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The Role of Subicular Place Cells In Spatial Information Processing: Effects Of Variations In Visual Information, Arena Size And Open Field Compartmentalisation.

By Jorge R Brotons-Mas

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

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Summary

The hippocampal formation is involved in different cognitive processes such as learning, memory and spatial navigation. One of the landmark findings in neuroscience was the discovery of hippocampal place cells (PCs) -neurons which seem to code for spatial location in rodents and humans. In previous research, it was found that "place cells" were also present in different substructures of the hippocampal formation such as the subiculum or the entorhinal cortex. During the last few years, studies of the properties of place cells in the hippocampus proper have been many but somehow the study of other relevant areas, like the subiculum, has been neglected.

The aim of this thesis was to better understand how the subiculum, as part of the hippocampal formation, processes spatial information under different conditions and how changes in the environment are integrated by PCs in the subiculum. Extracellular recordings of subicular neurons were implemented in freely-behaving animals under different environmental conditions in order to characterise in a better style the specific mechanisms underlying subicular PC activity.

Several lines of evidence indicate that the subiculum may have a more important role in spatial information processing in contrast to previous thinking which regarded the subiculum just as an output or simple relay structure of the hippocampus proper. More recent anatomical evidence suggests that it is actually strategically located within the hippocampal formation, receiving direct inputs from CA1 and the entorhinal cortex, among other important areas, receiving highly processed information form the hippocampus as well as direct information from the entorhinal cortex. This gives the subiculum the possibility of acting as an important integrator of spatial information from diverse cortical (and indeed subcortical) sources.

The objective of this thesis was to study the characteristics of subicular PCs under different environmental conditions. The first experiment was concerned with the

relevance of visual information in PCs firing. The second experiment was aimed to study how PCs in the subiculum codify physical changes of the environment such as size, and the final part of this thesis was to study how PCs do it with environment modification as well as how it codifies information in different or similar environments.

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I would like to thanks professor O'Mara for giving me the great opportunity to work with him. He gave me the chance to work in sciences and to achieve a personal dream. He is been of great support during the development of this work becoming also a great friend and guidance.

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Thanks to Rufo who was with me during the long nights of the summer, his presence was more than enough. I hope that everybody have such a good friend.

Thanks to Rosa, for staying with me, for waiting for me, for…

"…Caminante, son tus huellas

el camino, y nada más;

caminante, no hay camino,

se hace camino al andar.

Al andar se hace camino,

y al volver la vista atrás

se ve la senda que nunca

se ha de volver a pisar.

Caminante, no hay camino,

sino estelas en la mar…"

Antonio Machado.

Table of contents

Summary

Chapter 1: Introduction **18**

Chapter 2: Methods **60**

Chapter 3: Electrophysiology Of The Subiculum **80**

Chapter 4: Visual Modulation Of Subicular Place Cells **108**

Chapter 5: Scaling Properties Of Subicular Place Cells In Multiple Size Environments. **144**

Chapter 6: Effects Of Compartmentalisation Of A Familiar Environment In Subicular Place Cells **192**

Chapter 7: General Discussion **230**

Chapter 8: References **248**

Figures And Tables

Chapter 1: Introduction

Figures

Figure 1: Macroanatomy Of The Hippocampal Formation 27

Figure 2: Parahippocampal Area 29

Figure 3: Intrinsic Connections Of The Hippocampal Formation 30

Figure 4: Proximo-Distal Organisation Of The Subiculum 31

Figure 5: Grid Cells, Subicular Cells And Hippocampal PCs 40

Figure 6: McNaughton's Model 1996 50

Figure 7: Sharp's Model 1999 55

Figure 8: O'Mara's Model Of Subicular Function 56

Chapter 2: Methods

Figures

Figure 1: Acquisition System Oscilloscope 69

Figure 2: Cluster Cutting Techniques 72

Figure 3: Firing Field Size 76

Tables

Table 1: Anaesthetics 67

Table 2: Analgesia And Antibiotics 67

Chapter 3: Electrophysiology Of The Subiculum

Figures

Figure 1: Autocorrelograms, ISIH And Spike Waveforms For Bursting Units 93

Figure 2: Autocorrelograms, ISIH And Spike Waveforms For Fast Spiking, 94

None Bursting Theta Modulated And Regular Unit

Figure 3: Spike Width Distribution 95

Figure 4: Propensity-To-Burst Histograms 97

Figure 5: Bursting Units, Autocorrelograms And ISIH 98

Figure 6: Strong Theta Modulation Bursting Units, Autocorrelograms And ISIH 99

Figure 7: Fast Spiking Units, Autocorrelograms And ISIH 100

Figure 8: Non-Bursting Theta-Modulated, Units Autocorrelograms And ISIH 101

Figure 9: Regular Units, Autocorrelograms And ISIH 102

Figure 10: EEG Power Spectra Of 4 Different Recordings 104

Figure 11: EEG Trace And Spike Raster Plot 105

Tables

Table 1: Descriptive For Unit Type (Mean And S.E) 92

Chapter 4: Visual Modulation Of Subicular Place Cells

Figures

Figure 1: Protocol For Light-Dark Experiment 118

Figure 2: Anatomical Reconstruction Of Electrode Location 119

Figure 3. Firing Map Of Subicular PCs 120

Figure 4. Scatter Plots For Spatial Parameters 122

Figure 5: Firing Field Centre Of Mass 123

Figure 6: Scatter Plots For Distance Of Mass And Max Bin 126

Figure 7: Scatter Plots For Distance To The Centre Of Mass And Mean Correlation 127

Figure 8: Stability-Plasticity Index Sample Distribution 130

Figure 9 : Subicular PCs 130

Figure 10: Condition By Condition Stability-Plasticity 131

Figure 11. Example Of Plastic And Stable Units Across Different Conditions 134

Figure 12. Subicular PCs Across All Transitions 135

Figure 13: Plastic And Stable Units Activity Across Condition 137

Figure 14: Stable And Plastic Units, Skaggs And Firing Field Size 138

Tables

Table 1. Spatial Parameters 121

Table 2: Correlation Matrix For Stability Plasticity Measurements 125

Table 3: Descriptive Statistics Plastic And Stable Units Classified Using Correlation Index 128

Chapter 5: Scaling Properties Of Subicular Place Cells In Multiple Size Environments.

Figures

Figure 1: Behavioural Protocol 154

Figure 2: Anatomical Reconstruction Of Electrode Location 155

Figure 3: Scatter Plots For Spatial Parameters 157

Figure 4: Scatter Plots For Stability Parameters 162

Figure 5: Stability Index 162

Figure 6: Good Scaling Units 164

Figure 7: Imperfect Scaling Units 165

Figure 8: Scaling Remapping Units 166

Figure 9: Arena-Specific Units. 167

Figure 10: Firing Frequency Across Condition 171

Figure 11: Skaggs Index Across Conditions 174

Figure 12: Firing Field Size And Unit Type Across Condition 176

Figure 13.1: Grid-Like Unit 180

Figure 13.2: Grid-Like Unit 191

Figure 13.3: Grid-Like Unit 192

Figure 13.4: Grid-Like Unit 193

Figure 13.5: Grid-Like Unit 194

Figure 14.: Polar Plots Head Direction Cells 195

Tables

Table 1: Descriptive Statistics Spatial Parameters 156

Table 2: Frequency And Spatial Parameters Per Unit Class 159

Table 3: Correlation Matrix For Plasticity-Stability Parameters 161

Table 4: Mean Spatial Parameters Per Condition (Mean And S.E) 168

Table 5: Preferred Angle And Frequency Of Firing 179

Chapter 6: Effects Of Compartmentalisation Of A Familiar Environment On Subicular Place Cells

Figures

Figure 1: Standard Protocol For The Four Rooms 203

Figure 2: Anatomical Reconstruction 204

Figure 3: Grid-Like Cells And Subicular PCs 208

Figure 4: Firing Field Location 209

Figure 5: Different Firing Field Location 210

Figure 6: Units Recorded In 3 Conditions 211

Figure 7: Plastic And Stable Units 213

Figure 8: Units Recorded In The 5 Conditions 216

Figure 9: Units Recorded In The 5 Conditions 217

Figure 10: Firing Field Replication 218

Figure 11: Firing Field Replication And Complex Response To Arena Division 220

Figure 12: Units Recorded In The 3 Conditions 221

Figure 13: Firing Field Replication 222

Figure 14: Unit With No Firing Field Replication 223

Figure 15: Grid-Like Cells Response To Barriers 226

Tables

Table 1.1: Spatial Parameters For Classic SPCs And Grid-Like Cells 206

Table 1.2: Spatial Parameters For Classic SPCs And Grid-Like Cells 206

Table 2: Correlation And Distance Of Mass. Values For Plastic And Stable Cells Across Conditions 214

Abbreviations

Ab: Angular bundle

ACH: autocorrelogram

ANOVA: Analysis of variance

A-P: Antero posterior

B: Bursting unit

BSTM: Bursting strong-theta-modulated

BVC: Boundary vector Cell

CA1: Cornus Ammonis, subfield 1

CA3: Cornus Ammonis, subfield 3

DCM 1: Distance to the centre of mass type 1

DCM 2: Distance to the centre of mass type 2

DG: Dentate gyrus

DV: Dorso-ventral

EC: Entorhinal cortex

F: Fornix

FET: Field effect transistor

FF: Firing field

FFs: firing fields

FFT: Fast Fourier Transformation

FS: Fast spiking

Gl: Granular Layer

HD: Head direction

HPA: Hypothalamus adrenal axis

HPC: Hippocampal place cell

HPCs: Hippocampal place cells

IP: Intraperitoneal

ISIH: Inter-spike interval histogram

L: Lateral

LED: Light emitting diode

LIA: Large irregular activity

MEC: Medial Entorhinal Cortex

ML: Molecular Layer

M-L: Medio-lateral

mm: millimetres

msec: milliseconds

mf: mossy fibbers

µm: micrometers

NBTM : Non-bursting theta-modulated

Pcl: pyramidal cell layer

PoDG: Polymorphic layer of the dentate gyrus

POS: Position of the maximum value of the ISIH

PP: Perforant pathway;

PrS: Presubiculum

PaS: Parasubiculum

RS: Regular units

SC: Schaffer collaterals

SIA : Small irregular activity

Sl: stratum lucidum

Sl-m, stratum lacunosum-moleculare

So: stratum oriens

SPC: Subicular place cell

SPCs: Subicular place cells

Sr: stratum radiatum

SRA: Slow regular activity

STB: Strong-theta-modulated bursting units

VR: Virtual Reality

Chapter 1 : Introduction

Contents

1. Introduction To Spatial Navigation
    a. Spatial Navigation In Humans And Animals
    b. Allocentric And Egocentric Navigation
2. Anatomy Of The Subiculum And The Hippocampal Formation
    a. .Macro Anatomy Of The Hippocampal Formation
    b. Cytoarchitectonic Organisation Of The Subiculum
    c. Subicular Afferents And Efferents
    d. Theta Rhythm
    e. Electrophysiology Of The Subiculum
    f. Place Cells And Grid Cells
    g. Place Cells

i. Place Cells Properties

    1. Place Cell Stability And Plasticity
    2. Directionality: PCs And Head Direction Cells
    3. Place Cells And Goal Navigation
    4. What Does Affect PC Firing?
    5. PC Firing And Navigational Decisions

3. The Subiculum From Different Points Of View: A Model Review
4. Aims Of This Thesis

5.

1.1 Introduction To Spatial Navigation

1.1.1 Spatial Navigation In Humans And Animals

Animals and humans are obliged to move about in their environments in search for food, shelter or mates, actions which are basic for the survival of the individual and the species. The brain of every species has evolved to make individuals capable of navigating their environments in an efficient manner. From migratory birds to humans, we all have to be able to use different information to generate knowledge of our environments to navigate successfully to important places. O'Keefe & Dostrovsky (1971) suggested that the hippocampus was the main brain structure involved in spatial navigation, and it was the neuronal substrate which allows us to generate a "cognitive map" of space. This "cognitive map" would contain topographic information which would allow subjects to choose the best way to get to a goal by making different calculations based on the relationship between different environmental landmarks. Other strategies, which may be less flexible, such as egocentric navigation, could also be used to navigate and would be part of the general navigational tools used by individuals.

Birds with hippocampal lesions can fly during their migration using a compass strategy, but they get lost in their local area and are unable to generate a cognitive map (Bingman & Yates, 1992). The classic studies of migratory birds shed light on the way these expert navigators use different strategies to make use of different types of available information to orient themselves throughout long distance in their migratory flights or in their short trips in search for food. Watson & Lashley (1915) showed that if naïve migratory birds in their first flight were captured and transported in a perpendicular direction to where they were directed, they will miss the final destination by the amount of km they were transported. These birds were flying towards a fixed goal using a compass strategy (Griffin, 1955). On the other hand, if the same procedure was

implemented in experienced birds, they will correct the distance they were transported, successfully reaching the final goal. Experienced birds used a more elaborated approach to navigation involving knowledge of the environment and therefore some form of 'cognitive' mapping. Therefore, cognitive mapping would depend on experience and learning, ruling out the possibility of instinctive knowledge of the migratory routes. While using a compass strategy, birds can use three different sources of information like the sun, geomagnetism and the stars (Keeton, 1974). Experiments which manipulated the internal clock of birds demonstrated that these would use the sun to orientate themselves in respect to its internal clock. Keeton (1974) demonstrated that birds are capable of flying during bad weather not being affected by their internal clock. Also by applying magnets in the head of the animal this can be redirected towards a specific if the skies are overcastted. More recently, some studies have demonstrated that pigeons used different approach when navigating homewards. Lipp et al., (2004) observed that pigeons used highways and their exits in their fly to their nest as cues using compass adjustment during the middle part of the fly and a cognitive map when approaching the loft area.

