: 0800 – 2000 hours). All testing was carried out during the light phase. One of the rats used in this study was previously used in the gradient maze study.

Apparatus

The apparatus was based on that used by Poucet (1989). It consisted of a circular walled arena (diameter 180 cm, height 30 cm) resting on the floor of the experimental room. The floor of the arena was covered with sawdust. The experimental room was illuminated as usual by two 12 volt bulbs attached to the ceiling (for full details of the experimental room see Chapter 2). The arena was surrounded by numerous potential cues, including a black and white striped cue card was fixed just above the side wall in the same position throughout the experiment.

In each session, four objects were placed in a square formation at the centre of the arena approximately 50 cm apart; a fifth object was kept in

reserve for use later in the non-spatial change sessions (see below). A total of 25 different objects were used in this experiment (five objects x five sessions). Objects were carefully selected for their weight heavy enough not to be displaced by the animal), smoothness (so as not to catch the recording cable), and height (short enough not to obscure the LED on the headstage from the tracking camera). The experimenter was able to observe contacts rats made with each object from their position at the side of the arena.

Surgery

Electrodes were implanted using the standard method (see Chapter 2). In two rats electrodes were aimed at the following coordinates in dorsal subiculum: AP –6.8 mm (relative to bregma), L 4.0 mm, DV 2.4 mm (one left hemisphere, one right hemisphere). In the other two rats electrodes were aimed at the following coordinates in dorsal subiculum: AP –5.8 mm (relative to bregma), L 3.0 mm, DV 2.5 mm (both left hemisphere; all coordinates from Paxinos and Watson, 1996).

Following the experiment, rats were deeply anaesthetized using sodium pentobarbitol, then perfused. Brains were removed and stored in 4 % formaldehyde for several days. Brains were subsequently sliced on a vibrotome and all electrode placements were verified as being in dorsal subiculum.

Recording techniques

Two channels of neuronal activity were recorded during each session: the first channel acquired unit activity and was filtered with bandpass 300 – 5000 Hz and amplified by a factor of 10,000; the second channel acquired EEG and was filtered with bandpass 1 – 500 Hz and amplified by a factor of 1,000. Both channels were fed into the analogue-to-digital conversion system (DataWave Technologies, Colorado, USA) described in detail in Chapter 2. Unit activity output was also sent to a loudspeaker for auditory feedback.

An overhead video tracking system was used to follow the location of the rat in the maze. The system operated in one-spot (monochrome) tracking mode and detected a small LED attached to the headstage (see Chapter 2). The tracking system split the environment into 256 x 256 pixels and sent time-stamped positional data to the acquisition system.

After each session the electrodes were driven ventrally by approximately 50 μm with the hope of encountering new units.

Protocol

Three days before recording began, in order to familiarize the rats with the apparatus, rats were placed into the *empty* arena for six periods of 5 min separated by 3 min intervals. During the intervals the rat was

returned to a holding chamber close to the apparatus. One day before recording began, rats were placed into the empty arena for one period of 5 min, then four objects were placed into the arena (in square formation; these objects were not used during the recording sessions) and the rats were allowed to explore for a further two periods of 5 min; these three sessions were again separated by 3 min intervals.

Each recording session typically lasted approximately 45 mins and consisted of six trials, 5 min duration per trial and with a 3 min interval between trials. Neuronal activity was recorded during the entire 45 min session. At the start of each trial, the rat was taken from the holding chamber close to the arena and placed into the arena *at the same position in each trial* (close to the side wall, equidistant from and in the direction of objects C and D). At the end of each trial the rat was returned to the holding chamber and remained there until the start of the next trial. On day 1 of recording, each rat received a 5 min trial in the empty arena before the six main trials.

Of the six main trials, in trials 1 – 4 the objects occupied the same positions (as described above). During the interval between trials 4 and 5 either a spatial *or* a non-spatial change was made to the objects. The spatial change consisted of moving one of the objects by 55 cm to stand against the wall nearest to its original position; the non-spatial change consisted of removing one of the objects from the arena and placing the reserve object in exactly the same position. Trial 5 was therefore the

main test of reaction to a spatial or non-spatial change. Trial 6 was a repetition of the object configuration in trial 5. The object changed and the type of change (spatial or non-spatial) made in each session were both pseudorandomly varied between sessions. To eliminate the possible biasing factor of any olfactory cues, the experimenter handled every object in the arena between trials 4 and 5, although only one object, as described, was changed. In addition, the sawdust was raked between sessions. Figure 6.1 illustrates the task protocol.

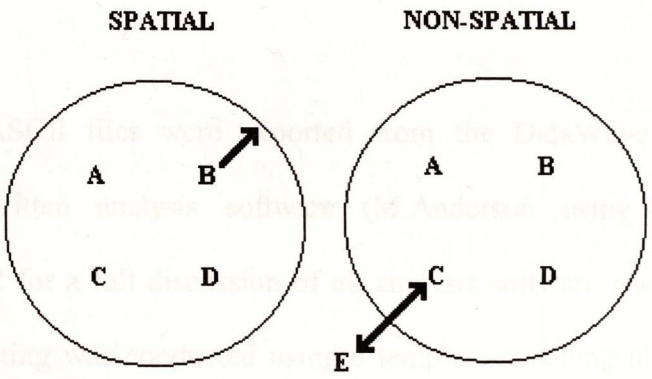


Figure 6.1. Diagram of the object exploration task used here. Sessions consisted of six trials, and either a spatial or a non-spatial change was made between trials four and five. The spatial change involved moving any one of the objects from its original position to stand against the nearest portion of wall; the non-spatial change involved removing any one of the objects and replacing it with a novel object in the same position. See text for discussion.

The experimenter pressed a key on the keyboard when the rat made contact or near-contact (~1 cm) with each object during each trial; a different key was used to represent contacts with each object. The key

was pressed once when initial contact was made. In early trials it was usual for rats to explore each object for a relatively long period of time. In this case, the key was pressed again when the rat made re-contact with the object *only if* (a) contact with the object had been broken and (b) the rat had turned away from the object. These *event flags* were saved to file together with a time-stamp. When a non-spatial change had been made, the same flag was used for the reserve object as had been used for the replaced object.

Data analysis

Output ASCII files were exported from the DataWave system into custom-written analysis software (M.Anderson using Matlab; see Chapter 2 for a full discussion of all analysis software and techniques). Spike sorting was conducted using a template-matching algorithm with conservative criteria for acceptance. EEG was visualized using a data replay plotter which also allowed the simultaneous viewing of spike times and event flags; as such, portions of the EEG trace could be cropped for further analysis. Continuous records of unit and EEG activity were also visualized.

After spike sorting, individual unit data were further processed to display inter-spike interval histograms (ISIHs), autocorrelation histograms (ACHs), peri-event time histograms (PETHs) based on the event flags, and raster plots (see Chapter 2). ISIHs, ACHs, and raster plots were used,

together with the electrophysiological characteristics of the units (spike duration and spike amplitude) in an attempt to classify each unit according to known cell subicular cell types. Simple mean rate (Hz) was also calculated for each unit by dividing the total number of spikes by the session time.

To determine the nature of the spatial signal, if any, carried by subicular units, the 'information content' measure was used (Skaggs *et al.*, 1993), which calculates the amount of information, *I,* contained in the firing of a unit, using the formula

$$I = \sum_i (\lambda_i / \lambda) \log_2 (\lambda_i / \lambda) P_i ,$$

where λ_i is the mean firing rate in pixel i, λ is the overall mean firing rate, and P_i is the probability of the animal occupying pixel i (which is simply the time spent in pixel i divided by the total trial time). For a fuller description of this measure see Chapter 2. Spatial information content is measured in 'bits of information per spike' (bits / spike).

Firing rate maps were constructed in Matlab to enable easy visualisation of location-specific firing, using the system described in *Main Methods*. Briefly, the maps are constructed by dividing the open-field environment into pixels (square measuring 16 x 16 cm). The number of spikes that fired when the rat occupied a pixel is divided by the total time the rat spent in that pixel (in seconds) to produce a firing rate for that pixel (in Hz). All the pixel firing rates are mapped as a contour plot using Matlab's in-built contouring algorithm. The maps are colour-coded in

increments of 20% of the peak firing rate for each individual map. The 'peak rate' of each map is equal to the rate of the pixel with the highest firing rate in each map. Other spatial measures were also calculated, based on the firing rate maps. 'Non-zero rate' represents the mean of all the pixels in a firing rate map displaying a rate above 0 Hz. This measure controls for the probability that units would not fire in all areas of the arena (i.e., in all pixels) but only in discrete locations, thus biasing the simple mean rate; it is probably closer to the true firing rate of the cell. Also, the size of the firing field for each cell was calculated by dividing the number of pixels with a firing rate of greater than zero by the total number of pixels visited in the environment.

To test for the effects of contact with each object a one-way ANOVA was conducted for each unit using the contact with each object pre- and post-change as the independent variable (eight levels: 2 periods x 4 objects). The independent variable used in the ANOVAs was the transformed (see below) firing rate of each unit during the 2 s period immediately after the time when contact was made with each object, and was calculated using the event-flag data.

Prior to analysis, firing rates were transformed using the following formula,

$$X' = (\sqrt{X} + \sqrt{X+1}) ,$$

since, as found in previous studies (e.g., Wiener *et al.*, 1995; O'Keefe and Speakman, 1987) and in Chapter 5, firing rates showed skewed

distributions. An assumption behind ANOVA is the normality of the data, and in agreement with previous studies (Wiener *et al.*, 1995; O'Keefe and Speakman, 1987; and see Winer, 1971) we found that this was the most effective transform.

EEG was analysed using discrete Fourier transforms. Fourier analysis is extremely useful for EEG analysis as it breaks down the signal into constituent sinusoids of different frequencies. Power spectra were plotted using this data; any dominant frequencies present in the EEG appear as peaks in the plot.

Statistical analysis

Where appropriate, differences between firing rates were analysed parametrically with one-way analyses of variance (ANOVA). For analyses of firing rates before and after the onset of a particular behaviour (as recorded with the event flags and displayed in the PETHs), paired t-tests were used since a plot of the differences pre-and post-flag showed them to be normally distributed. Statistics were calculated using the Statistical Package for the Social Sciences (SPSS) software, or MINITAB.

6.4 Results

Histological findings

The tracks of electrode bundles were reconstructed by visual inspection after histological processing (see Figure 6.2). The recording electrodes from all rats were positioned in dorsal subiculum. The electrode track from one animal could not be located but was included because because the units had very similar electrophysiological characteristics to units recorded from other animals.

Unit electrophysiological characteristics and spike train analysis

33 subicular units were isolated from the four rats. Examination of the ISIH and ACH, and unit waveform characteristics (amplitude and width), for each unit showed that, as expected from previous chapters (see chapters 3, 4, and 5), most units could be classified into one of four classes: a bursting unit class, a regular spiking unit class, a theta-modulated unit class, and a fast spiking unit class. For details of the defining characteristics of each class, see *Results* in Chapter 3. It is noted that all the units recorded here showed very similar ISIHs and ACHs and other electrophysiological measures to the units collected in the baseline experiment. Table 1 displays the firing characteristics of the classified units, separated by unit class.

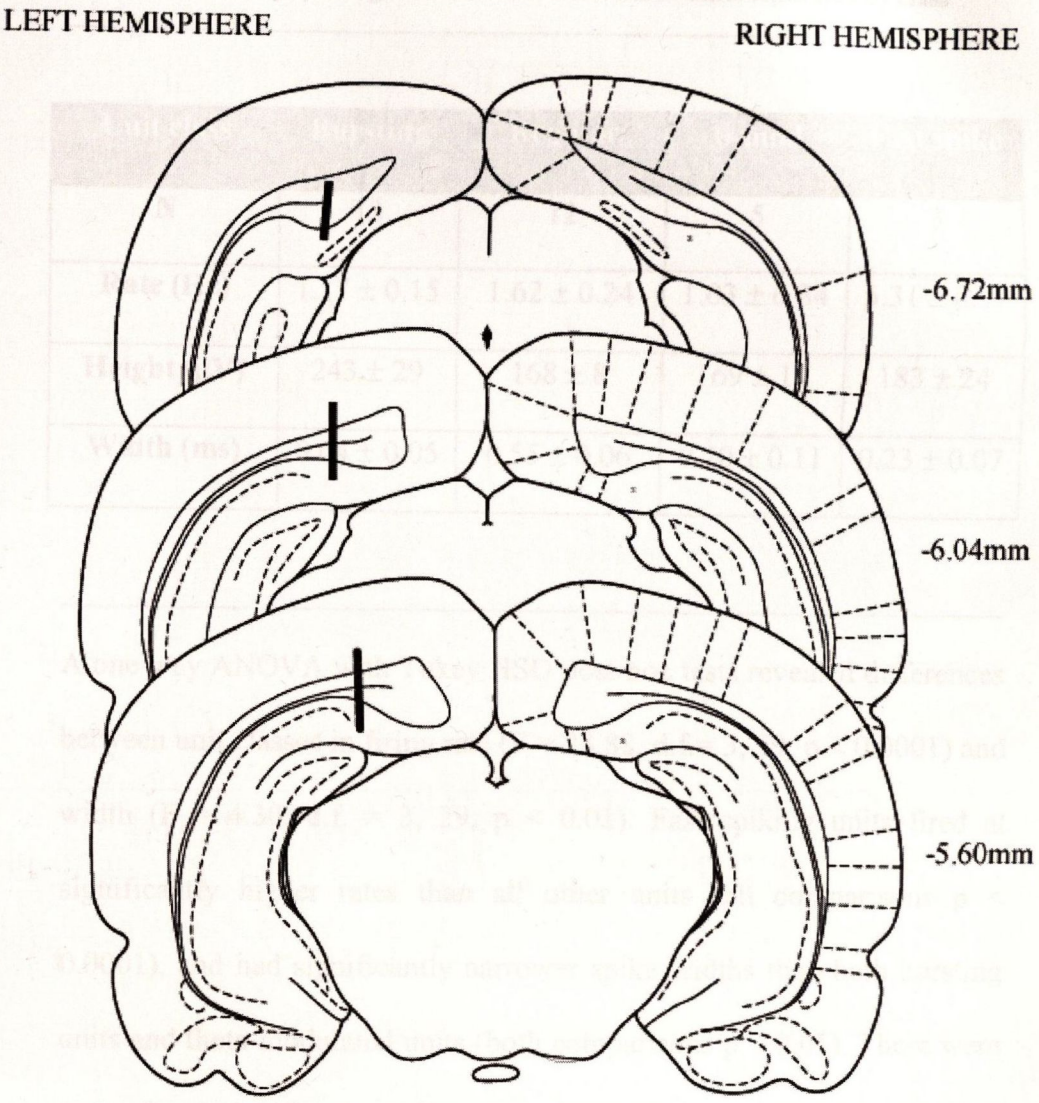


Figure 6.2. Results of the histological analysis showing the reconstructed electrode tracks from three of the four animals. The electrode track from the remaining animal could not be located but was included because the units had very similar electrophysiological characteristics to units recorded from other animals.. The heavy black line in each section represents the electrode track. Slice measurements are posterior to bregma (Paxinos and Watson, 1996).

TABLE 1. Electrophysiological measures of subicular units separated by class

Unit class	Bursting	Regular	θ-mod	Fast spike
N	13	12	5	3
Rate (Hz)	1.17 ± 0.15	1.62 ± 0.24	1.03 ± 0.34	5.31 ± 0.37
Height (μV)	243 ± 29	168 ± 8	169 ± 17	183 ± 24
Width (ms)	0.64 ± 0.05	0.55 ± 0.06	0.70 ± 0.11	0.23 ± 0.07

A one-way ANOVA with Tukey HSD post-hoc tests revealed differences between unit classes in firing rate ($F = 28.88$, d.f.= 3, 29, $p < 0.0001$) and width ($F = 4.30$, d.f. = 3, 29, $p < 0.01$). Fast spiking units fired at significantly higher rates than all other units (all comparisons $p < 0.0001$), and had significantly narrower spike widths than both bursting units and theta-modulated units (both comparisons $p < 0.05$). There were no other significant differences between unit classes on the electrophysiological measures displayed in Table 1.

Object contacts

Figure 6.3 displays the grand mean number of contacts rats made with each object in the arena across trials An increase in the number of contacts made with the changed object is evident in trials 5 and 6 as was expected following the object change. This is evidence that rats responded to the change with increased exploration of the changed

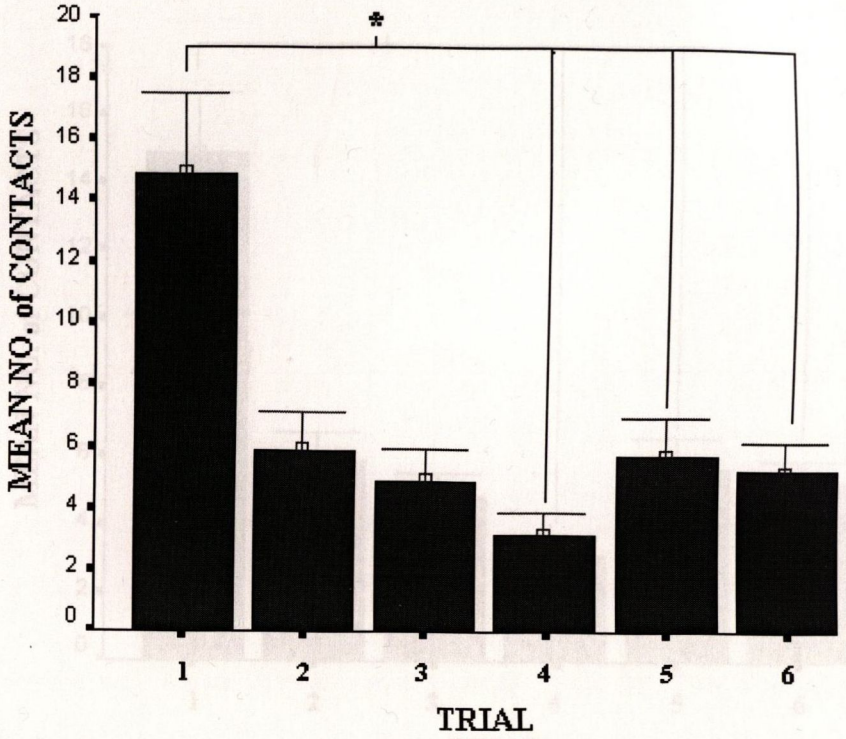


Figure 6.3. Chart displaying the grand mean number of contacts rats made with objects in the six trials of the object exploration task collapsed across all sessions. (* $p < 0.0001$). See text for discussion.