1.1.2 Allocentric And Egocentric Navigation

Two basic navigational strategies allow animals and humans to navigate successfully: Allocentric navigation enables humans and animals to generate topographical representation of their environments by using multiple relevant landmarks of their environments. Morris, Garrud, Rawlins, & O'Keefe (1982) using the watermaze proved that rats used multiple environmental cues available in the experimental room to find a hidden platform which allows the rat to escape from the water. Later, Schenk & Morris (1985) trained groups with different degrees of damage in the entire rostro-caudal extent of the lateral and medial entorhinal cortex, and included parts of the pre- and para-subiculum, angular bundle and subiculum to navigate in the watermaze searching

for the hidden platform using external cues. Rats had a strong degree of impairment in this version of the task but not in the visible platform version . Morris, Schenk, Tweedie, & Jarrard (1990) found in combined hippocampal and Subicular injured animal's impairment in the hidden platform version of the watermaze. Therefore these different structures of the parahippocampal complex are necessary for the allocentric navigation strategy, supporting the original suggestion of O'Keefe & Dostrovsky (1971). Similar evidence has been found in humans using FMRI techniques. Aguirre, Detre, Alsop, & (D'Esposito, 1996), during a functional magnetic resonance study in which human subjects had to navigate throughout a virtual reality environment, observed activation of the hippocampus and parahippocampal region.

The egocentric or path integration strategy implies using other available information such as internal cues, motor input, vestibular information and directional information. All these inputs allow the subject to calculate its present and its future position by summing all different movements and turns. The hippocampus and other areas such as the parietal cortex seem to be involved (Commins, Gemmell, Anderson, Gigg, & O'Mara, 1999; Save, Paz-Villagran, Alexinsky, & Poucet, 2005; Whishaw, Hines, & Wallace, 2001). Wolbers, Weiller, & Buchel, (2004) found evidence that the parietal cortex and other structures are involved in path integration while humans navigate in a virtual reality environment using a route strategy.

1.2 Anatomy Of The Subiculum And The Hippocampal Formation

So far, some of the neuronal bases of spatial navigation have been briefly described as the two basic strategies used during navigation. In the following sections a description of the hippocampal formation and especially of the subiculum is presented. The specifics of the hippocampal formation, its afferents and efferents can help us to understand better the flow of information through the neuronal circuits involved in spatial navigation.

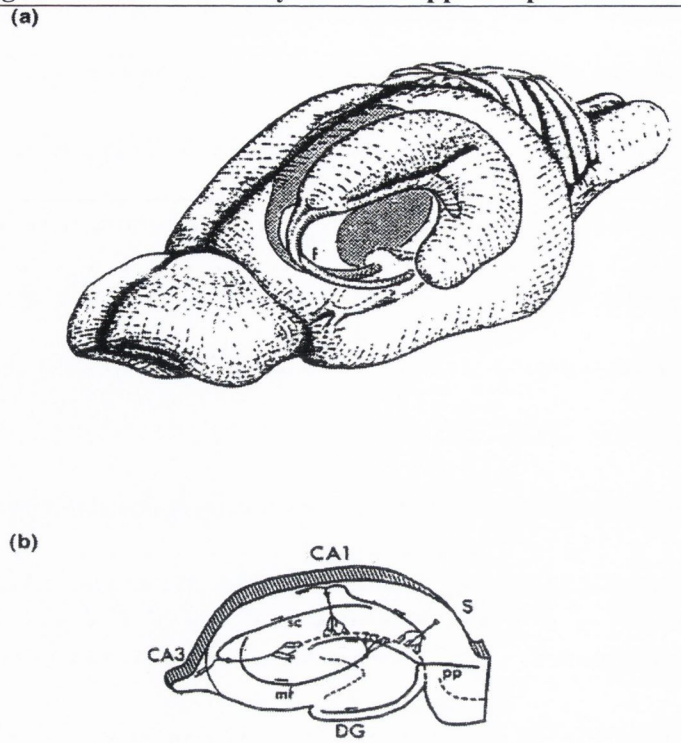
1.2.1 Macroanatomy Of The Hippocampal Formation

A distinction between the hippocampus proper and the hippocampal region must be made. The hippocampus proper, consisting in 2 interlocked cell layers with the shape of a C consisting in the dentate gyrus and cornus ammonis which comprehend the two main subfields CA1 and CA3 area. The parahipocampal region includes the entorhinal cortex (EC), the periallocortical area of the perirhinal area, the subicular complex including the presubiculum (PrS), parasubiculum and subiculum (PaS) (Witter, Groenewegen, Lopes da Silva, & Lohman, 1989). The parahippocampal region could be seen as the ultimate association area to which all different areas of cortex and subcortical inputs converge (Swanson, 1982). Therefore, the hippocampus is located in a strategic position within the brain to synthesise all different types of information from propioceptive to that coming from the external world.

The subicular complex, is composed by the subiculum proper, presubiculum and parasubiculum, being located between EC and CA1 ventrally and between CA1 and retrosplenial cortex dorsally, (Burwell, Witter, & Amaral, 1995). It has been debated the possibility of defining the postsubiculum as a fourth area within the subicular complex (Witter, Ostendorf, & Groenewegen, 1990), although other authors, described the postsubiculum as the dorsal area of the presubiculum (O'Mara, Commins, Anderson, & Gigg, 2001).

Further controversy about the subicular complex is related to its conceptualization as a functional and anatomical unit (Lopes da Silva, Witter, Boeijinga, & Lohman, 1990; O'Mara et al., 2001). From an anatomical point of view, the subiculum could be clearly differentiated from the parasubiculum and the presubiculum by their different afferents and efferents. While the subiculum receives direct input from CA1 area, presubiculum and parasubiculum receive direct input from neocortical areas. Although they receive input from the subiculum, this is not dominant when compared to neocortical input. Subiculum shares with the hippocampus proper the same allocortical organisation being composed by 3 layers, while presubiculum and parasubiculum are more similar to EC having continuous layers. Presubiculum and parasubiculum present direct strong connection with the anterior thalamic nucleus being a major different feature of this structure (Burwell et al., 1995).

Figure 1: Macroanatomy Of The Hippocampal Formation



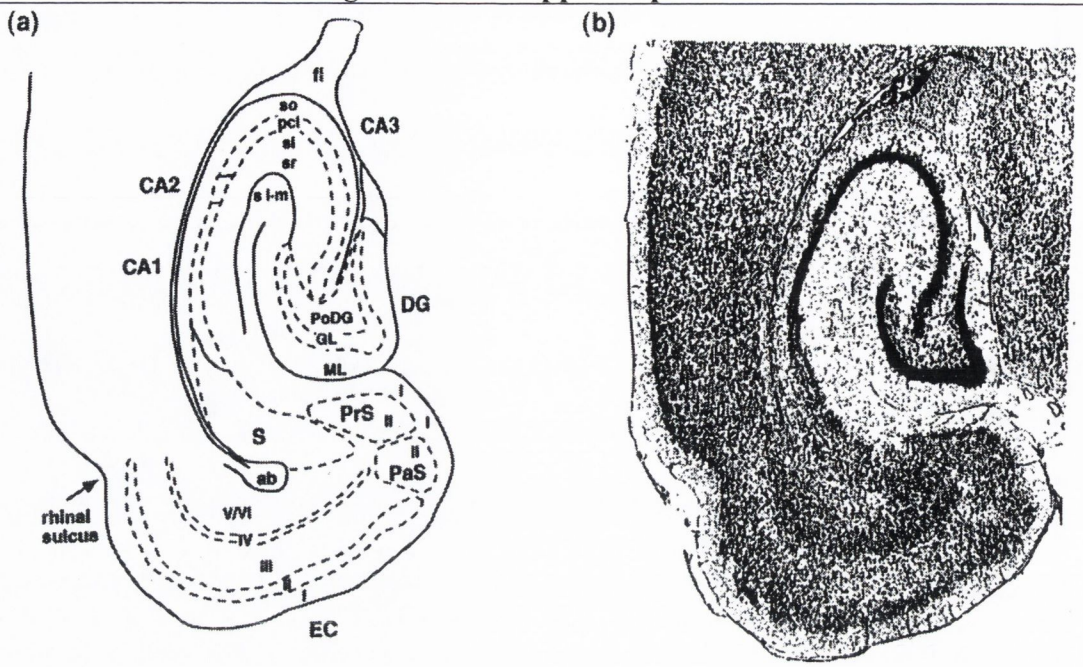
(a) A three-dimensional representation of the hippocampal formation in the rat brain. (b) Line drawing of a cross-section through the hippocampal formation illustrating the major areas and projections of the hippocampus. S, subiculum; CA1 and CA3, fields of the hippocampus; DG, dentate gyrus; pp, perforant pathway; mf, mossy fibbers; sc, Schaffer collaterals; f, fornix (Amaral & Witter, 1995).

1.2.2 Cytoarchitectonic Organisation Of The Subiculum

The subiculum is divided in three principal layers: molecular layer, pyramidal layer and polymorphic layer. The molecular layer is continuum to the strata-lacunosum moleculare and radiatum of CA1. The pyramidal layer is composed by large pyramidal neurons which present less density of packing that the pyramidal layer of the hippocampus proper. Also, smaller units are found in this layer being thought to be interneurons of the subiculum (Swanson & Cowan, 1977). Harris, Witter, Weinstein, & Stewart (2001) found that pyramidal units project their apical dendrites in the molecular layer and their basal dendrites in the deep areas of the pyramidal layer. Neurons in the

deeper layers of the subiculum have dendrites reaching the outer molecular layer. Since branch point was located in the superficial layers branching pattern depended on soma location. Superficial cell layer tended to have short or absent apical dendrites while deeper located units had larger apical dendrites. No difference was observed in basal dendrites between superficial and deep located neurons. Collateral axons differ between deep located units and superficial units (Harris et al., 2001). Deep located units have ascending axon which would reach the superficial areas staying in the area delimited by its dendrites while superficial units had axons reaching longer distances and they presented much more varicosities than observed in the hippocampus. Neurons projected to CA1, EC and presubiculum. Interestingly units in the deeper layer tended to be bursting units while units in the superficial layers tended to be regular spiking. In both cases neurons seemed to be projecting units. Subiculum seem to have some sort of columnar and laminar structure (Harris et al., 2001).

Figure 2: Parahippocampal Area



(a) A line drawing of a horizontal section through the hippocampal formation indicating various regions, layers and fibre pathways. (b) A Nissl-stained horizontal section of the hippocampal formation. ab, angular bundle; PaS, parasubiculum; PrS, presubiculum; ML,Gl, and PoDG, molecular, granular and polymorphic layers of the dentate gyrus; so, stratum oriens; pcl, pyramidal cell layer; sl, stratum lucidum; sr, stratum radiatum; sl-m, stratum lacunosum-moleculare; fi, fimbria; EC, entorhinal cortex (adapted from Amaral & Witter 1995).

Figure 3: Intrinsic Connections Of The Hippocampal Formation

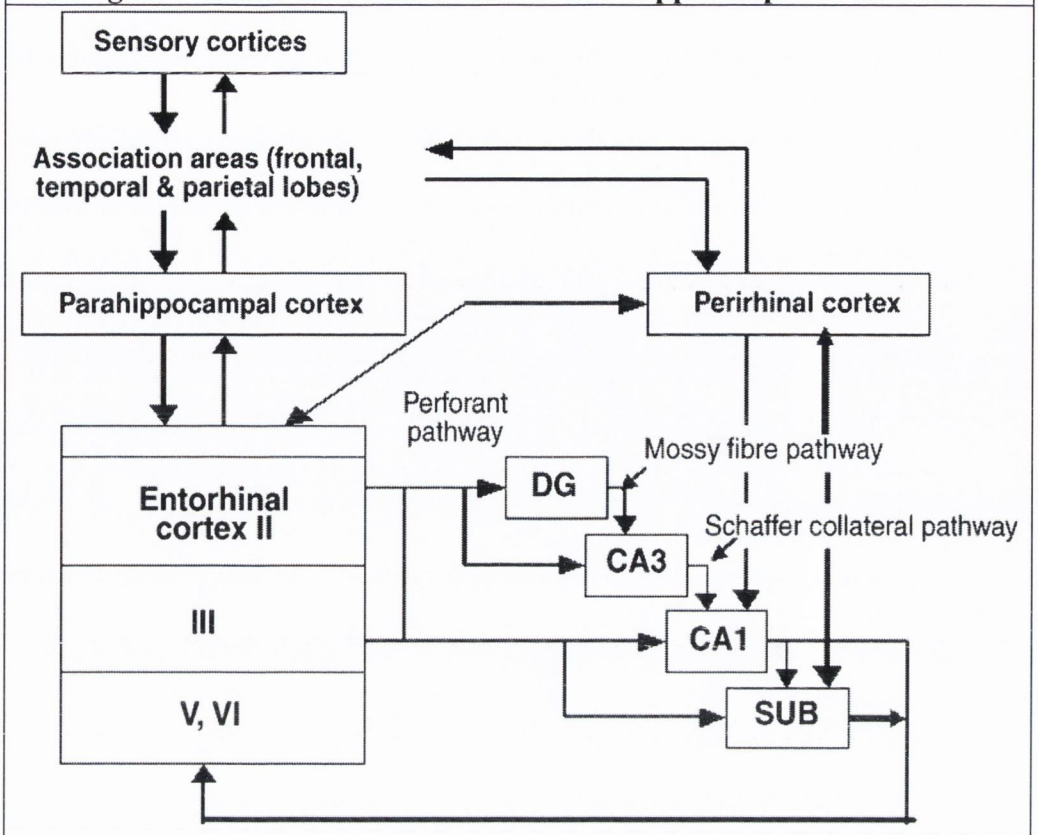
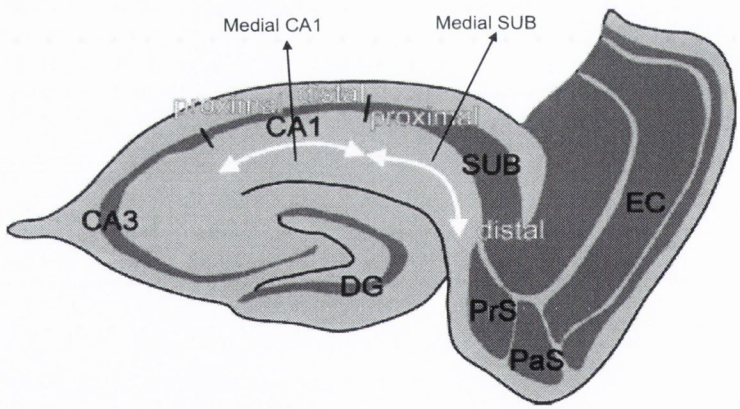


Fig. 3 Intrinsic connections of the hippocampal formation, including the recently discovered projection from perirhinal cortex to CA1 and subiculum. Adapted from (O'Mara, 2005).