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object. A repeated measures analysis of variance with trial as the within measure revealed a significant main effect of trial ($F(5,65) = 21.07$, $p < 0.0001$). Subsequent paired t-test analysis of this effect revealed that rats demonstrated normal exploration and habituation patterns from trial 1 to trial 4 ($t = 7.41$, df 13, $p < 0.0001$). However, no significant differences were observed between trial 4 and trial 5 and trial 4 and trial 6. Figures 6.4 and 6.5 display the grand mean number of contacts rats made with each object in the arena across trials, separated by whether the change was spatial or non-spatial. Here we can see that rats were slower to

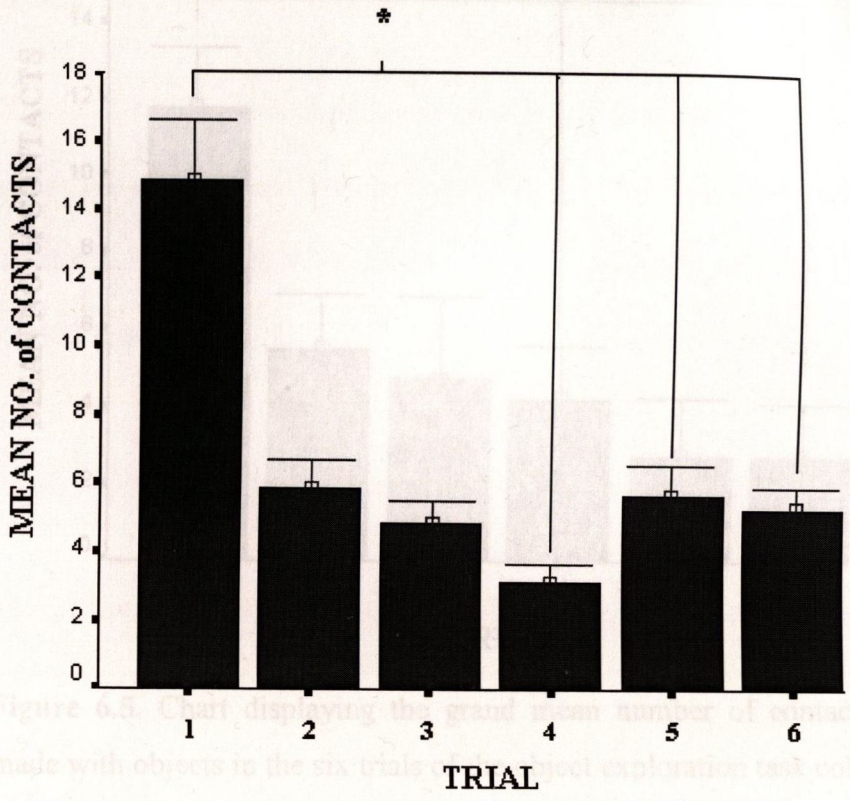


Figure 6.4. Chart displaying the grand mean number of contacts rats made with objects in the six trials of the object exploration task collapsed across all sessions *for spatial changes only* (* $p < 0.005$). See text for discussion.

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respond to non-spatial than to spatial change: spatial changes produced increased exploration in both trials 5 and 6 whereas non-spatial changes actually produced *decreased* exploration in trials 5 and 6. Again, repeated measures analysis of variance with trial as the within measure revealed a significant main effect of trial for the non-spatial change sessions ($F(5,15) = 4.37$, $p < 0.05$), and for the spatial change sessions ($F(5,30) = 9.73$, $p < 0.0001$). Subsequent paired t-test analysis again revealed no significant differences between trial 4 and trial 5 and trial 4

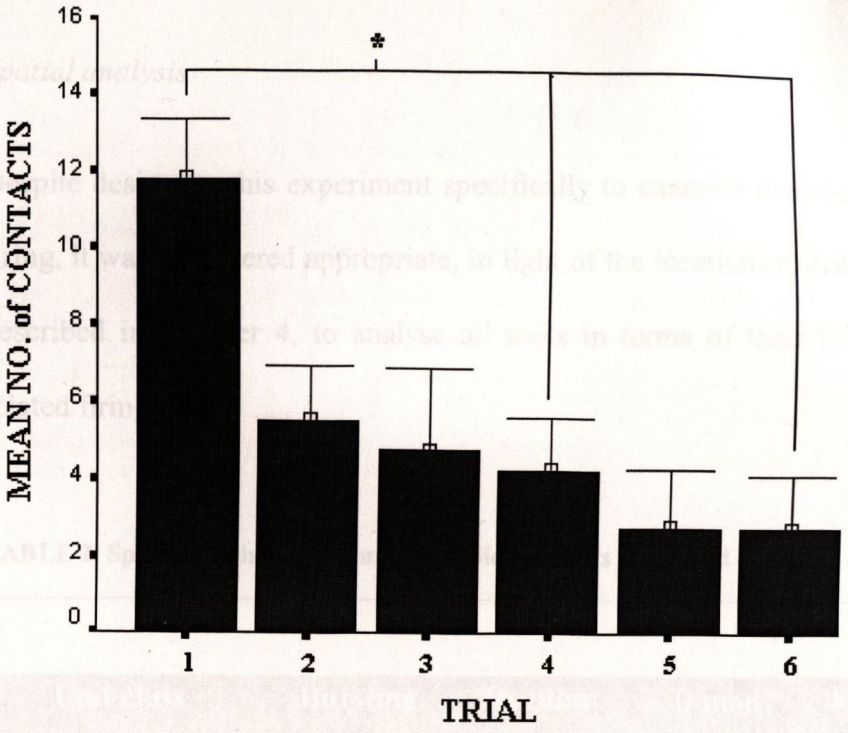


Figure 6.5. Chart displaying the grand mean number of contacts rats made with objects in the six trials of the object exploration task collapsed across all sessions *for non-spatial changes only* ($p < 0.005$). See text for discussion.

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and trial 6.

The unit firing ANOVAs revealed very few differences between post-contact firing rates for either spatial or non-spatial change sessions. Four units showed significant differences in the spatial change sessions, and three units showed significant differences in the non-spatial change trials. Post-hoc tests that revealed significant differences between pairwise comparisons showed that none of these differences were related to the changing, either spatially or non-spatially, of objects; indeed, only one of these significant comparisons was between the same object in the pre- and post-change periods, and this was an object that did not change between these periods.

Spatial analysis

Despite designing this experiment specifically to examine object-related firing, it was considered appropriate, in light of the location-related firing described in Chapter 4, to analyse all units in terms of their location-related firing.

TABLE 2. Spatially-related measures of subicular units separated by class

Unit class	Bursting	Regular	θ-mod	Fast spike
N	15	22	2	2
Field size	0.72 ± 0.03	0.80 ± 0.02	0.78 ± 0.04	0.93 ± 0.01
Peak rate (Hz)	2.60 ± 0.38	2.18 ± 0.27	1.82 ± 0.34	7.42 ± 1.11
Non-zero rate (Hz)	1.17 ± 0.15	1.63 ± 0.24	1.04 ± 0.34	5.32 ± 0.47
Spatial info. c.* (bits / spike)	0.36 ± 0.07	0.16 ± 0.02	0.20 ± 0.04	0.13 ± 0.02

*Spatial information content

Table 2 displays the spatially-related firing characteristics of subicular units recorded in the object maze. A one-way ANOVA with post-hoc tests on each measure revealed significant differences between unit classes on all three measures. On field size ($F = 5.40$, d.f. = 3, 29, $p < 0.005$), bursting units had significantly smaller place field sizes to fast spiking units ($p < 0.005$); on peak rate ($F = 17.39$, d.f. = 3, 29, $p < 0.0001$), fast spiking units had significantly higher peak pixel rates than all other unit classes (all comparisons $p < 0.00001$); on non-zero rate (F

= 29.22, d.f. = 3, 29, $p < 0.00001$) fast spiking units again had significantly higher non-zero rates than all other unit classes (all comparisons $p < 0.00001$); and on spatial information content ($F = 3.57$, d.f. = 3, 29, $p < 0.05$) bursting units signalled significantly more spatial information than regular spiking units ($p < 0.05$). No other comparisons on these three measures reached significance.

Firing rate maps and paths were constructed to visualize location-related firing. Firing rate maps were collapsed across trials 1 – 4 to form a 'pre-change' map, and across trials 5 - 6 to form a 'post-change' map, since individual trials did not provide a good sampling of the maze surface (it is noted that the 'post-change' path plots do not show ideal sampling of the arena surface). Ten units (30 %) showed strong evidence of location-related firing when maps of the pre- and post-change collapsed trials were compared. Six of these units are displayed in Figures 6.6, 6.7, and 6.8: four were recorded during spatial change sessions (Figures 6.6 and 6.7) and two were recorded during non-spatial change sessions (Figure 6.8).

PETH and raster analysis

Individual PETHs and rasters were visualised for all units using the event flag data. Hence, for each unit, four PETH and raster plots were made corresponding to the time of contact with the four different objects.