1.2.3 Subicular Afferents And Efferents

The subiculum is the major output structure of the hippocampus, receiving major afferences from CA1 region (Amaral & Witter, 1989). This projection is organised following a structured organisation reaching all areas of the subiculum. Areas of CA1 bordering CA3, regarded as the proximal portion of CA1, projects to the distal area of the subiculum, area bordering the presubiculum. Distal CA1, area bordering the subiculum, projects to the proximal subiculum, area bordering the distal CA1. The medial portion of CA1, located between proximal and distal, projects to medial subiculum (Amaral & Witter, 1989). Also a back-projection from the subiculum to CA1 appears to exist (Seress, Abraham, Lin, & Totterdell, 2002), a suggestion supported by electrophysiological data (Commins, Aggleton, & O'Mara, 2002).

Figure 4: Proximo-Distal Organisation Of The Subiculum



Coronal section through hippocampus and adjacent entorhinal cortex (EC). White line indicates transverse axis of hippocampus. This axis runs in the transverse plane from the hilar region of the dentate gyrus towards EC, within each of the subfields CA3, CA1, and subiculum. Regions closest to the dentate gyrus are termed proximal, while regions closest to the entorhinal cortex are termed distal, which results in different portions in each subfield of the hippocampus. CA, cornu ammonis; DG, dentate gyrus; EC, entorhinal cortex; PaS, parasubiculum; PrS, presubiculum; SUB, subiculum. Adapted from Naber et al (2001a)

Pre- and parasubicular backprojections have been proved weak, (Kohler, 1985). However, there is a strong projection from the subiculum to pre- and parasubiculum. This connexion seems to follow a topographic organisation with the dorsal subiculum projection to the dorsal and caudal parts of the pre- and parasubiculum, while more ventral subicular areas project to rostral and ventral pre- and parasubicular areas (Swanson & Cowan, 1977; Witter et al., 1990).

Subiculum-Entorhinal Cortex

Subicular to entorhinal cortical efferents have been characterised in different species (Beckstead, 1978; Sorensen & Shipley, 1979; Milner & Amaral, 1984; Vermeulen-Van der Zee, te Kortschot, & Witter, 1987; Kohler, 1985; Tamamaki & Nojyo, 1995). Subiculum efferents to the EC seem to reach the deep layers of the entorhinal cortex in both the medial entorhinal cortex and the lateral entorhinal cortex, reaching layer four and generating an important input entrance from the subiculum to the EC. There are also weak projections to the superficial layers of the EC (Kohler, 1985; Witter et al., 1989). Witter et al., (1990) proposed a division of the subiculum in proximal, distal, and medial due to their different subcortical afferents. In fact, they found that only proximal subiculum projected to the EC. Tamamaki & Nojyo, (1995) found that projecting neurons to the EC could be found throughout the whole proximal-distal axis of the subiculum. More recently, it has been found that proximal subiculum projects mainly to the lateral entorhinal cortex while the distal part of subiculum terminates in the medial entorhinal cortex. In this way, information flow could be separated from the differing components of entorhinal cortex (Kloosterman, Witter, & Van Haeften, 2003). EC-subiculum pathway, seem to target the molecular layer of the subiculum in different species (Van Hoesen, Pandya, & Butters, 1972; Steward, 1976; Witter & Groenewegen, 1984; Wyss, 1981). Witter & Groenewegen, (1984) found in the rat that projection from the EC target the outer two-thirds of the molecular subicular layer. Efferents to the

subiculum have their origin in layer II and III of the EC (Steward & Scoville, 1976), with cells in layer two innervating subicular cells in all layers, making synapse in dendrites and therefore driving subicular cell firing. Although it was proposed that EC input to the subiculum via CA1 and direct EC-subicular connection could be integrated in the same units (Tamamaki & Nojyo, 1995), in a recent work it was proved that for both EC division, medial and lateral, to CA1 and subiculum as well as projections from CA1 to the subiculum were divergent and only few portions of CA1 and subiculum receiving input from the same EC area are also interconnected (, Lopes da Silva, & Witter, 2001a). This suggests possible functional divergence for EC-CA1 and EC-Sub circuits.

Subiculum-Perirhinal Cortex

Subiculum is also connected to the perirhinal cortex. It receives direct input from the perirhinal cortex by receiving direct input from dorsal and ventral areas which project to the molecular layer of the subiculum (Kosel, Van Hoesen, & Rosene, 1983) Dorsal injections of anterograde tracer labelled the lateral subiculum proximal to the hippocampal proper while the ventral injection labelled the medial subicular area. Amaral & Witter, (1989) found that caudal perirhinal cortex project to proximal subiculum while more rostral perirhinal areas projected to more distal areas of the subiculum. It seems that projections from the perirhinal cortex arrived to the subiculum via the perforant pathway (Kosel et al., 1983). Naber, Witter, & Lopes da Silva (2001b) found that perirhinal injection of anterograde tracer labelled fibres of the border area of CA1 and subiculum. Similarly Witter, Wouterlood, Naber, & Van Haeften, (2000) demonstrated that perirhinal cortex projects to the distal area of the subiculum. The postrhinal cortex also sends projections innervating the distal CA1 and the proximal Subiculum, areas which are interconnected too, the stronger connection being between

postrhinal cortex and subiculum, while projection from perirhinal cortex seems to be more equally distributed (Witter, et al., 2000).

Projections from the subiculum to the perirhinal cortex have been also found across different species (Kohler, 1985; Sorensen & Shipley, 1979; Swanson, 1982; van Groen, van Haren, Witter, & Groenewegen, 1986). Proximal dorsal subiculum projects to the deep layers of the perirhinal cortex, and distal areas of the subiculum seem to project to retrosplenial cortex and postrhinal cortex. Projections from the distal subiculum seem to target ventral retrosplenial cortex (Witter & Groenewegen, 1990). No afferents have yet been found from ventral subiculum or proximal subiculum to retrosplenial cortex. It also seems that connections between the subiculum and retrosplenial cortex are unidirectional since no labelling has been found in the subiculum after injections of an anterograde marker in the retrosplenial cortex (Wyss & Van Groen, 1992). Although retrosplenial cortex sends projections to the hippocampal formation, it seems that none of them reach the subiculum but instead, they connect to the presubiculum and parasubiculum (Wyss & Van Groen, 1992)

Subiculum-Prefrontal Cortex

Restricted areas of the subiculum send projections to the prelimbic and medial orbital areas in a similar way to CA1. Fibres coming from half of the proximal subiculum reach the orbital cortex and the prelimbic cortex with similar distribution pattern to CA1 projections. Finch (1993) presented evidence of ventral subicular projections to the prelimbic, infralimbic and cingulated cortex. Connections between the subiculum and the prefrontal cortex are of interest due to the involvement of the prefrontal cortex in complex cognitive process (O'Keefe & Nadel, 1978; Gemmell & O'Mara, 1999).

Subiculum-Subcortical Structures

The subiculum has also particular relations with subcortical structures projecting and receiving projection from different relevant areas; in particular, the ventral subiculum

projects to the ventromedial nucleus of the hypothalamus (Witter & Groenewegen, 1990). Canteras, Simerly, & Swanson, (1992b) found that the ventral premammillary areas also send projections to the ventral subiculum. Witter et al., (1990) found that injections anterograde marker in the dorsal subiculum bilateral labelling of the mamillary nuclei. Ventral subiculum projects to the amygdala innervating the posterior basomedial nucleus and the posterior basolateral nucleus (Canteras & Swanson, 1992). In the same way, the subiculum seems to receive input from the amygdala complex. Fibres with origins in the posterior amygdalar nuclei innervate the ventral subiculum and adjacent CA1 region (Canteras, Simerly, & Swanson, 1992a). Further connections are also found between the septum and the subiculum, receiving input from both dorsal and ventral subicular areas (Namura, Takada, Kikuchi, & Mizuno, 1994). The anatomical relationship between both structures seems to be bi-directional. Septal areas innervate all different areas of the hippocampus, specifically the subiculum receives information from the diagonal band of Broca (Lopes da Silva et al., 1990). Nucleus accumbens is innervated by neurons located in the proximal dorsal subiculum but not by the distal portion (Witter et al., 1990). The authors also reported ventral subicular projections to the nucleus accumbens.

The nucleus reuniens of the thalamus receives input from the ventral subiculum (Witter et al., 1990). Wouterlood, Saldana, & Witter, (1990) found terminal labelling is most dense in the molecular layer of the ventral part of the subiculum, following anterograde injections in the nucleus reuniens. Witter et al., (1990) demonstrated that irrespective of the proximo-distal location of the injections in the ventral subiculum, anterograde labelling in the anterior nuclei of the thalamus or in the interanteromedial nucleus is extremely weak or absent.

It can be seen how the subiculum communicated with different cortical and subcortical structures receiving and sending afferent and efferent fibres. It is clear that the

subiculum is strategically located not only within the hippocampal formation but also between cortical and subcortical structures.

1.3 Theta Rhythm

The electrophysiological rhythm most often implicated in sensory and motor integration is in the theta frequency range when recorded in the hippocampus (Bland & Oddie, 2001). Theta activity is characterised by a regular sinusoidal activity between 4 Hz-8 Hz. Its changes in amplitude and frequency are directly related to sensory inputs reflecting changes in any sensorial pathway and also changes in motor behaviour, (Whishaw & Vanderwolf, 1973; Kramis, Vanderwolf, & Bland, 1975; Bland & Oddie, 2001). The fact that hippocampal PCs firing is related to the theta rhythm, (O'Keefe & Recce, 1993), strengthens the idea that this sensory and motor integration process would convey some essential information for spatial navigation. Theta rhythm has been also found in humans while navigating a virtual maze (Bischof & Boulanger, 2003). This relationship was further related to the difficulty of the maze (Kahana, Sekuler, Caplan, Kirschen, & Madsen, 1999) and it has been clearly dissociated from other components of the task, being clearly related with navigation (de Araujo, Baffa, & Wakai, 2002).

1.4 Electrophysiology Of The Subiculum

Differences in vitro work suggested the predominance of bursting units in the subiculum (Stewart & Wong, 1993; Behr, Empson, Schmitz, Gloveli, & Heinemann, 1996). Bursting units are neurons capable of firing trains of more than 2 spikes in an interval of 10 msec to 20 msec. This mechanism has been proposed as a way of improving information transfer to different areas and seems to be dependent on Ca^{+} currents (Jung, Staff, & Spruston, 2001). Staff, Jung, Thiagarajan, Yao, & Spruston, (2000) proposed that subicular units belong to the same cell type, varying in their propensity to burst.

Previous work (Behr et al., 1996) found two basic cell types in relation to their response to orthodromic stimulation, bursting units, and regular spiking units which responded with single action potential after stimulation. Stewart & Wong (1993) classified units as bursting units and non-bursting units. They also determined that bursting units could be shifted to a non-bursting state by lowering their membrane action potential. Similarly Taube (1993) found bursting and regular units. In more recent work (Staff et al., 2000) classify units in 3 basic cell types, strong-bursting units, weak bursting units and regular spiking units. Menendez de la Prida, Suarez, & Pozo (2003) in an in-vitro preparation recorded units from the ventral subiculum finding further cell types. Units were classified as 54%, regular spiking 26% bursting units, 11% fast spiking, 9% unclassifiable and 6% of units presented membrane potential oscillations probing that the subiculum is an electrophysiological-more complex structure than previously proposed.

Sharp & Green (1994) studied spatial information coding by subicular units. In vivo in the freely-behaving animal, extracellular recordings were performed, and a tentative classification was proposed. Units were classified as bursting units, depolarised bursting, non-bursting units and theta cells. Bursting and non-bursting units were related as those bursting and non-bursting units mentioned by others. Depolarised bursting were associated with the capability observed by Stewart & Wong (1993) of subicular bursting units to shift from bursting to non- bursting mode by lowering the membrane potential. Anderson & O'Mara (2003), also using in vivo freely-behaving techniques, found high percentage of bursting units and also described theta units, neurons which tended to increase firing whenever theta was present in the EEG.

1.5 Place Cells And Grid Cells

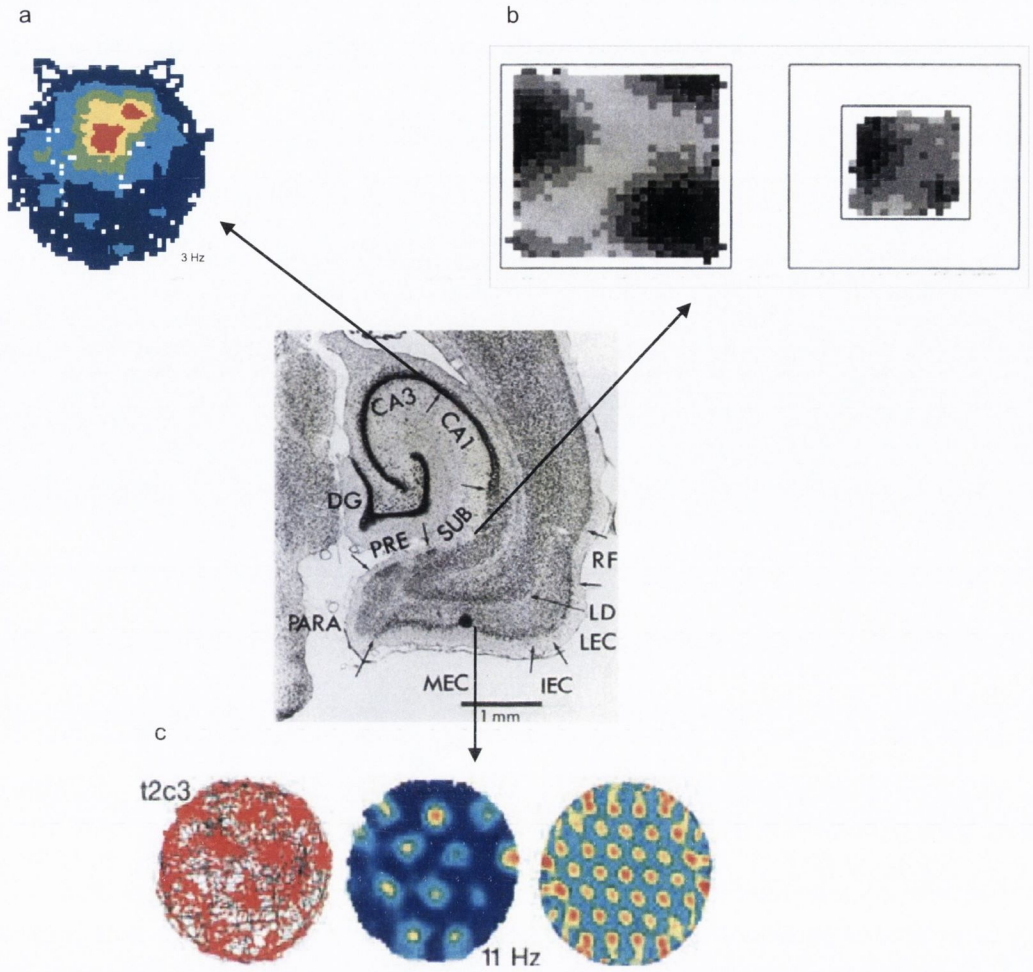
O'Keefe & Dostrovsky (1971) recorded single neurons in the hippocampus in chronically implanted rats which foraged freely for food in a small arena. They found a

group of cells whose firing increased whenever the animal was in a discrete location of the environment, firing field. The firing of these neurons seems to be independent of other variables such as view, direction or speed of movement, being location the best predictor of their firing. Later research supported the original finding, (Muller, Bostock, Taube, & Kubie, 1994; Poucet, Lenck-Santini, Paz-Villagran, & Save, 2003). PCs were seen as the first objective measurable neuronal base involved in spatial navigation. The study of PCs has generated a broad body of investigation and research but while easy to record in rodents space or-place-related activity requires special recording conditions to be detected in primates (O'Mara, 1995; S. M. O'Mara, Rolls, Berthoz, & Kesner, 1994). It was questionable if this same mechanism would be also present in the human brain. Recently Ekstrom et al. (2003) recorded from subcortical implanted electrodes in epileptic patients, finding that cells in the human hippocampus fire strongly in specific locations while the subject navigated a virtual reality environment, probing the existence of PCs in humans.

Place cells have been recorded in different areas of the hippocampal formation. For example Barnes, McNaughton, Mizumori, Leonard, & Lin (1990) recorded units in all different areas of the hippocampus proper as well as in the subiculum. Later, Sharp & Green (1994) confirmed the existence of units in the subiculum which seemed to code for spatial information. Quirk, Muller, Kubie, & Ranck (1992) also studied the characteristics of medial entorhinal cortex (MEC) which seemed to share different characteristics with subicular PCs. More recently Hafting, Fyhn, Molden, Moser, & Moser (2005) discovered a specific unit type which presented different properties to those previously described. Units of the dorsal MEC presented regular multi-peak activity. The firing pattern generated was characterised for having a grid-like structure in which firing fields would be constantly spaced and where angular relation was always the same. Theses units seemed to produce more firing fields in larger arenas

keeping the distance and angular relationship between them and therefore keeping stable the grid-like structure. Completing these findings Sargolini et al. (2006) discovered the existence of grid cells, grid by direction cells and head direction cells in deep layers of the EC, while in the superficial layers only grid cells were found. It has been proposed that grid cells could be the neuronal bases of path integration (McNaughton, Battaglia, Jensen, Moser, & Moser, 2006).

Figure 5: Grid Cells, Subicular Cells And Hippocampal PCs



Unit a represents the typical place cell recorded in CA1. Units in this area display sharp unique firing fields in each environment. Adapted from (Lever, Wills, Cacucci, Burgess, & O'Keefe, 2002). Unit b recorded in the subiculum across two different size arenas. On the contrary to Ca1 units, subicular place cells normally fire in multiple locations of the environment. In this case this was observed in both arenas, multiplying the number of firing peaks in the larfer arena which also scaled up in this condition (Sharp, 1999a). Unit c Grid cells recorded in the medial entorhinal cortex. It can be seen how firing field created a grid-like structure. Left figure represents animal track and spike position, middle firing field map with multiple firing peaks. Left graph, temporal autocorrelogram verifying the grid structure. Adapted from (Hafting et al., 2005)

1.5.1 Place Cells Properties

Different parameters have been studied to understand better how PCs code for spatial information among which we can highlight stability, directionality of PCs firing, sensory information and cue control of PCs firing, PCs and navigation towards a goal.

1.5.1.1 Place Cell Stability And Plasticity

PCs tend to fire in stable manner if no spatial or other manipulation is implemented in the recording chamber. Thompson & Best (1990) recorded a cell for 153 days using the same recording arena. Hill (1978) suggested that PCs firing field (FF) is generated as quickly as the animal explores the environment. Posterior studies have demonstrated that PCs learn to for salient cues in the environment. Kentros et al (1998) found that blockade of NDMA receptor impaired PCs firing stability in new environments. Lever et al. (2002) studied the progressive differential representation of two different arenas by hippocampal PCs. Although firing patterns where similar in both arenas, at the beginning the spatial representation diverged after repetitive exposure. New representation of space was stable one month later for each of the environments. Therefore, although FF can be stable across long period of time, they also reflect spatial and neuronal plasticity. On the other hand, McNaughton et al., (1996) and. McNaughton, Barnes, Meltzer, & Sutherland (1989) postulated that place cell firing depends not only upon a learning process but also on being relatively hard-wire characteristic of the hippocampus related to brain development. This would facilitate mapping in new environments.

1.5.1.2 Directionality: PCs And Head Direction Cells

McNaughton, Barnes, & O'Keefe (1983) found that PCs firing frequency was higher when the animal was running in inwards direction in a radial arm maze. Previous research suggested that PCs directional firing was related to the recording chamber physical characteristics and to the task demands. Markus et al. (1995) found that

directional firing of PCs was higher in the radial arm maze and also in an open field arena whenever the animal had to move in a linear trajectory to retrieve a reward. Muller et al. (1994) studied the directionality of place cell (PC) confirming previous findings which suggested that physical restrain of the recording chamber can make FF look directionally polarised. Taube, Muller, & Ranck (1990) described a cell type whose firing coded for head direction (HD) firing only whenever the animal head is pointed to a specific direction. This type of cell, is found in different structures of the parahipocampal complex as well as in other subcortical structures (Taube et al., 1990; Taube, 1995). The firing of these neurons conveys information about where the animal's head is pointing. They seem to use environmental cues to calibrate their directional firing (Goodridge, Dudchenko, Worboys, Golob, & Taube, 1998) and they depended on vestibular input without which, its firing disappears. Cacucci, Lever, Wills, Burgess, & O'Keefe, (2004) demonstrated that a group of cells firing, in the presubiculum and parasubiculum, codes for location and direction. The authors suggested that this type of cells could synthesise spatial information and direction information being the bridge between both systems. This interaction between head direction system and location information system seems to be necessary for efficient spatial navigation. Head direction system would facilitate information to place cells to set their firing in relation to distal external cues, facilitating in this way efficient navigation under specific conditions. This interaction is clearly reflected in all proposed models of spatial navigation (McNaughton et al., 1996; Redish & Touretzky, 1997; Sharp, 1999b; McNaughton et al., 2006). This link between place cell location and head direction system seems to be of great relevance as demonstrated by different experiments. Calton et al., (2003) produced lesions in different brain areas implicated in the head direction system, anterior dorsal nucleus of the thalamus and postsubiculum. They found significant difference among animals with a lesion and the control animals

in spatial coding capabilities of their PC. Thus PCs were able to keep their place firing but the spatial signals were clearly diminished. This result indicates that head direction system is not the only source of directional information. Previous experiments demonstrated CA1 area also would be integrating place as well as head direction (Leutgeb, Ragozzino, & Mizumori, 2000).

1.5.1.3 Place Cell And Goal Navigation

An efficient navigational system must be capable of representing place significance. O'Keefe and Nadel (1978) suggested in their model that PCs don't code for goals or hedonic aspects of navigation. On the other hand some authors have suggested that place cells are related to the meaning of a place (Breese, Hampson, & Deadwyler, 1989). Speakman & O'Keefe (1990) found that goal location change did not alter the location of FFs in a radial arm maze. The fact that FF of place cells are stable while the rats forage for pellets of food thrown to random locations in the recording chamber suggests they are not coding for the goal aspect of navigation, (Muller, Kubie, & Ranck, 1987). O'Mara (1995) suggested that the prefrontal cortex could be the structure involved in goal-coding during navigation; later work has proved its role in goal navigation coding (Hok et al, 2005). Other structures such as the amygdala could also be involved in place preference learning due to its role in place significance learning (White & McDonald, 1993). Hollup, Molden, Donnett, Moser, & Moser (2001) trained animals to escape using a hidden platform in a ring shaped watermaze. They observed a strong concentration of PCs near the escape platform, suggesting that areas of space of behavioural significance could be over represented by the hippocampus. Martin & Ono (2000) found that subiculum and nucleus accumbens cells firing predicted reward administration and also coded for spatial location. In the same line of experiments Kobayashi, Tran, Nishijo, Ono, & Matsumoto (2003) demonstrated that PC firing changed due to task demand modifications. Unit remapping correlated with efficient

performance. Ekstrom et al. (2003) found cells in the human hippocampus which coded for location while navigating in a virtual reality (VR) city, but they also recorded cells in the parahippocampal region and also cells throughout the frontal and temporal lobes responded to the subject's navigational goals and to conjunctions of place, goal and view. Similarly Hok, Save, Lenck-Santini, & Poucet (2005) found cells in the prefrontal cortex of the rat which coded for goal location. Previous research had shown that prefrontal cells differentiated between high and low frequency rewarded arms in the radial arm maze (Pratt & Mizumori, 2001).

Hippocampal place cells representation is susceptible to change in relation to new task demands or reward location changes. Besides, hippocampal place cells could over represent important areas of space. It could well be that all of these changes could be due to the integration of place significance in other areas of the brain such as the nucleus accumbens, amygdala and prefrontal cortex, areas which are all strongly interconnected with the hippocampus (O'Mara et al., 2001; Witter et al., 1989)

1.5.1.4 What Does Affect PC Firing?

Environmental cues help animals and humans to make navigational decisions, to locate themselves and to calculate different trajectories to reach relevant goals (O'Mara, 1995). Muller & Kubie (1987) introduced different manipulations of the recording arena to study the different effects on PC. Recordings were carried out in a cylindrical arena with a cue card attached at the wall acted as a distal cue. Rotation of this visual cue produced a rotation of the FF keeping the same angular relation as in the original configuration. Removal of this cue card produced FF to rotate to unpredictable positions. Manipulations of the cue size did not affect FF. Placing a small barrier over the location of a previously recorded FF was enough to make the FF disappear. Doubling the size of the area and wall height produced some cells to expand their FF in relation to the new size although most cells generated new FFs, producing what has

been called remapping (Bostock, Muller, & Kubie, 1991), in the same way that if the arena shape was changed from a cylinder to a square, cells also remapped. Sharp (1997, 1999b) recorded PCs in the subiculum following similar manipulations. Subicular firing fields seem to display more similarities across the different manipulations of the environment. Sharp, Kubie, & Muller (1990) recorded place cells in a cylindrical arena where one white card acting as visual cue was on attached to the wall. A second card was introduced generating a symmetrical environment. If visual cues were driving PCs, then cells should generate symmetrical FF. Most of the cells did not show a symmetrical FF; most of them kept an asymmetric FF which rotated about 30° with the introduction of the second card. Only a few cells displayed a symmetrical FF in some of the sessions. Hetherington & Shapiro (1997) studied the effect of cue removal on hippocampal PC firing fields. They observed that removal of a cue proximal to the FF reduced the size of this, but removal of a distal cue would produce an enlargement of FF size. Jeffery & O'Keefe (1999) manipulated a visual cue either when the animal was present or before he was placed in the recording arena. PCs did not rotate their FF if the cue was moved in their presence but if the cue was rotated while away, then FF would also rotate. Rats learn to rely in egocentric information when the visual cue was not reliable. Cressant, Muller, & Poucet (1997) placed objects centrally in the recording chamber. They found that this configuration did not exert any control on the FF. On the contrary, if these objects were placed against the recording chamber, then they were exerted control on FF location. Shapiro, Tanila, & Eichenbaum (1997) rotated in different directions proximal and distal cues observing that some of the cells rotated with the distal cues and others with the proximal cues. Hill & Best (1981) recorded PCs in animals blindfolded and deafened. PCs of these animals were stable in spite of their sensory deprivation. . Quirk, Muller, & Kubie (1990) found that although the firing of PCs in a darkened chamber depended on if the animal had previously had available visual information,

their PCs had coherent firing fields in the darkness. Save, Cressant, Thinus-Blanc, & Poucet (1998) suggested that rats early blinded had reliable PCs of similar characteristics as normal animals. Using objects as cues located against the walls and rotating these made FFs rotate with them in similar way as PCs in control rats did. On the contrary, animals with striate visual cortex lesions had PCs with lower spatial coherence and where object rotation exerted a quite poor effect (Paz-Villagran, Lenck-Santini, Save, & Poucet, 2002). It is then clear that another mechanism other than allocentric information is being used by the animals. PCs would be using some sort of path integration to keep their firing stable (McNaughton et al., 1996). Rivard, Li, Lenck-Santini, Poucet, & Muller, (2004) found what they called barrier cells. They recorded PCs while animals forage for food in an open field where a high barrier was located. Barrier cells would fire around this and their FF would move with the barrier if this was moved. The barrier would exert similar control if the animal was located in a second new environment. Previous research found that the geometry of the arena exerts a quite strong control on PCs firing (O'Keefe & Burgess, 1996; Lever et al., 2002). Anderson & Jeffery (2003) hypothesized hippocampal place cells, not only process spatial information, but also contextual spatial information. Place cells would be then sensory modulated by geometric cues and also by other informational element. This could explain why subtle changes in the environment could generate extreme changes in the established firing field of place cells.