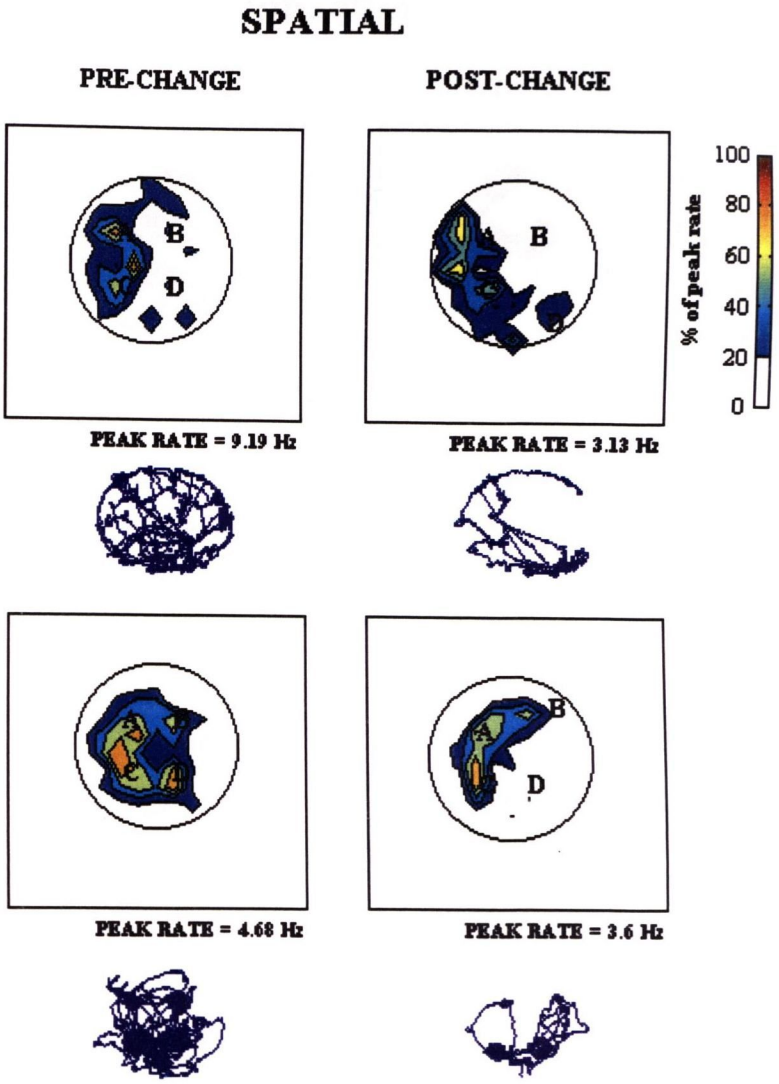


Figure 6.6. Firing rate maps constructed from collapsed trials both before and after the time of the object spatial change (two maps side by side for each unit). The positions of the objects are shown. The paths taken by the animal for the collapsed trials are presented below their corresponding firing rate maps. Even though parts of the arena were not visited in the 'post-change' trials, the maps illustrate consistent location-related firing for these units despite the object movement. See text for discussion.

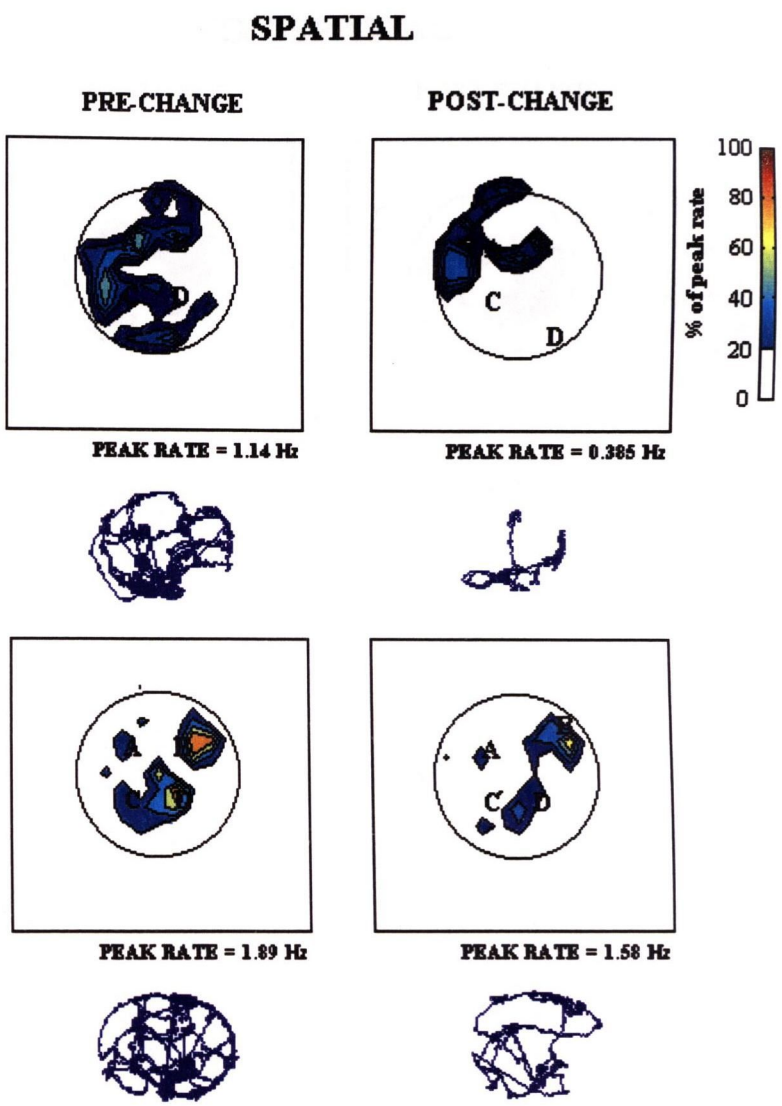


Figure 6.7. More firing rate maps constructed from collapsed trials both before and after the time of the object spatial change (two maps side by side for each unit). The positions of the objects are shown. The paths taken by the animal for the collapsed trials are presented below their corresponding firing rate maps. Even though parts of the arena were not visited in the 'post-change' trials, the maps illustrate consistent location-related firing for these units despite the object movement. See text for discussion.

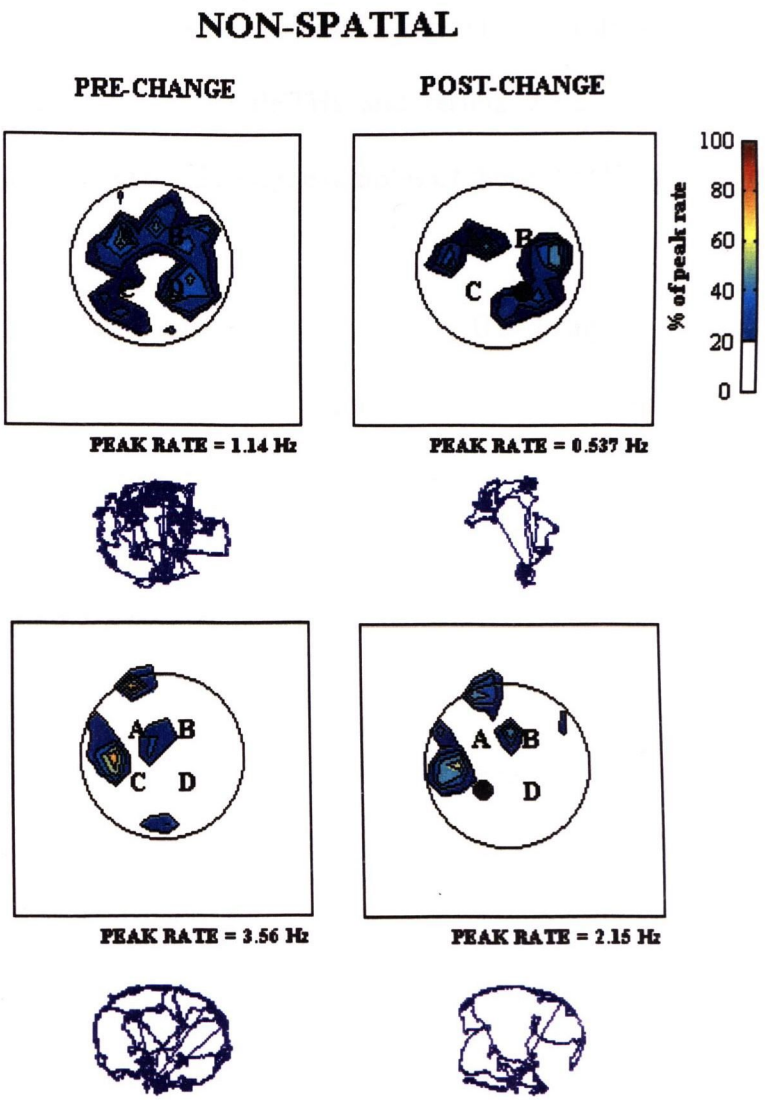


Figure 6.8. Firing rate maps constructed from collapsed trials both before and after the time of the object non-spatial change (two maps side by side for each unit). The positions of the objects are shown. The paths taken by the animal for the collapsed trials are presented below their corresponding firing rate maps. Even though parts of the arena were not visited in the 'post-change' trials, the maps illustrate consistent location-related firing for these units despite the object change. See text for discussion.

Given that the one-way ANOVA revealed no differences related to the changed object, the PETHs and rasters were collapsed across all six trials. Figure 6.9 displays examples of these PETHs and rasters.

Paired t-tests on pre- and post-event flag firing in the PETHs revealed that 23 units (70 %) showed significant differences between pre- and post-event flag firing rates for contacts with at least one of the objects (at either 2, 5, or 10 s pre- and post-flag intervals; $p < 0.05$). Interestingly, all of these PETHs and rasters displayed a remarkable similarity to the *F-correlate* PETHs and rasters displayed in the gradient maze chapter in this thesis (*F-correlate* PETHs and rasters used the animal's arrival at the food reward as the zero reference - see Chapter 5). Both examples of the *F-correlate* responses were encountered here: PETHs showing increases in firing immediately *before* contact had been made with the object, followed by a significant decrease in firing relative to the pre-contact baseline firing rate (as *F-correlate increase/decrease* response units do); and PETHs showing significant increases in firing rate relative to the pre-contact firing rate *after* contact had been made with the object (as *F-correlate increase* response units do). Given that subicular units did not respond to object changes we believe that this is further evidence of the influence of movement-related features on subicular unit firing, identical to the influence described in the gradient maze chapter.