Visual cues are relevant for PCs when setting their FF within the recording arena. It is also clear that objects can exert similar effects on FF whenever these are located against the wall, acting in a similar way as distal visual cues. On the contrary, blind rats and rats in the dark can keep their FF. Place cells can be affected by proximal cues and geometry of the arena and also size. Similarly, the fact that hippocampal PCs display different firing location in different environments (Sharp, 1999a) could be seen as an evidence

that the hippocampus is coding spatial and non spatial aspects of the environmental context. On the contrary, the subiculum and entorhinal cortex PCs tend to represent different environments in similar ways, therefore appearing to act as a more generalised spatial representation. Different studies found that cells in the hippocampus code for non spatial information and this could be the key to explaining why PC firing shows some context specificity. Deadwyler & Hampson, (2004) found that hippocampal and subicular firing are involved in processing different aspects of the same task which were not exclusively spatial elements. Eichenbaum, Dudchenko, Wood, Shapiro, & Tanila (1999) suggested that the hippocampus codes spatial and non-spatial information automatically. Frank, Brown, & Wilson (2000) found that neurons in the entorhinal cortex fired in a specific location, but depending on where the animal had come from as well as where, implicating the entorhinal cortex in trajectory recognition through space. Furthermore, PCs in different areas of the parahippocampal region code spatial information in an apparently different way. This is coherent with the anatomical description of this region. It has been often argued that hippocampus would code more than spatial information while other arenas of the region would be less sensitive to these aspects. Therefore it is of great interest to elucidate how different areas of the parahippocampal region and the hippocampus code different aspects of space and context.

1.5.1.5 PC Firing And Navigational Decisions

If PCs firing represent some sort of spatial map (O'Keefe & Dostrovsky, 1971) then the firing of these cells must be related with navigational decision and performance of rats involved in a spatial task. Lenck-Santini, Save, & Poucet (2002) trained rats in three different navigational tasks while recording PCs in the dorsal hippocampus. Animals had to locate a goal in three different conditions. In the "far condition", animals had to navigate towards a goal located far form a visual cue, using an allocentric strategy. The

two other conditions did not imply this navigational strategy. Manipulations of the cues in the presence of the rat produced FF to stay at the original location while hidden manipulation generated FF to turn in coherent way to the cue manipulation. In all conditions, there was found a disruption of the relationship between FF and cue whenever the animal was present, but performance was most impaired in the far condition. Therefore, the animal always had to use a map strategy. Similar results were obtained using other experimental protocols (Lenck-Santini et al., 2001). In the same vein, experiments with humans suggest that activation of right hippocampus was related to accuracy while navigating in a virtual town (Maguire et al., 1998).

1.6 The Subiculum From Different Points Of View: A Model Review

Unit recording in the freely moving animal has produced a wide set of data which have facilitated the development of different theories and models of the hippocampal formation and its role in spatial navigation (O'Keefe & Dostrovsky, 1971; John O'Keefe & Nadel, 1978; McNaughton et al., 1996; Redish & Touretzky, 1997; Sharp, 1999b; Hartley, Burgess, Lever, Cacucci, & O'Keefe, 2000; Barry et al., 2006). Although in all these theories multiple roles have been assigned to the subiculum, they have been biased towards explaining the role of the hippocampus being developed under two major approaches: path integration (McNaughton et al., 1996, 2006; Redish & Touretzky, 1997; Sharp, 1999b) and allocentric navigation (John O'Keefe & Nadel, 1978 ; O'Keefe, 1999; Barry et al, 2006).

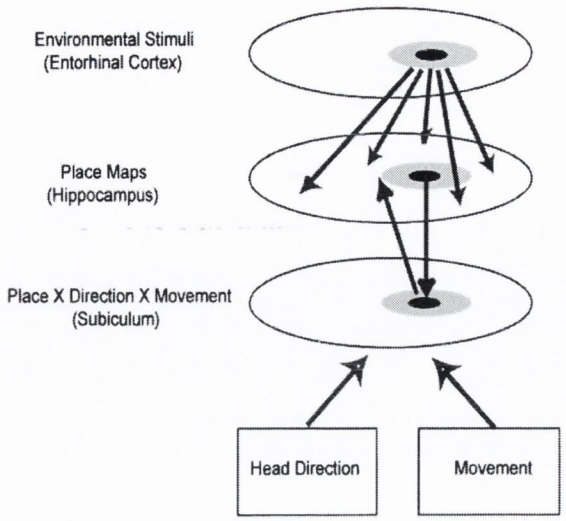
1.6.1 Path Integration Based Models

1.6.1.1 McNaughton's Model

McNaughton et al. (1996) proposed that the hippocampal place cells and head direction cells were part of a preconfigured network that generates an internal abstract representation with independence of the external allocentric cues. The model suggests

that external cues are necessary to initialise this idiothetic representation of space (Markus, Barnes, McNaughton, Gladden, & Skaggs, 1994; Quirk et al., 1990). Contrary to previous views of the hippocampus (O'Keefe & Nadel, 1978) a model was proposed, based in a partial preconfigured representation of space relaying on, at least partially, hard wire network anatomically located in CA3. PCs firing would represent distance and bearings, a notion shared by other models not based in path integration (Hartley et al., 2000; Barry et al., 2006). Repeated association between idiothetic representation and spatial landmarks would allow PC firing to correct the path integration system preventing PC firing to drift. (Knierim, Kudrimoti, & McNaughton 1995). Motor information is one of the cue informational sources of the system since elimination of motor input produced head direction cells and PCs to stop firing (Foster, Castro, & McNaughton, 1989; Basset et al, 2005). More recent work suggests that motor input, vestibular input and visual information contribute to the spatial signal of PCs in a specific manner (Terrazas et al., 2005).

Figure 6 : McNaughton Model 1996



McNaughton model as described by Sharp (1999b). The entorhinal cortex integrates sensory information from different brain areas interacting with the internal map in the hippocampus which receives directional and motor information from the subiculum. In this way, the subiculum is seen as the bridge between different subsystems.

McNaughton et al. (1996) suggested that units in the hippocampal CA3 area function as and autoassociative network, producing different states defined as the distribution of firing rates over the population. States would not depend on learning but on synaptic weights, preconfigured probably through development. Different subset of units activated simultaneously would produce different path integration references frames, where connections between neurons would represent the relation of neighbourhood, coding for space. The updating of the system would depend on the interaction of different subsystems. In this way, head direction cells would provide with directional information need by the system to continuously update. The model also requires a subsystem to integrate movement, place information and direction. The subiculum was proposed as the structure which could perform this role. This was supported by the fact that subicular units seem to code for the 3 elements (Sharp & Green, 1994). In the same

way direction by place cells have been found in other areas of the hippocampal formation (Cacucci et al., 2004).

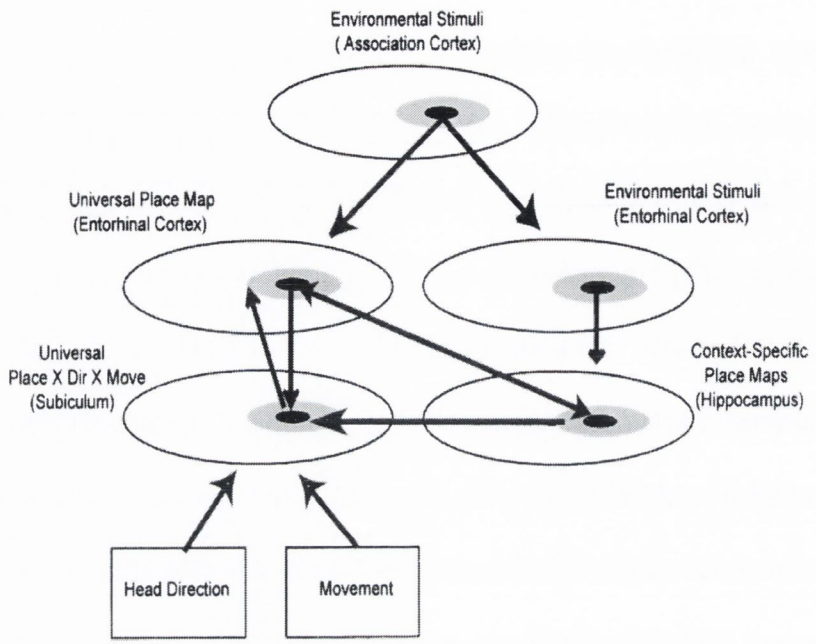
In the last formulation of the model, (McNaughton et al. 2006) assumed similar theoretical premises to the original model. The head direction is represented as a circular attractor network of units under global inhibition in which proximity of units would indicate strength of connections. Being a circular network, periodic boundaries are assumed and activity would rely on maximal excitatory input, movement detection and direction by vestibular or visual information. Similarly a two-dimensional attractor network is assumed for the locational system. This network would be connected to a secondary layer coding for the same location, specific direction and speed of movement. In this way, a back-projection to the location matrix would be offset in accordance with the preferred direction of this second layer. Grid cells, grid cells x direction, and head direction cells in EC seem to fit perfectly with the proposed model (Hafting et al., 2005; Sargolini et al., 2006).

1.6.1.2 Sharp's Model

Sharp (1999b) proposed a model based on that suggested by McNaughton et al., (1996). Similar theoretical assumptions are made. Specific locations are represented by a subset of units which would be distributed in a theoretical two dimensional matrix not corresponding to a real topographical representation. In this way, units located in different areas of the matrix could represent the same real locations. Remapping indicates that alternative subset of units would participate in the representation of different environments. The model accepts that the same unit could be involved in the mapping of multiple spatial contexts (McNaughton et al., 1996). The path integration models rest on the assumption that future position could be calculated from the present position. In this way and as explained above (McNaughton et al., 1996) the subiculum

could perform path integration. Sharp (1997, 1999a) proved that subicular PCs seem to generate similar firing field across different environments, a characteristic that was also present in entorhinal cortex (EC) PCs (Quirk et al., 1992). Information on position, direction and movement could facilitate a universal representation transferable to different environments. In a key difference to the model proposed by McNaughton, Sharp (1999b) suggested that EC would also be composed by a 2-D matrix network attractor capable of path integration through the reciprocal connection with the subiculum. EC would represent the environment in a unique way which would be also transferred to different environments, by sending sensory and spatial information from the universal map to the hippocampal area, where a subpopulation of units would be activated and associated with sensory information, allowing the system to align the internal and the external representation. Feedback from CA1 to the subiculum would explain subicular firing field rotations after visual cue manipulations, which in agreement with McNaughton et al. (1996) demonstrates that visual cues in familiar environments can override idiothetic information.

Figure 7: Sharp's Model 1999



In Sharp's proposal of hippocampal functioning, the entorhinal cortex and the subiculum are proposed as the anatomical bases of the universal spatial map. The subiculum integrates directional and motor input which is used by the system to implement path integration extrapolating similar spatial representation across different environments. Adapted from Sharp (1999b)

The recent discovery of EC grid cells, (Hafting et al., 2005; Sargolini et al., 2006) suggests, as proposed by Sharp (1999b), the existence of both a universal map in the EC and the existence of an internal representation of space mainly independent from the external world (McNaughton, 1996). On the other hand, the existence of head direction cells, grid x direction x cell and grid cells in the entorhinal cortex (Sargolini et al., 2006), suggests that the subiculum could share similar functions with other brain structures, at least partially.

1.6.1.3 Redish And Tourezky Model

Redish & Touretzky (1997) suggested that the subiculum in combination with parasubiculum and the superficial entorhinal cortex would be the basic structure in

charge of path integration. They suggested that a system performing path integration should respect the following criteria:

i) they had to be able to represent the position of the animal collectively; ii) receive input from the head direction system, iii) receive information about self-motion from motor and vestibular system, iv) update the representation as the animal moves around and v) send output to the area associated with the PC. As suggested by Sharp (199b), the subiculum needs to interact with other system to generate the universal map and path integration.

1.6.2 Allocentric Centred Models

Boundary Vector Cell (BVC) model proposes that firing of PCs in the hippocampus depends on inputs tuned to fire at certain distance to the boundaries of an environment and at certain allocentric angular position to the barrier. In this way firing of a unit would be tuned in relation to two Gaussian curves, one directional and one referred to distance, (Hartley et al., 2000; Barry et al., 2006). On the contrary to McNaughton et al. (2006) model which doesn't rely on the existence of barriers, the BVCs model predicts that insertion or elimination of barriers can generate expectable firing field changes. This is supported by experimental evidence demonstrating the relevance of boundaries and geometrical information of the environment to set PCs firing (O'Keefe & Burgess, 1996; Lever et al., 2002). It has been that removal of environmental barriers induces not only PCs remapping but also firing cessation, in the same way barrier insertion produces firing field duplication at the same distance and angular bearing both in the original and in the new barrier (Barry et al., 2006).

Barry et al. (2006) proposed that subicular units could be the hypothesized. If subicular firing is modulating CA1 place cells, a strong anatomical connection has to be described. So far, Seress et al. (2002) has described a weak back-projection from the

subiculum to CA1 and Commins et al. (2002) found electrophysiological evidence of a back-projection from subiculum to CA1. Thus, it is possible that subiculum-CA1 interaction is produced through an in-direct pathway involving the EC as it is supported by anatomical evidence (Kloosterman et al., 2003). Supporting this idea, it has been demonstrated that specific spatial task engages the subiculum and the entorhinal cortex but not the hippocampus (Jenkins, Amin, Harold, Pearce, & Aggleton, 2003).

1.6.2.1 Non-Spatial Approaches

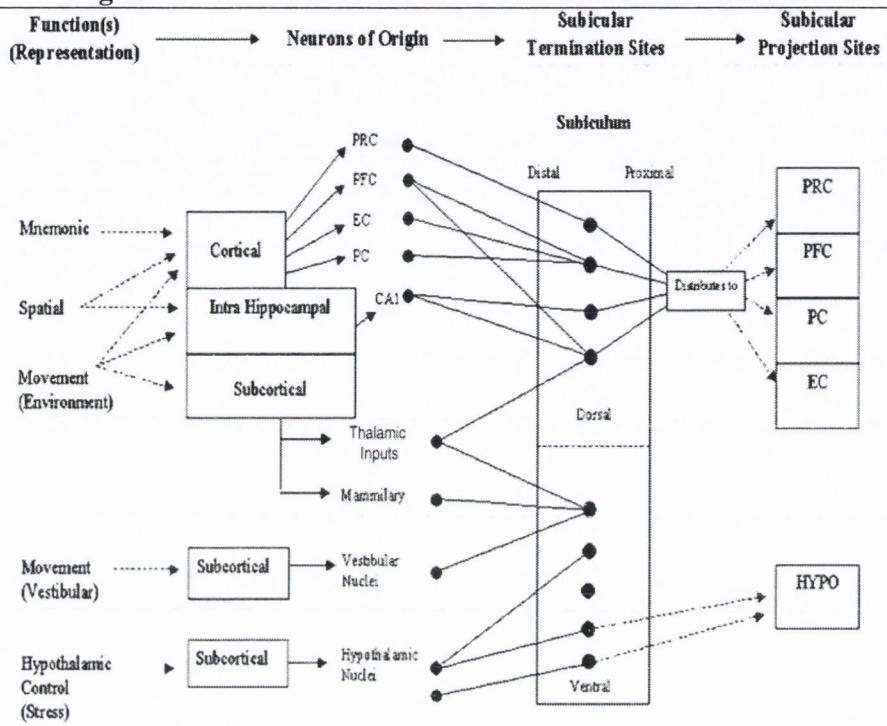
Other authors suggested that the hippocampus is not only involved in spatial information processing, but also in other aspects involved in the task performed by the rat (Eichenbaum et al., 1999). Deadwyler & Hampson (2003, 2004) found that units in the hippocampus not only fired in relation to spatial location but also in relation with the different demands of the task. They found that the hippocampus and the subiculum had complementary roles in the performance of a delayed-nonmatch-to-sample short-term task (Hampson et al., 2000). Similarly, other authors found that the hippocampal neurons are activated in relation in different task types (Eichenbaum et al., 1999).

1.6.3 Functional Segregation In The Subiculum

O'Mara (2005) proposed a model based on the anatomical description of the hippocampal formation and the inputs and outputs of the subiculum. The dorsal subiculum would be involved in spatial information processing while the ventral subiculum could be related to the control of the HPA axis. The model assumed similar to other authors, (Lowry, 2002), arguing that ventral subiculum exerts a dynamic and inhibitory influence on the HPA axis, and therefore substantially orchestrates the stress response. The model rests under the hypotheses that there is a dorso-ventral segregation of function within subiculum, and that the particular pattern of convergence of inputs to

subicular neurons determines the response properties of single subicular neurons. The limit of this model is that the neuroanatomy is yet underdetermined.

Figure 8 : O'Mara's Model Of The Subicular Function



A model of subicular functions. Here, synaptic transmission and anatomical connectivity runs from left to right (a deliberate simplification); information of differing types (mnemonic, etc.) derives from various cortical and subcortical circuits and is projected to the subiculum, converging in particular patterns and thereby giving rise to differing neuronal response types. For simplification, no details of distal-proximal distribution of fibers are provided (but these do vary). PRC _ perirhinal cortex; PFC _ prefrontal cortex; EC _entorhinal cortex; PC _ parietal cortex; Hypo _ hypothalamus Adapted from (S. O'Mara, 2005)

1.7 Aims Of This Thesis

The subiculum is still a poorly understood brain region. Its anatomical position and relationship to other areas put it in a strategic place within the hippocampal formation. Different models have tried to explain its role in spatial navigation and specifically in path integration.

The aim of this work is to understand how the subiculum integrates different environmental changes with the aim of studying PCs response in relation to the different models previously described. It is also aimed to understand the electrophysiological characteristics of SPCs:

1. Following previous work which has used electrophysiological techniques in freely-behaving animals (Sharp & Green, 1994; Anderson & O'Mara, 2003) this work aims to clarify the electrophysiological characteristics of subicular neurons.

2. Redish & Touretzky (1997) model suggests that the subiculum would be part of the path integrator system and therefore stable PC firing should be sustained during darkness.

3. Previous work demonstrated the capability of subicular units to expand their firing fields across different size arenas under very restricted size changes (Sharp, 1999a) while others suggest that BVCs could be found in the subiculum (Barry et al., 2006). To study the relevance of arena barriers in subicular place cells different protocols were implemented.

4. As subicular units have demonstrated similar characteristics to EC units and due to the strong input from the entorhinal cortex (Witter, Naber et al., 2000; Naber et al.,

2001a), it was hypothesized that grid-like characteristics or other properties could be detectable in larger arenas.

Chapter 2 : Methods

Contents

1. Summary

2. Methods Description

    a. Subjects

    b. Microdrives

    c. Electrodes

    d. Surgical Electrode Implantation Techniques

    e. Recording Techniques

    f. Data Analysis

2.1 Summary

Male adult lister hooded rats were implanted with two or four tetrodes in the dorsal subiculum (see each chapter for specific stereotaxic coordinates and hemisphere). Each tetrode was made of one bundle of four microwires. The electrodes were mounted on a microdrive which allowed for dorsal to ventral movement of the tetrodes to search for single-unit activity. Tetrode technology was chosen with the objective of improving unit separation. Before each experimental session, tetrodes were screened for neuronal activity (single-unit activity and electroencephalography/EEG) and if spikes were detected, then the experimental protocol was implemented. The behavioural activity and the different environmental manipulations were designed to study the role of the subiculum. Unit activity was related to the observed behaviour to determine if neurons activity was related with the codification of spatial information. At the end of each experimental session, the acquired data was analysed and interpreted (see below) in the light of the environmental manipulations to better understand how subiculum codes for the different environmental manipulations. After the full protocol was implemented, electrodes were lowered between 25 μm and 50 μm in search of more neurons. The objective was to sample the activity of as many neurons as possible. Finally the animals were sacrificed via an overdose of general anaesthetic, the brains sectioned and electrodes tracks analysed to verify the actual location of tetrodes.

Note: This chapter provides general information about the main experimental and electrophysiological methods. For more information the methods section of each chapter should be consulted.

Different custom written scripts (matlab) were created during the development of this work for correct data processing and analysis.

2.2 Methods Description

2.2.1 Subjects

A total of nine lister hooded males weighting between 300 g on arrival were used. Before surgery, rats were housed in groups of 5 animals in standard cages of 50 cm x 30 cm and 35 cm high and kept on a 12:12 Light-Dark cycle; food and water ab libitum and temperature was controlled. After electrode implantation rats were housed individually and food consumption controlled.

2.2.2 Microdrives

The microdrives were supplied by Axona (Axona, St. Albans, UK). Microdrives consisted of a vertical post located in parallel to a screw. A small lever was attached so turns of the screw were possible. Each complete turn was equivalent to a vertical displacement of about 200 μm, but it was possible to move the electrodes in steps as accurate as 50μm. The tetrodes were threaded through a 25 gauge cannula and protected with a 21 gauge cover and connected by a set of wires to gold pin connectors. Tetrodes and guide cannula composed a unit which was fixed to a small nut located in the screw, which allowed for electrodes to be lowered with each anticlockwise movement of the screw. The whole set of microdrive, cannula, cover and tetrodes were as light as < 2g.

2.2.3 Electrodes

The recording electrodes consisted in two or four bundles of four platinum-iridium wires (90% platinum/10% iridium; HM-L insulated; 25 μm bare wire diameter; California Fine Wire Ltd, California, USA) twisted together. The bundles were cut in a small angle of about 10° so the tips would be separated by a small distance. Tetrodes were thread through a guide cannula, 25 gauge, and protected with 21 gauge cover. Although in previous research, it was found that tetrodes techniques did not improve single-unit separation in the subiculum (Sharp, 1999a), preliminary testing carried our in our laboratory, proved otherwise. Recordings were done in reference mode so each

electrode was referenced to the most silent electrode of the preparation, in this way background noise commonly present in all channels was eliminated. One of the tetrodes channel was used to record electroencephalography. In this case the electrode was not referenced to any other channel of the preparation. Tetrodes techniques appeared to improve unit separation.

2.2.4 Behavioural Training

Animals were trained in the chasing pellet task. Rats were food-deprived up to 80% of their original weight. After this, behavioural training was initiated. Rats were placed in the open field and grains of sweetened rice were thrown in every 20 sec to random locations within the open field, in this way animals were in continuous locomotion, thereby allowing for complete sampling of the environment.

2.2.5 Surgical Electrode Implantation Techniques

Anaesthesia was induced using intraperitoneal injections of ketamine (Imalgene100mg/kg), xylazine (10mg/kg Rompun, Bayer), and atropine (0.05mg/kg Bimeda, Dublin, Ireland). Gas anaesthesia, (Isoflourane 2%) was used to keep anaesthesia during surgery. A protocol of complementary drugs was used during surgery and the recovery period. Enrofloxacin (10mg/kg Baytril, Bayer), Buprenorphine (0.05mg/kg, Buprex), Metilprednisolone (10mg/kg, Urbason), antibiotic powder (Cicatrin, Wellcome Ireland Ltd., Dublin, Ireland) were administered to reduce infection, inflammation and to induce analgesia. Animals were place in a stereotaxic apparatus, (Kopf Instruments, Tujunga, CA, USA), and the skull was exposed. A trephine was used to make a small craniotomy 1.8 mm diameter over the desired location, at co-ordinate 6.8 A-P and 4 M-L. Seven jeweller screws were fixed to the skull, one of which acted as a grounding point. Once the screws were fixed, the tetrodes were aimed at the dorsal subiculum.

The dorsal subiculum was chosen for different reasons: 1. Most of the previous research done in the hippocampus proper has been done in the dorsal hippocampal area which is known to be strongly connected with the dorsal subiculum. This could help us to understand better our results and the relationship of these two areas. 2. It is also known, that dorsal hippocampus is spatially more relevant than the ventral area of this structure (Jung, Wiener, & McNaughton, 1994; O'Mara, 2005). 3. The coordinates were chosen with the objective of facilitating the correct placement of electrodes in the subiculum. After electrode insertion in the brain, an electrode cover was lowered so the length of the exposed electrodes was protected. The craniotomy was then covered with Vaseline to protect the exposed brain surface and the microdrive base cemented with dental acrylic (Associated Dental, Swindon, UK). After surgery, the exposed tissue area was dusted with antibiotic powder (Cicatrin, Wellcome Ireland Ltd., Dublin, Ireland) and the rat was left to recover under observation. For the next five days the animal was maintained under an antibiotic and analgesic protocol to ensure full recovery and to facilitate animal wellbeing. Experiments started after a minimum of seven days of recovery.