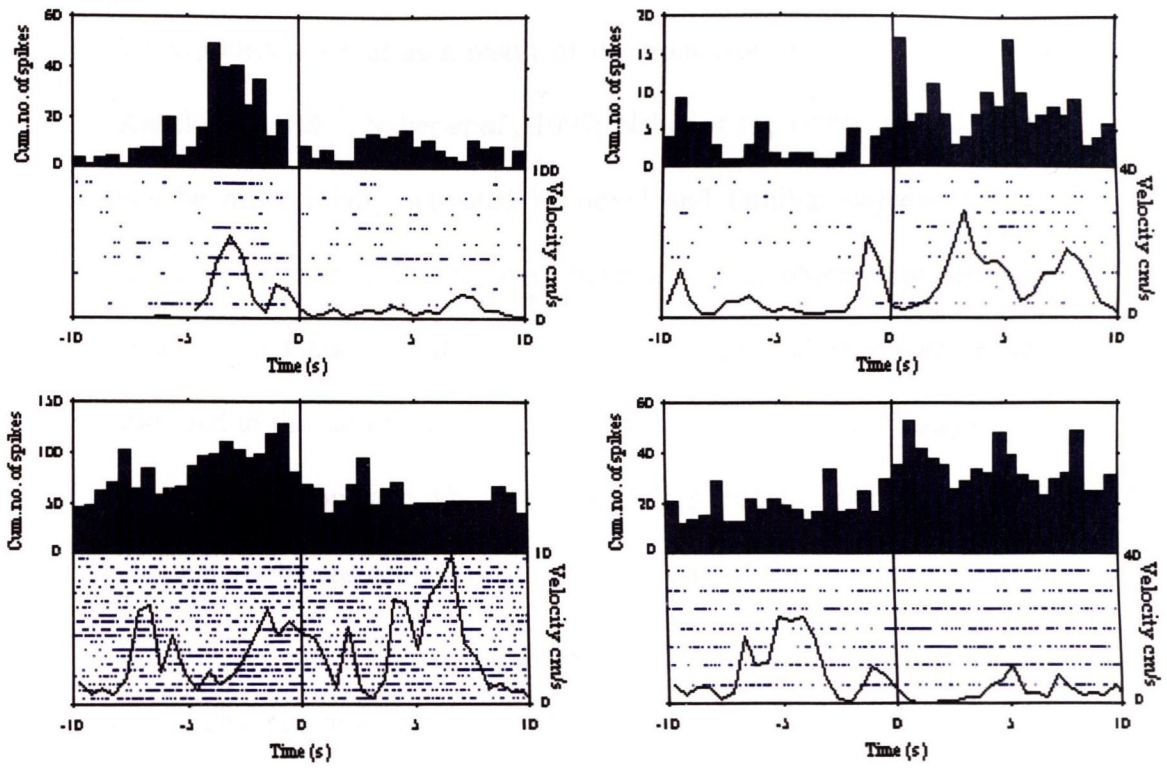


Figure 6.9. PETHs and raster displays with concurrent velocity shown here to illustrate the similarity between unit firing immediately pre- and post-object contacts with unit firing pre- and post-reward arrival in the gradient maze chapter (see Chapter 5). The two units in the first column respond very similarly to the *F-correlate increase/decrease* response units in the gradient maze, while the two units in the second column respond like the *F-correlate increase* response units. See text for discussion.

6.5 Chapter discussion

We hypothesised that as a result of its input from the perirhinal cortex (Kosel *et al.*, 1983; Naber *et al.*, 1997; Naber *et al.*, 1999) subicular units may be differentially activated by novel and familiar objects. Indirect evidence that the subiculum may have a role in object representation comes from Galani *et al.* (1998) who showed that rats with lesions restricted to the subiculum failed to respond to non-spatial changes in an object exploration task whereas they did respond to spatial changes (a non-spatial change consisted of replacing one of the familiar objects in the environment with a novel object *positioned in the same place;* a spatial change consisted of moving one of the familiar objects to a new position in the environment). To test this hypothesis, we recorded subicular unit activity in an object exploration task, similar to the task developed by Poucet (1989) and used by Galani *et al.* (1998 – see below). This task was chosen for its simplicity and its ecological validity. It also, in previous work, has a clear behavioural effect of an increase in object exploration following changes to the object task environment which can be used to verify that each rat is responding to the object changes.

Our findings were compromised by the facts that, despite charts demonstrating an increase in object exploration following both spatial and non-spatial changes to the environment, this increased exploration failed to reach significance for both trial types. It may be that the objects used in the current experiment were not salient enough to induce

significant behavioural differences when changes were made to the environment. As such, it is impossible to state with confidence that the animals did indeed either perceive or respond to these changes. Given, however, that this doesn't eliminate the possibility that subicular unit firing would still show differential effects on contact with familiar and novel objects in the environment, analysis of subicular unit firing was continued.

Our findings suggest either (1) that the perirhinal input to the subiculum may be too weak or diffuse to influence subicular unit firing in the present task; or (2) that the rats failed to notice the changes; or (3) that recognition of the objects does not necessarily occur at the time of contact. While four units showed differences in firing rate between objects after contact was made with these objects in spatial change sessions, and three units showed differences in firing rate between objects after contact was made in non-spatial change sessions, not one of these differences was between the changed object *before and after the change* (i.e., no units increased or decreased their firing on contact with the changed object). As such, these significant differences are probably correlated with other features of the task environment, such as the spatial location of the animal ('place' correlates).

Firing rate maps constructed from the pre- and post-change periods (i.e., two maps, one for trials 1 – 4 and one for trials 5 – 6) showed reliable location-related firing for 30 % of the units. It appears that changing

objects in the open field, whether the change is spatial or non-spatial, has little effect on the firing of subicular units. For the spatial change maps, this result fits neatly with a study of hippocampal place cells in which objects placed centrally in the open field, at a distance apart from each other, fail to exert control over place fields (Cressant *et al.*, 1997). Place fields in the current experiment occupied the same proportion, or more, of the arena surface as they did in the pellet-chasing experiment (see Chapter 4) indicating that subicular place fields scale according to the current environment: this finding fits with a study showing that increasing the size of an arena while keeping the shape the same results in a scaling-up of subicular place fields (Sharp, 1999).

Interestingly, the majority of units showed significant differences between pre- and post-event flag firing rates which were reminiscent of the differences shown by units in the gradient maze task to arrival at the food reward (so-called *F-correlate* responses: see chapter 5). We believe that this is further evidence that an important correlate of subicular unit firing are movement-related features. Similar to the *F-correlate* findings, while unit firing did appear to be related to velocity, the relationship between unit firing and movement appears to be a complex one: it may be that there exists a 'higher-order' relationship between unit firing and velocity, such that changes in velocity and/or acceleration ('inertial cues') are represented. Further analyses are required to elaborate this finding.

Chapter 7

Main discussion

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7.1 Introduction

7.2 Summary of the main findings from the current work

7.3 Theoretical implications

7.4 Synthesis

7.1 Introduction

The subiculum is a poorly-understood structure and yet one which forms a key part of current competing theories of hippocampal formation function (Sharp, 1997; Redish and Touretzky, 1997). Discovering how and why subicular cells fire will aid our efforts to describe the functioning of the subiculum, and the wider role of the hippocampal formation.

Using the technique of recording from single units in freely-moving animals, this thesis work aimed to describe the firing characteristics of subicular units (chapter 3), their spatial correlates (chapter 4), and their correlates with explicit experimental manipulations, chosen to test specific hypotheses concerning subicular function (chapters 5 and 6).

7.2 Summary of the main findings from the current work

The main findings from the current work are:

1. Subicular units can be separated into *at least* four classes on the basis of the electrophysiological characteristics of their firing rate, spike duration, relationship with simultaneously recorded EEG, and spike train time characteristics. These classes are defined as: bursting, regular spiking, theta-modulated, and fast spiking. Fast spiking and theta-modulated units are most likely interneurons, probably of distinct types.

2. In line with the findings of a recent patch-clamping study (Staff *et al.*, 2000) we find that subicular bursting units show large variation in their 'propensity to burst'.

3. In the open field, subicular units show reliable location-related firing. In restrictive environments, subicular place fields can be directional.

4. Bursting units signal the most spatial information; fast spiking units signal the least spatial information.

5. Subicular units represent 'place' in both a qualitatively and quantitatively different manner to hippocampal units:

subicular place fields are larger, have more peaks of firing within a more diffuse area of lower rate firing, and transmit less spatial information per spike than hippocampal place cells do.

6. Subicular place fields multiple peaks of activity suggest that disparate areas of the current environment are being connected together into multiple representations on single subicular units.

7. Subicular unit firing covaries reliably with mean arm velocity in a four-arm maze. This correlation may be better described as a movement-related or 'inertial' correlate: the relationship between subicular unit firing and velocity appears to be complex.

8. Subicular units often have multiple correlates: many units are significantly correlated with both location and velocity.

9. Body angle in the vertical plane has a negligible influence on subicular unit firing.

7.3 Theoretical implications

The details of the main findings from this thesis work were discussed in each corresponding experimental chapter (see the *Discussion* sections of Chapters 3, 4, 5, and 6) and will not be reiterated here. Rather, the theoretical implications of the findings reported above will be discussed briefly in relation to current theories of subicular function.

After characterising subicular single unit responses in a baseline task, we conducted experiments to test specific hypotheses of subicular function. First, in an effort to confirm and extend previous work (Sharp and Green, 1994), we analysed in detail the spatial representations of subicular units in a pellet-chasing task. Second, with hypotheses generated from prior empirical evidence of possible non-spatial correlates of subicular unit firing (reward representation hypothesis - Martin and Ono, 2000; object representation hypothesis - Galani *et al.,* 1998), we observed subicular unit firing in a gradient maze, and in an object exploration task, specifically to look for non-spatial as well as spatial correlates

The two major correlates of subicular unit firing, demonstrated by the present work, are (1) the location of the animal, and (2) what we wish to term 'movement-related factors' for reasons discussed below. Our findings confirm and extend the results of earlier studies (Barnes *et al.*, 1990; Muller *et al.,* 1991; Sharp and Green, 1994; Sharp, 1997; Sharp, 1999a,c). In general terms, therefore, we suggest that the current work

lends support to spatial / navigational models of hippocampal formation function (e.g., O'Keefe and Nadel, 1978; Redish, 1999).