Table 1: Anaesthetics

Anaesthesia	**Dose**	**Via**
Isuoflorane	2%	gas
Xilazyne (Rompun)	10 mg/kg	IP
Ketamine (imalgene1000)	100 mg/kg	IP
Atropine	0.05 mg/kg	Subcutaneous

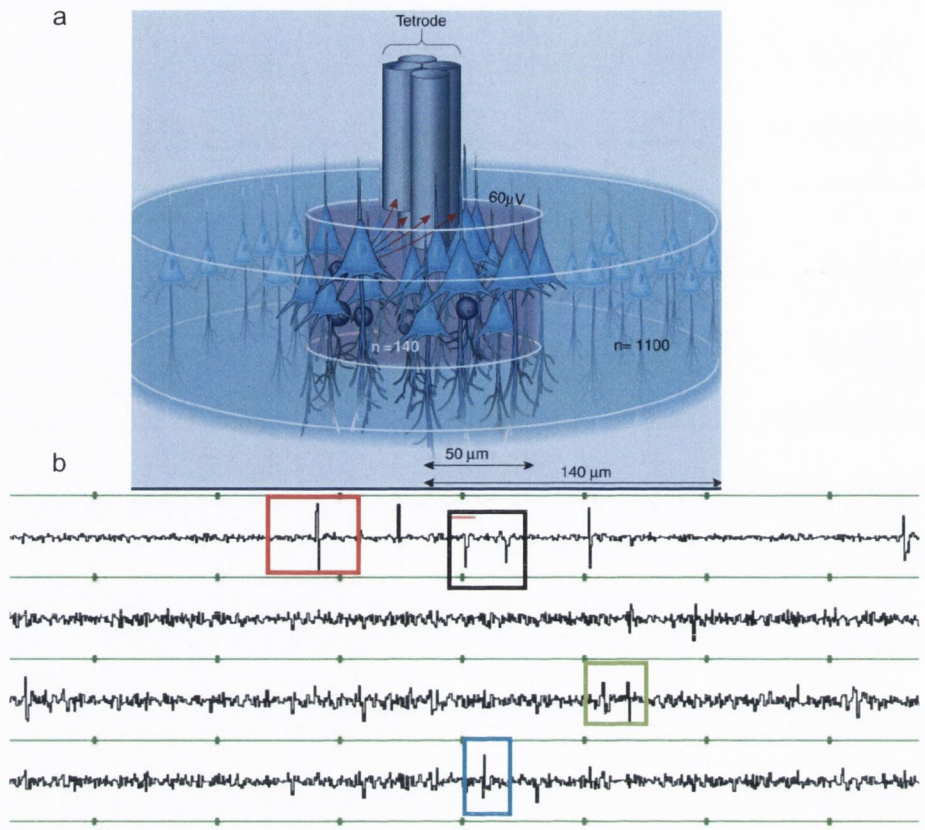
Table 2: Analgesia And Antibiotics

Drug	**Dose**	**Protocol**	**Via**
Metilprednisolone (Urbason)	10 mg/kg	Surgery	IP
Buprenorphine (Buprex)	0.05 mg/kg	Surgery Post surgery: 12h	Subcutaneous
Enrofloxacin (Baytril)	10 mg/kg	Surgery Post surgery: 12h	Subcutaneous

2.2.6 Recording Techniques

A sixteen channel headstage (Axona, St. Albans, UK) was connected to each microdrive. The headstage was a miniature, unity-gain buffer with AC-coupled inputs. A Field effect transistor, (FET), was connected to the microdrive and by means of flexible low-noise wire the signal was feed to a preamplifier (Axona, St. Albans, UK). Then the signal was sent to Axona acquisition system, (Axona, St. Albans, UK), which used differential amplifiers. Signal was amplified between 10000 to 40000 and band-pass filtered between 380 Hz and 6 KHz for single-unit detection. Spikes were recorded whenever the signal exceeds a predefined user threshold. Waveforms were sampled at a frequency of 50 KHz. Each waveform stored had duration of 1 msec (200 μsec before threshold and 800 μsec after threshold detection). Electrodes were referenced to other channels with similar background noise and no activity. EEG was continuously stored at a frequency of 250 Hz and bandpass between 0.34-125 Hz.

Figure 1: Acquisition System Oscilloscope



Tetrode techniques allow for improved unit separation by simultaneous activity comparisons across multiple channels. This technique is based on the different distance from each detected unit to each electrode in the tetrode which generates different amplitudes across all multiple channels, see a above (adapted from Buzsaki, 2004). This can be observed on the oscilloscope, see b, (recording obtained in this thesis). In channel one two different units are detected, highlighted in the red and black square. While in channel 3 and four two more units were also present, green and blue squares respectively. In this case at least 4 different neurons were recorded at the same time as confirmed by off line unit separation.

The headstage was provided with a small infrared light- emitting diode, (LED). In this way animal movements could be recorded by the tracking system consisting in an overhead camera which detected the infrared light emitted by the LED. The accuracy of sampled tracking was measured and improved until the tracking was as accurate as possible. If positions were lost during recording the tracking system interpolate the

animal's position by calculating movement using the last tracked position and the next sampled position. Direction could be also calculated by the motion of the animal. Position was sampled at 50 Hz so the animal movement track was accurately recorded and digitally stored. The tracking system divided the arena in a matrix of 64 bins were pixels position were allocated. The camera was calibrated so 332 pixels would correspond to 100 cm. The X and Y coordinated was time stamped and recorded while the animal was foraging for food in the arena this information would be used later to construct firing fields maps.

The Axona acquisition system made analogue-to-digital (AD) conversion of single-unit activity, electroencephalography and position of the experimental animal.

2.2.7 Data Analysis

Once data was acquired, off-line analysis was carried out. The Axona system produced a set of different files containing the pertinent information. For each recording session one file was generated for each active tetrode. These files contained the spike waveform and the time stamps while a file was also generated for the recorded EEG. The position was also stored containing information about the position of the animal during the recording session in the X and Y coordinates. General information about the recording settings were always recorded and stored.

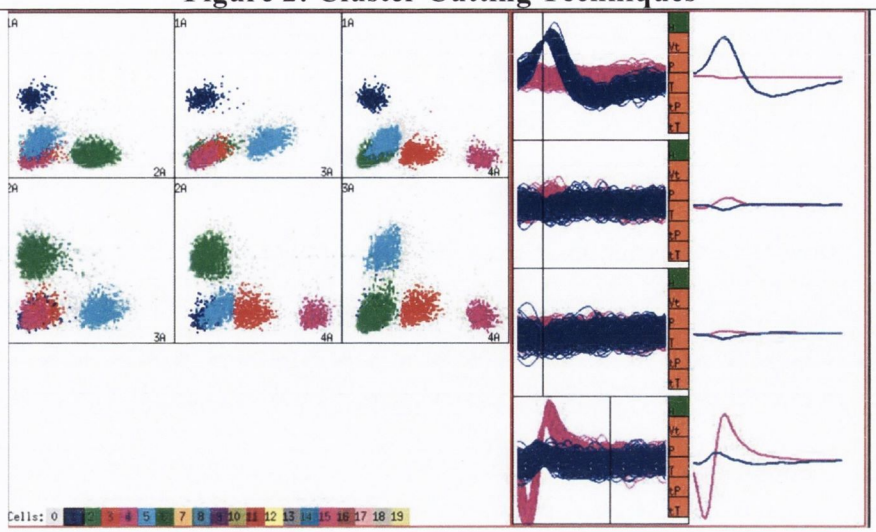
Data analysis proceeded in the following order:

1. Unit Separation.

Single-unit activity was isolated using cluster cutting techniques developed for data acquired with tetrodes. Although previous studies described difficulties using tetrode technology in subicular recordings (Anderson & O'Mara, 2003; Sharp, 1994) this technique was chosen due to its stronger potential to separate cells in other brain areas (Gray, Maldonado, Wilson, & McNaughton, 1995). Cluster cutting techniques were implemented using specific software: TINT (Axona, St. Albans, UK) allowed multiple

parameters comparisons across different electrodes. Amplitude of spike in electrode one was compared to amplitude of spike in electrode two, three and four. Also the rest of the electrodes were compared to each other using a maximum of 6 parameters simultaneously. Unit spikes tended to cluster around a central point when data was plotted using parameters described above, thus facilitating the use of a graphic interface. Units were isolated by assigning spikes to different clusters. Peak-trough distance, voltage at time t, peak height, trough, time of peak and time of trough in each electrode were used to plot spikes, see figure 2 below.

Figure 2: Cluster Cutting Techniques



Unit firing tend to cluster around a hypothetical centre generating as many clusters as units were present in the recordings. Units were separated using a graphic interface which allowed for multiple comparisons across different electrodes. The six graphs on the left depict amplitude values for each of the electrodes. Different units are assigned to different clusters. For example unit 1, blue spike on the right, corresponded to the blue cluster. In the same way, waveform unit four in pink, on the right, represented the pink cluster. It can be seen how some units can be better discriminated in some of the graphs, unit A, in blue is better discriminated in graphs in the first row, where spike amplitude in electrode 1 is used. On the right it can be seen how this unit had higher amplitude in electrode 1. Similarly Unit B, in pink had higher amplitude in electrode 4 and it is clearly discriminated from the rest of units when electrode 4 is use to plot amplitude against other electrodes.

Recorded data had to meet several electrophysiological criteria for inclusion in the analysis:

i. After preliminary analysis, units with dissimilar waveforms were eliminated unless these dissimilar waveforms could be satisfactorily reassigned to other units. If not, they were regarded as noise and discarded from analysis.

ii. The average spike had to be clean of noise which: random electrical interference included those produced by electrical apparatus nearby the preparation, mobile

phones, chewing artefacts or other movement artefacts which could interfere with the signal

iii. Autocorrelation histograms (ACHs) and the inter spike interval histograms (ISIH) were plotted continuously during the unit isolation process. The objective was to obtain neurons with a clear refractory period of 1 msec minimum (Taube, 1993). If a neuron had spikes in the first msec bin after cluster separation, it was not accepted for further analysis; ACHs were also used to determine if the unit was contaminated with other types of noise.

1. Inter-spike interval histograms (ISIH): shows the timing relationships between spikes fired by the same unit. They were constructed by summing the time intervals between consecutive spikes. The x-axis represents the length of the time interval and the y-axis the number of intervals.

2. The position of the maximum value of the ISIH (POS) was obtained to determine bins with maximum firing intervals. This was used as an indicator to localise peaks of activity.

3. Propensity to burst defined as the percentage of intervals that occurred in the burst ISIH range (1–9 ms) was calculated to study bursting properties of units.

4. Autocorrelograms were constructed by summing the number of spikes occurred within 0-1000 msec period given the occurrence of a spike at time 0 msec. Both histograms indicate rhythmicity in the firing of a unit (Sharp & Green, 1994)

iv. Spike amplitude and waveform was compared across electrodes to verify that each unit was different in at least one of the electrodes. This is one of the advantages of tetrode techniques, since spikes are compared across different electrodes, unit isolation accuracy increases. First, because unit activity would be in general of different amplitude on all four tetrodes, and second, because spikes of equal amplitude are very unusual, and therefore spikes represented with equal amplitude

in all four electrodes are normally noise resulted from movement, chewing or other artefacts.

v. Spike width and amplitude was obtained using Frate, (Axona, St. Albans, UK).

2. Spatial analysis. Once each unit was clearly separated firing rate maps were constructed to detect spatial firing:

i. Firing Rate Maps.

Firing rate maps allow for visual inspection of neurons preferred areas of firing. They were constructed by normalising the number of spikes which happened in specific pixels coordinates by the total trial time the animal spent in that coordinate. This produces maps which depict the preferred areas of firing of each cell in Hertz. The pixel map was converted in to a 64 x 64 matrix. Firing map were plotted as a contour map by TINT. The firing map reflected the frequency of firing calculated from the top firing bins down to the lowest firing area by scaling them in decreasing intervals of 20% of the peak firing. During the construction of firing frequency maps, TINT used a smoothing algorithm.

ii. Spatial Information Content.

Skaggs index (Markus et al., 1994). This index calculates the spatial information load per spike.

$$I=\Sigma_i (\lambda_i/\lambda)\ log2(\lambda_i/\lambda)P_i$$

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*.

iii. Spatial Coherence.

It consists of a spatial autocorrelation, in which a correlation is calculated between the rate for each bin, and the average rate of the eight surrounding bins. Thus, high positive values resulted if the rate for each pixel could be better predicted by knowing the firing frequency of the neighbour location (Quirk et al., 1990; Sharp & Green, 1994)

iv. In-Field Maximum Frequency.

It was compute as the maximum firing field of the firing map.

v. Mean Frequency.

The total number of spikes divided by the total recording, time was taking as the average session firing rate and was expressed in Hz.

vi. Mean Non-Zero Frequency.

Since it was expected to find spatial modulation in unit firing it could be expected that the mean firing frequency could be distorted by spatial specificity. Therefore, dwelling time in bins which no firing were discarded and frequency calculated again.

vii. Spatial Selectivity.

Spatial selectivity was calculated as the ratio between the maximum firing frequency, and the mean frequency of firing for each recording session. This provides information about the signal-to-noise ratio in subicular units.

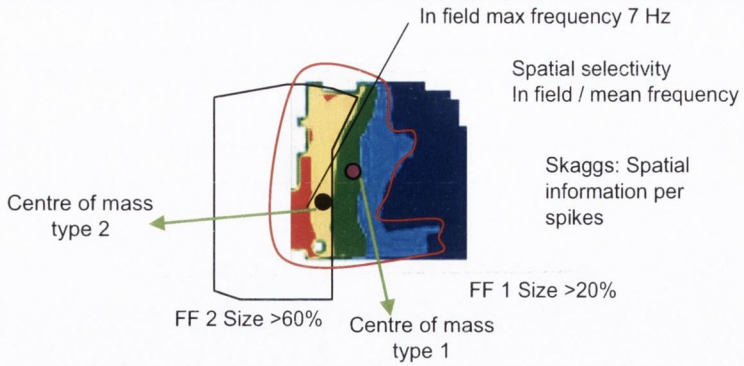
viii. Firing Field Size.

Different definitions of firing field are found in the literature (Sharp & Green, 1994; Hollup, Molden et al., 2001; Brun et al., 2002; Anderson & O'Mara, 2004). In this work firing field size was computed in two different ways:

Firing field size 1: Similar to other authors (Brun et al., 2002; Hollup, Molden et al., 2001) firing field size was compute as the percentage of the arena area in which the firing field was above 20% of the maximum firing frequency.

Firing field size 2: size was computed as the percentage of arena area in which firing frequency was above 60% of the maximum firing field. See figure 3 below for a graphic description.

Figure 3: Firing Field Size



Subicular units tend to display lower spatial specificity that hippocampal place cells. To define the size of each firing field, two different approaches were taken: First, firing field size was compute as the % of the arena in which the firing frequency was above 20% of the maximum firing frequency, area demarked by the red line above. The second definition excluded all areas of the firing map in which firing frequency was below 60% of the maximum frequency, area delimited b the back line. Centre of mass type one, purple spot, and type 2, black spot are also shown.

ix. Firing Rate Map Stability.