In fact, we find no evidence of non-spatial correlates of subicular unit activity. We suggest that the *F-* and *T-correlate* responses described in Chapter 5 and observed also in Chapter 6 are strong evidence that the 'reward-related' firing that Martin and Ono (2000) describe for subicular units in a random and predictable reward task owes, in fact, to a movement-related correlate (and the stronger evidence comes from the *T-correlate* responses which are not directly associated with reward); we have suggested experiments that would help decide between the two hypotheses. In relation to this movement-related correlate, while there is a strong relationship between velocity and unit firing in the gradient maze, it is our suspicion that this relationship is more complex than a direct one between velocity and firing rate and for this reason we refer to this correlate as 'movement-related'. Future analysis should attempt to elaborate the precise nature of this relationship.

What role might a structure that encodes both location and movement be playing? It is plausible that such findings support the path integrator model of subicular unit activity. If an animal is to maintain a *coherent* internal representation of where it is in an environment it needs a representation of where it currently is, some means of tracking changes in its position, a representation of head direction, and a means of feeding its computations back into the system responsible for maintaining the

current position. This is in fact a reiteration of the five criteria that Redish (1999) states a path integrator must meet (see Chapter 1). We have, therefore, from this thesis, further evidence that the subiculum meets at least two of these criteria; hence, if Redish's criteria are valid, it is reasonable to propose that the subiculum may form at least part of a path integration system. At this stage, however, it is impossible to choose between the models presented by Sharp (1999b) and Redish and Touretzky (1997) (also Redish, 1999). The fact that the subiculum does not meet all of the criteria above does not in fact rule out Sharp's model, as Redish suggests (Redish, 1999), because Sharp's model also includes the entorhinal cortex (Sharp, 1999b) which sends a strong projection to the hippocampus (e.g., Amaral and Witter, 1989) (Redish states that Sharp's model does not meet the criterion which states that the proposed structure 'must send [its] output to the area associated with the place code'). Careful lesion studies are required to decide which model, if either, best describes path integration processes. For example, lesions to the parasubiculum will abolish path integration according to Redish (1999) but not according to Sharp (1999b). In relation to this point, Alyan and McNaughton (1999) reported that rats with hippocampal lesions performed as well as control rats on a path integration task performed in darkness: they conclude that neuronal circuits sufficient for computing a homing vector using path integration are located outside the hippocampus. Indeed it may be true that the hippocampus is not required by the path integration system, or that the other structures in the path integration system can cope with its loss; however, a further lesion study

analysing the effects of subicular lesions alone and combined hippocampal and subicular lesions on a path integration task would be useful.

Our analysis of the spatial correlates of subicular unit firing may provide clues as to the neural embodiment of the calculations required to path integrate. Subicular place fields are typically multi-peaked, with distinct areas of lower firing between the multiple peaks, suggesting that *multiple CA1 cells may synapse onto single subicular cells*. If the subiculum connects together disparate areas of the environment at the single unit level, then the CA1-subiculum pathway may provide the neural basis for the calculation of paths between the disparate locations that are represented.

Lesion studies show that the deficits which result from lesions restricted to the subiculum only or to the hippocampus only are qualitatively different from each other (Morris *et al.*, 1985): while hippocampal lesioned rats showed stereotyped circling patterns of swimming in their attempts to find the hidden platform, subicular lesioned rats showed patterns of searching which 'closely resembled those of normal rats who do not know where the platform is located.' This is strong evidence that these two structures support different representations. One possible explanation for the behaviour of the subicular lesioned animals is that, based on the idea above, they lack the neural mechanisms required to calculate paths.

It will be of interest in future to simultaneously record from multiple subicular units while rats undergo a clear, well-defined test of their path integration abilities. By correlating unit firing with specific aspects of such a task, it may be possible to discern a more precise role of the subiculum in this process at the single unit level. Thirty years on from the discovery of place cells (O'Keefe and Dostrovsky, 1971), the spatial theory of the hippocampal formation (O'Keefe and Nadel, 1978), and its later refinements and additions (e.g., Redish, 1999), remains the most appealing description of the functioning of these structures.

7.4 Synthesis

At the end of the introductory chapter it was stated that the dorsal subiculum sends a strong locational signal to each of the areas to which it projects and that one role of the subiculum may be to transmit information about the animal's current location in space so that it may be used for various navigation-related functions, such as path integration.

To this we can now add that the subiculum also transmits robust movement-related or 'inertial' information to its target structures. We have shown that the firing of many subicular units is correlated with movement-related processes; and that subicular units often have multiple correlates, not just a single spatial correlate. The fact that many units are significantly correlated with location and velocity we feel favours an interpretation of the subiculum as part of a path integration system.

Appendices

Appendix 1. Subicular unit separation: problems and solutions

'A number of workers in the field have verbally reported difficulty in single unit isolation in this region due apparently to the fact that many cells are simultaneously active with relatively high rates (O'Keefe, personal communication).' Barnes *et al.,* 1990, p. 289

The quality of single unit separation in freely-moving recording work is an area of active debate. Belief in the tools which give the most effective separations varies between laboratories (Wheeler, 1998). It is apparent from the literature that subicular unit separation in particular presents problems not usually encountered during hippocampal unit separations (Barnes *et al.,* 1990; Sharp, 1997). The quotation above from Barnes *et al.* (1990) illustrates this problem. In their study of the spatially-correlated firing of neurons in EC, CA1, CA3, and subiculum, they find that '[because] of the overall higher discharge rates, the reduced tendency towards bursting and the frequent occurrence of rhythmic modulation, we were unable except for a few cases to make clear distinctions between CS [complex spike] and theta modes of firing in either entorhinal cortex or subiculum. Thus, all cells in these areas are presented as a single class.' (Barnes *et al.,* 1990, p. 296). They display autocorrelation histograms of individual subicular units which show patterns of firing intermediate between theta and the complex spike patterns of the CA fields. They state

that while the use of a stereotrode 'represents a substantial improvement in the ability reliably to isolate neurons...compared to the single microwire techniques' that 'even this method is subject to a variable error in regions where high overall levels of population activity lead to a substantial probability that spike signals from different cells overlap in time. Such overlap degrades the quality of isolation.' (Barnes *et al.,* 1990, p. 298). They go on to say that for this reason 'subicular cells have been notoriously difficult to isolate' (Barnes *et al.,* 1990, p. 298).

The most detailed analyses of subicular unit activity so far have come from the laboratory of Patricia Sharp and colleagues (Sharp and Green, 1994; Sharp, 1997; Sharp, 1999a; Sharp, 1999b; Sharp 1999c). They also report difficulties in separating subicular unit activity: '...it has been our experience that it is extremely difficult to obtain electrical isolation of single cells in the subiculum. It is not clear why this is true, although the difficulty may result from the fact that the cells in the subicular principle [sic] layers are somewhat close together [but see discussion in Taube (1993) – 'Although the subicular pyramidal cell layer is continuous with the CA1 pyramidal layer, the cell packing is considerably *less dense* in subiculum than CA1' – my italics], and many of the cells show high, tonic firing rates in the freely moving animal. Thus, the electrical signal from electrodes placed in the subiculum often consists of large amplitude (100-300 μV), multiple cell activity, with no characteristic, individual waveform(s) which are clearly discriminable.' (Sharp, 1997). They also report that using stereotrodes and tetrodes does not improve the ability to

separate unit activity in the subiculum because 'these multiple-wire probes record only multiple-unit activity when used in the subiculum.' (Sharp, 1999a). Instead, using single wire electrodes, they report that 'it is occasionally possible to obtain waveforms which are clearly distinguishable from the multiple unit background' (Sharp, 1999a).

In this thesis, all single unit data was collected using single electrodes; stereotrodes were briefly tested but no improvement in unit separation was obtained. The problems described above (Barnes *et al.,* (1990); Sharp, (1997); Sharp, 1999a) were apparent throughout data collection.

Our initial attempts at subicular unit separations were made with DataWave's Spike Sort software (DataWave Technologies, Colorado, USA) and Autocut, a spike separation program that works in tandem with the DataWave system. All spike separation is grounded on the belief that the action potentials ('spikes') produced by a single neuron will have the same characteristic waveform shape, with occasional small changes over time due to slight drifts in electrode position (changes which may be tracked). Both DataWave programs plot points in x-y coordinates of one waveform parameter against another (parameters for single electrode recordings include peak amplitude, valley amplitude, spike width, peak time, etc.). The aim is to form distinct clusters of points with clear boundaries; each cluster is assumed to be produced by waveforms from a single neuron. User-controlled boxes are then drawn around each cluster

so that only waveforms with parameter values falling within the boundaries of the box are assigned to that cluster. This method has been successful at separating hippocampal waveforms in to several clusters from a single electrode. Both programs also support multiple electrode separations, plotting their unique parameters (e.g., peak phase angles). We, however, could not produce satisfactory separations of subicular unit activity using either program: not only did overlaid plots of individual waveforms clearly contain waveforms of varying shapes, later histogram plots (inter-spike interval histograms and autocorrelation histograms) showed that very many 'separations' contravened the refractory period constraint at the core of spike separation theory in that the 0 - 1 ms bin of both histogram types contained counts, i.e., neurons apparently fired during their own refractory periods. Since we know that typical neurons have refractory periods of at least 1 ms, and that subicular neurons in particular have refractory periods of around 1 - 3 ms (Taube, 1993), such separations cannot be trusted. Typically, Spike Sort and Autocut produced one large cluster, with no apparent boundaries even between portions of this large cluster. Attempts to refine the cluster separation were unsuccessful.

The spike sorting for all data collected in this thesis was performed using a custom-written template-matching algorithm (M. Anderson). A template-matching solution was chosen because it is considered to be the most effective method: 'No linearly derived feature set can outperform template matching, provided that the [background] noise is white and

uncorrelated with spike class' (Wheeler, 1998). This program, written in the Matlab programming environment (MathWorks Inc. Colorado, USA), is discussed in detail in the *Main Methods* chapter.

While we believe that this template-matching algorithm is more successful than other spike sorting methods, further attempts were made to improve confidence in our unit separations. Muller (1997) argues that the degree of stringency of unit separations should be determined by the aims of the study. By tightly defining the separation parameters, there is a high probability that every waveform assigned to a unit belongs to that unit, and thus also, an increased probability of excluding waveforms that belong to that unit (false negative). Alternately, by loosely defining the cutting parameters, there is a high probability of assigning every waveform to a discriminated unit, and thus also, an increased probability of including waveforms that do not belong to discriminated units (false positive). Rejecting waveforms that in fact were caused by the firing of the target neuron is analogous to type I error in statistics (the separation parameters are too conservative; hence the size of the rejection region on a distribution of waveform parameters is too large) and classifying waveforms from other neurons (or artefact) as spikes from a target neuron is analogous to type II error (the separation parameters are too liberal; hence the size of the rejection region is too small). If one accepts that few unit separations are perfect, one can argue that there are times when it is preferable to make a type I error instead of a type II error and vice versa. Given that subicular unit separations are problematic, we

decided to compare both stringent and less stringent separations of the same unit waveforms; the aim was to decide if it would be preferable to accept type I errors (rejecting waveforms from our target neuron), given that subicular single unit separations under less conservative conditions appear to contain waveforms from more than one neuron. Our hope was that for the purposes of our studies (e.g., examining spatially-correlated firing), type I errors would not effect our conclusions.

Another issue that supports our decision to make separations under stringent conditions comes from the quoted section from Sharp (1999a) above: 'it is occasionally possible to obtain waveforms which are clearly distinguishable from the multiple unit background'. There is a sampling issue at stake here: if only the largest and most easily separable units are included in a study then, by rejecting the majority of recorded units, one is introducing a bias in to the sampling. If one can only 'occasionally' separate units from the multiple unit background activity then these units may represent special cases, or at least that the tissue in the immediate vicinity of these neurons may differ from the structural average in terms of electrical conductance or cell packing. Similarly, if one records only very large amplitude spikes there may also be a sampling bias. One could argue, as theory suggests, that the very large waveforms are produced by neurons whose cell bodies are close to the electrode tip and hence one would equally as likely record from one cell type as another; on the other hand, one could argue that the very large waveforms were produced by neurons whose membrane characteristics, for example, differed from the

majority of neurons in the area. *If one aims to describe comprehensively the firing characteristics of neurons in a structure it is reasonable to propose that these neurons are sampled randomly.*

In conclusion, we accepted all units if (a) we could clearly separate a large number of spikes from the multiple unit background activity, and (b) such separations led to satisfactory histogram analyses, paying particular attention to the refractory period axiom. A consequence of this method is that overall histogram shapes are sometimes rather 'bitty' in their appearance, because, in making type I errors, we are not able to plot *all* spike intervals; this, in turn, does not make attempts to classify units as particular neuronal types (e.g., bursters or non-bursters) an easy task. However, we believe that the benefits gained from a fuller sampling of subicular units outweighs these disadvantages.

Appendix 2. Matlab analysis software

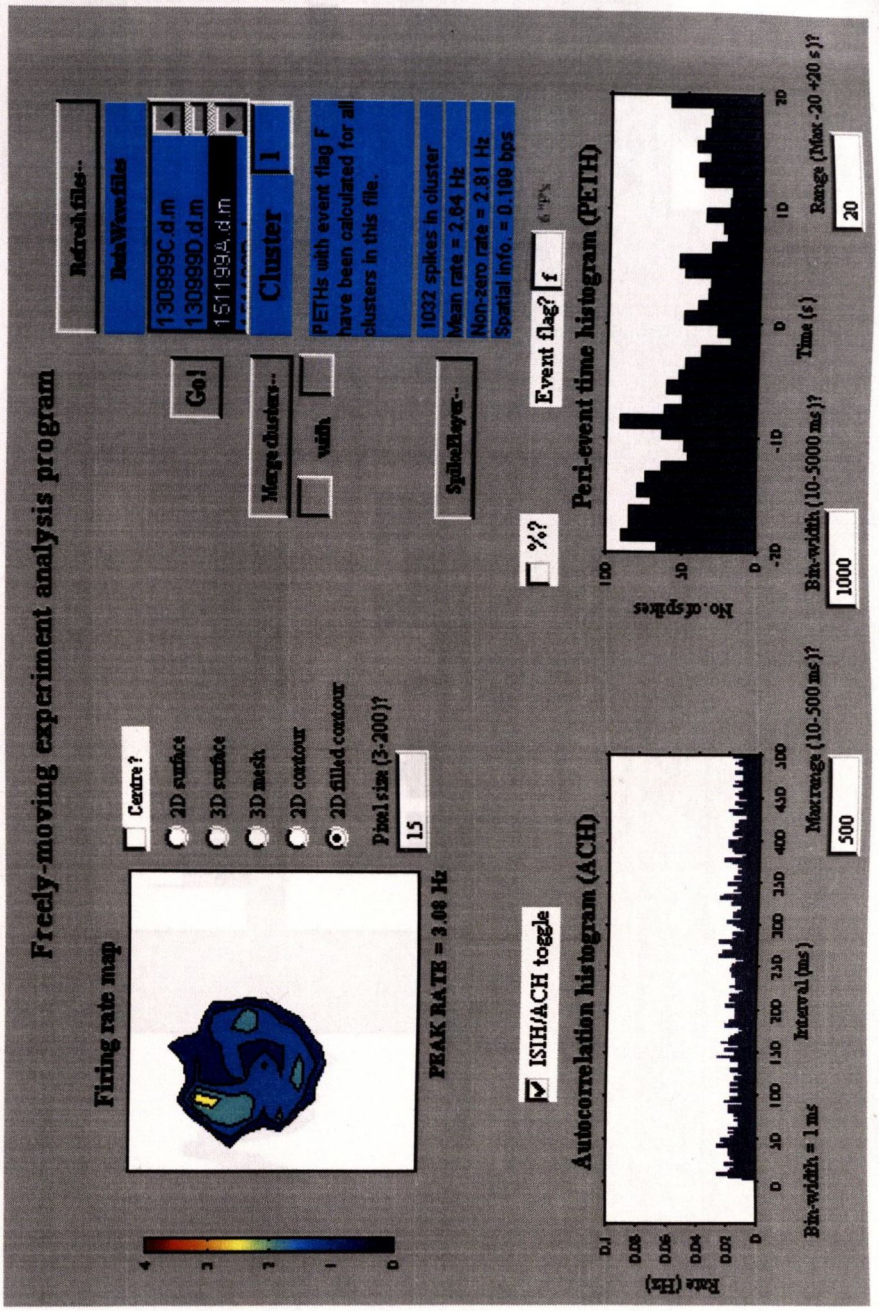


Figure 1. A print-out of the main analysis screen from the custom-written analysis software written using Matlab. It is printed here to illustrate the simultaneous production of firing rate maps, ISIHs, ACHs, and PETHs.

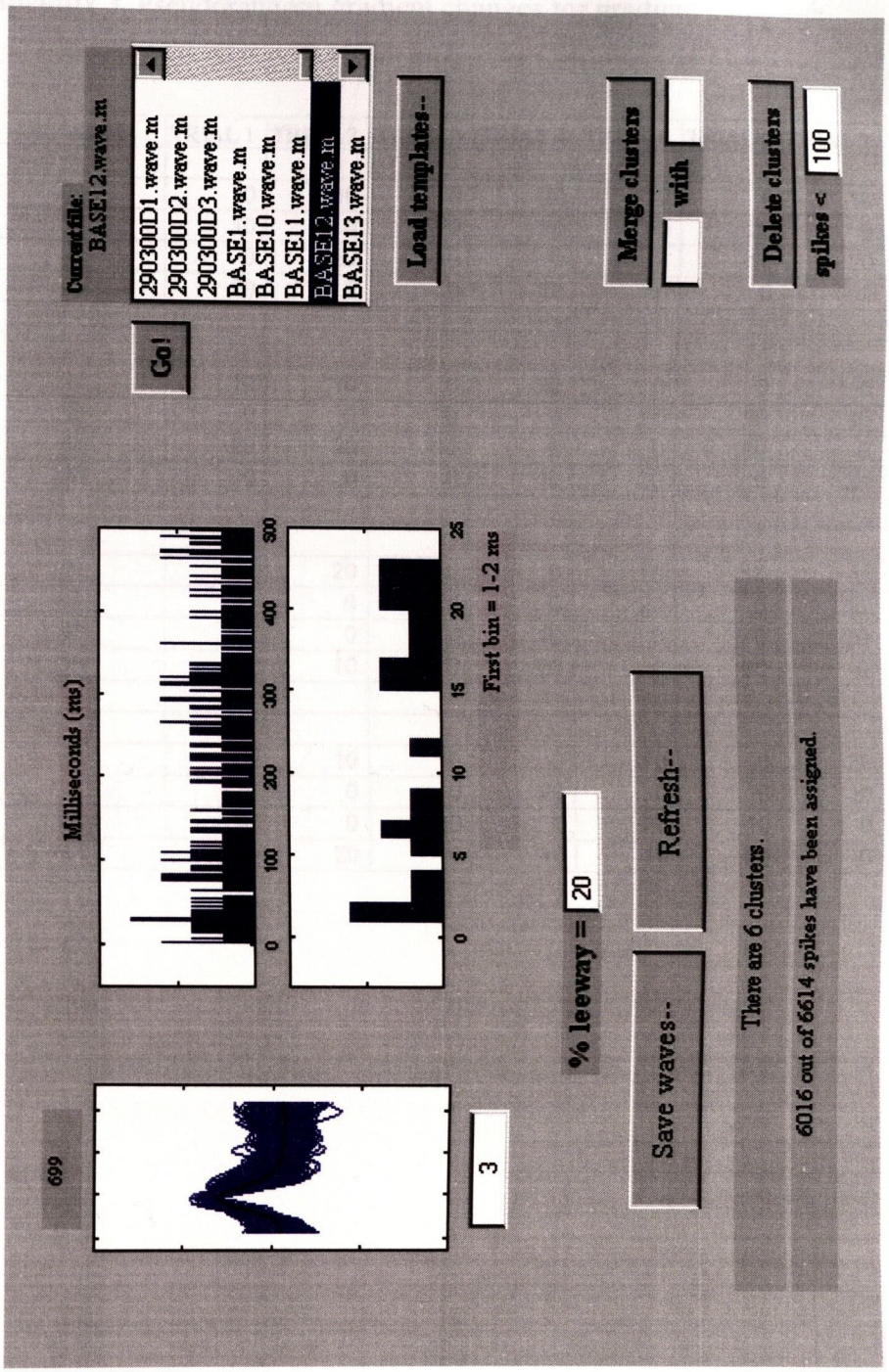


Figure 2. A print-out of the spike separation analysis screen from the custom-written analysis software written using Matlab. It is printed here to illustrate the simultaneous production of overlaid waveforms and ACHs which facilitate accurate separations.

Appendix 3. Pseudorandom gradient changes for gradient maze task

BATCH		ARM	TRIAL 1	TRIAL 2	TRIAL 3	TRIAL 4	TRIAL 5	TRIAL 6	TRIAL 7	TRIAL 8
1		N	0	10	0	20	20	0	10	0
		S	10	0	20	0	0	20	0	10
		E	0	20	10	0	0	10	20	0
		W	20	0	0	10	10	0	0	20
2		N	10	10	0	20	0	0	0	20
		S	0	0	20	0	10	20	10	0
		E	20	20	10	0	0	10	0	0
		W	0	0	0	10	20	0	20	10
3		N	20	20	0	0	10	10	0	0
		S	0	0	20	0	20	0	10	10
		E	0	0	10	10	0	0	20	20
		W	10	10	0	20	0	20	0	0
4		N	0	10	10	0	0	0	20	20
		S	20	0	0	20	0	0	10	10
		E	10	0	20	0	20	10	0	0
		W	0	20	0	10	10	20	0	0

Appendix 4. Significant main effects of arm location on unit firing rate in the gradient maze task (with any significant post-hoc comparisons [Tukey's HSD])

Unit 1: $F = 3.08$, d.f. = 3, 36, $p < 0.05$

(Post-hoc tests: S vs. W arms, $p < 0.01$)

Unit 2: $F = 3.8$, d.f. = 3, 36, $p < 0.05$

(Post-hoc tests: S vs. W arms, $p < 0.01$)

Unit 3: $F = 7.48$, d.f. = 3, 36, $p < 0.001$

(Post-hoc tests: N vs. W, $p < 0.001$; S vs. W arms, $p < 0.005$)

Unit 4: $F = 2.98$, d.f. = 3, 36, $p < 0.05$

(Post-hoc tests: E vs. W arms, $p < 0.05$)

Unit 5: $F = 8.05$, d.f. = 3, 36, $p < 0.0001$

(Post-hoc tests: E vs. S arms; N vs. S arms; N vs. W arms, all $p < 0.05$)

Unit 6: $F = 3.44$, d.f. = 3, 36, $p < 0.05$

(Post-hoc tests: S vs. W arms, $p < 0.05$)

Appendix 5. Significant main effects of trial on unit firing rate in the gradient maze task (with any significant post-hoc comparisons [Tukey's HSD])

Unit 1: $F = 4.42$, d.f. = 7, 36, $p < 0.005$

(Post-hoc tests: trial 1 vs. trial 8, $p < 0.0005$)

Unit 2: $F = 2.3$, d.f. = 7, 36, $p < 0.05$

Unit 3: $F = 4.95$, d.f. = 7, 36, $p < 0.005$

(Post-hoc tests: trial 2 vs. trial 5, $p < 0.05$; trial 3 vs. trial 5, $p < 0.05$; trial 3 vs. trial 6, $p < 0.01$)

Unit 4: $F = 2.28$, d.f. = 7, 36, $p < 0.05$

(Post-hoc tests: trial 2 vs. trial 8, $p < 0.05$)

Unit 5: $F = 2.93$, d.f. = 7, 36, $p < 0.05$

Unit 6: $F = 2.73$, d.f. = 7, 36, $p < 0.05$

Unit 7: $F = 2.44$, d.f. = 7, 36, $p < 0.05$

Unit 8: $F = 2.53$, d.f. = 7, 36, $p < 0.05$

Unit 9: $F = 2.67$, d.f. = 7, 36, $p < 0.05$

Appendix 6. Significant covarying effects of velocity on unit firing rate in the gradient maze task (with any significant post-hoc comparisons [Tukey's HSD])

Unit 1: $F = 4.44$, d.f. = 1, 36, $p < 0.05$

Unit 2: $F = 23.4$, d.f. = 1, 36, $p < 0.0001$

Unit 3: $F = 8.36$, d.f. = 1, 36, $p < 0.01$

Unit 4: $F = 8.66$, d.f. = 1, 36, $p < 0.01$

Unit 5: $F = 46.8$, d.f. = 1, 36, $p < 0.0001$

Unit 6: $F = 14.15$, d.f. = 1, 36, $p < 0.005$

Unit 7: $F = 9.58$, d.f. = 1, 36, $p < 0.005$

Unit 8: $F = 23.3$, d.f. = 1, 36, $p < 0.0001$

Unit 9: $F = 18.36$, d.f. = 1, 36, $p < 0.0001$

Unit 10: $F = 18.01$, d.f. = 1, 36, $p < 0.0001$

Unit 11: $F = 9.1$, d.f. = 1, 36, $p < 0.01$

Unit 12: $F = 12.68$, d.f. = 1, 36, $p < 0.005$

Unit 13: $F = 6.55$, d.f. = 1, 36, $p < 0.05$

Unit 14: $F = 10.14$, d.f. = 1, 36, $p < 0.005$

Unit 15: $F = 7.17$, d.f. = 1, 36, $p < 0.05$

Publications resulting from this thesis work

Abstracts

1. O'Mara and Anderson (2000). Single unit recordings in the subiculum of the freely moving rat in the open field and radial arm maze. *Soc. Neurosci. Abstr.*, Vol. 26, Part 1, p.472, 2000.

2. Anderson and O'Mara (2000). Single-unit recordings in the subiculum of the freely-moving rat during a pellet-chasing task. *European Journal of Neuroscience*, 2000.

Papers

1. O'Mara, S.M., Commins, S., and Anderson, M. (2000) Synaptic plasticity in the hippocampal area CA1-subiculum projection: implications for theories of memory. *Hippocampus* **10(4)**, 447-56.

2. O'Mara, S.M., Commins, S., Anderson, M., Gigg, J. (2001) The subiculum: a review of form, physiology and function. *Progress in Neurobiology* **64(2)**, 129-55.

Papers in preparation

1. Anderson, M., and O'Mara, S.M. (2001). A classification of subicular single units recorded in freely-moving rats.

2. Anderson, M., and O'Mara, S.M. (2001). The subicular representation of space: quantitative and qualitative analyses.

3. Anderson, M., and O'Mara, S.M. (2001). Single unit recordings of the subiculum on a gradient maze.

4. Anderson, M., and O'Mara, S.M. (2001). Single unit recordings of the subiculum during an object exploration task.

5. Anderson, M., and O'Mara, S.M. (2001). When do subicular single units fire?

Other publications resulting from my postgraduate work

Abstracts

1. O'Mara, S.M., Commins, S., Gigg, J., & **Anderson, M.** (1998). Long-term potentiation of the projection from hippocampal area CA1 to the subiculum. *Neuropharmacology,* S37, 2.08.

2. Commins, S., Gigg, J., **Anderson, M.,** & O'Mara, S.M. (1998). Long-term potentiation of the projection from hippocampal area CA1 to the subiculum *in vivo*. *European Journal of Neuroscience,* S10, 21.

Papers

1. **Anderson, M.,** Commins, S., & O'Mara, S.M. (2000). The effects of low frequency and two-pulse stimulation protocols on synaptic transmission in the CA1 - subiculum pathway in the anaesthetized rat. *Neuroscience Letters,* 279 (3), 181-184.

2. Commins, S., **Anderson, M.,** Gigg, J., & O'Mara, S.M. (1999). The effects of single and multiple episodes of theta patterned or high frequency stimulation on synaptic transmission from hippocampal area CA1 to the subiculum in rats. *Neuroscience Letters*, 270 (2), 99-102.

3. Commins, S., Gemmell, C., **Anderson, M.,** Gigg, J., & O'Mara, S.M. (1999). Disorientation combined with parietal cortex lesions causes path integration deficits in the water maze. *Behavioural Brain Research,* 104, 197-200.

4. Commins, S., Gigg, J., **Anderson, M.,** & O'Mara, S.M. (1998). Interaction between paired-pulse facilitation and long-term potentiation in the projection from hippocampal area CA1 to the subiculum. *NeuroReport,* 9, 4109-4113.

5. Commins, S., Gigg, J., **Anderson, M.,** & O'Mara, S.M. (1998). The projection from hippocampal area CA1 to the subiculum sustains long-term potentiation. *Neuroreport*, 9, 847-850.

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