To evaluate the stability of the spatial firing of a neuron across different conditions different measurements were implemented.

1. Firing field maps were compared by eye across conditions.
2. Spatial correlation: bin-by-bin correlation was calculated between different firing maps, high and positive correlation values would indicate firing field similarity (Quirk et al., 1990; P. E. Sharp & Green, 1994; Anderson & O'Mara, 2004)
3. Distance between the centres of mass.

To determine if the distribution of two different firing frequency maps were similar the centre of mass for each distribution was calculated. Then the distance between both centres of mass was obtained.

$$x_{CM} = \frac{\sum_{i=1}^{N} m_i x_i}{\sum_{i=1}^{N} m_i}$$

$$y_{CM} = \frac{\sum_{i=1}^{N} m_i y_i}{\sum_{i=1}^{N} m_i}$$

Centre of mass of particles is computed as the ratio of the sums of each element mass, m_i by its position xi, y_i divided by the total mass Σm_i. Frequency maps distribution are bi-dimensional bodies therefore Centre of mass was calculated for the Coordinates and abscises axis. For the firing field distribution, each bin frequency was regarded as an element of the two dimensional object, its position would be defined by the X,Y coordinates and it mass by the frequency of firing of a unit in that bin.

City block distance between the two centres of mass was calculated. Basically, the absolute value of the sum between the difference of the x coordinates in map 1, x_1, minus x coordinate in map 2, x_2, and the difference between y value in map 1, y_1 , and y value in map 2, y_2.

$$DC=|x_1-x_2| + |y_1-y_2|$$

a. Distance To The Centre Of Mass Type 1: the centre of mass was calculated including all bins of the firing map. Then, the distance between firing maps generated in two different conditions by the same units was calculated.

Distance to the centre of mass type 2: the centre of mass was calculated only for bins which firing frequency was above 60% of the maximum firing rate. See figure 3 above.

4. Distance Between The Two Maximum Firing Frequency Bins.

The distance between maximum firing rate bins in each condition was calculated to determine firing field drift. X and Y coordinates for the maximum firing frequency bin

were calculated for each firing map and then city block distance between the two maximum firing bins was calculated.

$$DC=|x_1-x_2| + |y_1-y_2|$$

3. EEG Analysis.

Simultaneous EEG acquired was analysed using Fast Fourier Transformation (FFT) and peridograms were plotted to identify dominant frequencies in the EEG, (Neuroexplorer, Nex Technologies).

4. Histological Analysis.

Once no activity could be detected, animals were deeply anesthetised using sodium pentobarbital, and transcardially perfused with saline and paraformaldehyde (4%), the brain removed and store in paraformaldehyde. Brains were sliced using a vibrotome and Nissl stained. Electrode tracks were studied under the microscope to verify electrode position.

5. Statistical Analysis.

Appropriate descriptive and inferential statistic techniques were used. All statistical analysis was performed using SPSS.

Chapter 3 Electrophysiology Of The Subiculum

Chapter Content

1. Summary
2. Introduction
3. Methods
4. Results
5. Discussion

3.1 Summary

The subiculum has been regarded as a relay station between the hippocampus and cortical and subcortical structures (Amaral & Witter, 1989). Using similar methodology as in previous studies (Anderson & O'Mara, 2003, 2004; Sharp & Green, 1994), unit recordings were performed in freely moving animals. A classification is presented after unit's spike trains analysis. A total of 4 types, further divided in 5 groups, were obtained: bursting units, strong-theta-modulated bursting units, fast spiking, non-bursting strong-theta-modulated and regular spiking. Cell type followed a continuous of propensity-to-burst in this same order. In agreement with previous work (Staff et al., 2000) a high propensity of units to burst was found.

3.2 Introduction

The subiculum is located in a strategic position within the hippocampal formation, receiving input from the superficial layers of the entorhinal cortex, CA1 and back-projecting to these structures and to other cortical and subcortical structures (Amaral & Witter, 1989). Also it is involved in epilepsy (Dreier & Heinemann, 1990), and spatial information processing (Sharp & Green, 1994). It has been regarded as a gating structure which could help seizures to propagate via GABA mechanism (Benini & Avoli, 2005), but also as a relevant structure which could convey spatial information to different cortical and subcortical structures (Sharp & Green, 1994). The subiculum has been classically regarded as a bursting structure in which most cells tend fire in bursts, defined as 2-6 action potentials in a interval of 5msec (Stewart & Wong, 1993). This has been suggested as an efficient mechanism to increase information fidelity (Jung et al., 2001). Stewart & Wong (1993) using intracellular recordings in vitro found two main cell types in the subiculum: bursting units (79%) and non-bursting (21%). Bursting units would respond with a train of spikes after antidromic or orthodromic stimulation, and no bursting unit responded with single action potentials to stimulation. Bursting cells, shifted to a non-bursting state if the resting membrane potential was lowered, a result not found in any other in vitro study. Behr et al. (1996) using intracellular recordings found two main cell types in the subiculum: bursting units (54%) and regular spiking (46%) units. Bursting cells had a higher resting membrane potential and could accommodate their firing during sustained current injection. Similarly, Taube (1993) found bursting and regular spiking units. Staff et al. (2000) using patch clamp in vitro defined 3 different cell types: strong-bursting (51%), weak bursting units (17%) and regular spiking neurons (32%) which could be shifted to a bursting mode after 4-aminopyridine was applied to the bath. They proposed that all

subicular neurons could belong to the same group of cells varying in their propensity-to-burst.

Recent work challenged the classical view of the subiculum as a predominant bursting structure. Menendez de la Prida et al., (2003) recorded neurons from the ventral subiculum labelling them with neurobiotin for later morphological analysis. Results suggest a more complex classification than those obtained from previous experiments. Four main groups were obtained which could be further sub-divided: Bursting units (26%), which were divided in strong-bursting units, which were able of responding with more than one burst after orthodromic stimulation, and weak bursting units which only generated one burst after stimulation. Regular spiking (54%) units which were further divided in tonic and accommodating neurons. Fast-spiking neurons (11%), and membrane oscillation neurons (6%), whose intrinsic membrane potential was not stable. Fast-spiking units were then identified as local interneurons while all the rest were projecting cells. Harris et al., (2001) implemented recordings in the subiculum combined with anatomical techniques. They found that bursting units were more frequently located in the deep layers while fast-spiking seemed to be recorded from more superficial layers.

Although the subiculum could be composed by different cell types it seems that bursting units have a predominant role in its function and electrophysiology. Recordings in freely-behaving animals found that subicular neurons were spatially tuned (Sharp & Green, 1994). Unit's classification was performed finding bursting units (34%), non-bursting units (41%) depolarised bursting units (14%) and theta cells (11%). The authors suggested equivalence between the cells types they found and those described by Steward et al (1993). Sharp & Green (1994) did not found significant differences for spatial-related firing across different cell types, a result which can have different theoretical interpretations. Anderson & O'Mara (2003) recorded dorsal subicular units

obtained a slightly different classification. Bursting units (40%), regular spiking (19.6%), theta modulated (31.1%) and fast spikes (8.1%). As in Sharp work, bursting and fast-spiking units were theta modulated. Theta units were those which presented higher frequency of firing whenever theta was presented in the EEG.

Hippocampal EEG has been characterised by three different pattern of activity. Large irregular activity (LIA) defined as irregular large amplitude activity and small irregular activity (SIA) both appearing at low frequencies, 1-4 Hz. Slow regular activity (SRA). SRA, theta, is characterised as being regular activity with a sinusoidal waveform which ranges between 4Hz-12 Hz. LIA seems to be the result of sharp waves which produces an increase of excitability of the intra hippocampal circuit (Buzsaki, 1986). Typically, hippocampal EEG is dominated by theta rhythm, a sinusoidal waveform, which has been involved in different behaviours. Whishaw & Vanderwolf (1973) found a clear correlation between animal behaviour and theta amplitude and frequency. Bland & Oddie (2001) suggested the possible role of theta in sensory and motor integration.

In this, work data gather across all different protocols was analysis and units classified after spike train analysis. It was expected to find a larger percentage of bursting units.

3.3 Methods

For complete description of methods refer to chapter 2. Variations in methods used are described below.

Subjects

Data obtained from all nine animals involved in the experiments described in the following chapters were included for analysis.

Electrode Implantation

After training animals were anesthetised with ketamine (90 mg/kg) and xylazine (10 mg/kg). Anaesthesia was maintained using Isoflourane at 2%. Rats were implanted with two or 4 tetrodes consisting of four 25μm twisted coated platinum-iridium (California Fine Wire) wires mounted in a microdrive (Axona Ltd) aimed at the dorsal subiculum. The dura was removed and the electrodes lowered into the brain 1mm which allowed the electrodes subsequently to be lowered in steps of 25-50μm. Animals were left to recover for 7 days.

Electrophysiological Recording

Signals were fed through a unity gain preamplifier connected to the microdrive, feeding the signal to the acquisition system (Axona, Ltd). Single-unit signals were amplified between 10-40000 times and band-pass filtered between 380 Hz and 6 KHz. One channel was used for EEG recording. Signals were filtered between 0.34-125 Hz and sampled at 250 Hz. A notch filter set to 50 Hz was used when necessary to avoid noise interference in the signal. Recording sessions lasted from 8 min in the small arenas up to 30 min in the large arena.

Data Analysis And Unit Isolation

Data was analysed with cluster cutting technique (Tint, Axona Ltd). Units were classified by being assigned to different clusters. Then text files were exported and experiments were analysed using matlab custom written programs. Non-normalised

inter-spike interval histograms and autocorrelograms were constructed and used to classify cell as well as for isolating units. Units had to have no spikes in the first bin msec of the ISIH to be included in the data set. Maximum values for the ISIH were obtained and used as orientation criteria for cell classification. Inter spike interval histograms (ISIH) provides information of unit activity profile; similarly, autocorrelograms were used to determine the existence of time modulation activity in the spike train obtained after unit isolation.

Unit waveform parameters were obtained using Frate (Axona, Ltd, London). Waveform height and width was calculated while the mean frequency was obtained dividing the number of spikes by trial time. A restrictive criterion was used to select units for further analysis. Units had to have mean frequencies above 1 Hz per session.

Spike Train Analysis

Inter-Spike Interval Histograms showing the time relationships between spikes fired by the same unit were constructed by summing the time intervals between consecutive spikes. The x-axis represents the length of the time interval and the y-axis the number of intervals. The refractory period of 1 msec minimum was always respected (Taube, 1993). The position of the maximum value of the ISIH (POS) was obtained to determine bins with maximum firing intervals. Although not a very robust parameter it was used as an indicator to localise peaks of activity.

Autocorrelograms were constructed by summing the number of spikes occurred within 0-1000 msec period given the occurrence of a spike at time 0 msec. Both histograms indicate rhythmicity in the firing of a unit (Sharp & Green, 1994).

Propensity to Burst defined as the percentage of intervals that occurred in the burst ISIH range (1–9ms) was calculated to study bursting properties of units.

EEG

To evaluate EEG fast Fourier transformations (FFT) were used, (Neuroexplorer). FFT allows the determination of dominant frequencies in the EEG. Peridograms were plotted to visualise dominant frequencies in the EEG.

Histological Analysis

Once no more units could be isolated animals were anesthetised and transcardially perfused with saline and paraformaldehyde at 4%. Brains were extracted and store for slicing in a cryostat and stained for electrode location.

Apparatus

The recording apparatus consisted of an open field of 50 cm x 50 cm and 50 cm height, painted black and with a grey washable floor and a similar apparatus of 100 cm x 100 cm only for units recorded in the last experiment. The apparatus was cleaned between trials and between light-dark transitions to eliminate possible cues generated by the animal. Differences in arena size should not impact on single-unit activity since behaviour and intensity of behaviours which could be affected by exploration were not observed to be different between the differing arenas.

3.4 Results

A total of 225 cells were recorded in 9 different animals across all experiments. After experiments were finished, the animals were sacrificed and their brains processed for histological analysis. Electrode positions were reconstructed by visual inspection and located in the dorsal subiculum. Only cells which did not present waveform anomalies and which respected refractory periods in their inter-spike histogram were included in analysis. If the ISIH presented anomalies, then cells were discarded. Units which presented mean frequencies above 1 Hz were included and the rest discarded from electrophysiological analysis as it was thought necessary to have enough spike sampling for being able of defining cell activity and general characteristics. 181 cells were included for electrophysiological analysis and unit classification of which 164 were classifiable in relation to defined criteria. Anatomical reconstruction of electrode traces can be found in the result section of each chapter.

Unit Classification

Unit exploration suggested the existence of different cell types as expected. Initial data examination suggested a clear unit differentiation based in the spike width. After inter-spike interval histograms and autocorrelation histograms were constructed, cells were classified in four different types: bursting units, which could be further divided in bursting and strong theta modulated bursting units, fast-spiking units, non-bursting theta-modulated units and regular spiking units. Cells were classified using the following criteria.

Bursting Units: they had clear peaks in the short time intervals of the ISIH. These units normally had a maximum ISIH value below 15 ms bins and not later than 30 ms bins. Also, they had clear theta modulation in the autocorrelogram histogram. As mentioned above, this category was further subdivided in two types of units. The second group, Strong-Theta-Modulated Bursting units was similar to the first group described above.

They had clear peaks in the short range of ISIH but also a second dominant peak in the range of theta rhythm with peaks below 30 msec bins and 130 msec bins. See figure 1.

Fast-Spiking Units: for a unit to be assigned in this category it had to present a spike width below 0.300 msec. It was observed that this group of units had clear peaks in the ISIH range, revealing a clear tendency to fire in burst, finding that the maximum value of the ISIH below 30 msec bins. Also strong theta modulation was observed in the autocorrelogram histogram, see figure 2. unit a.

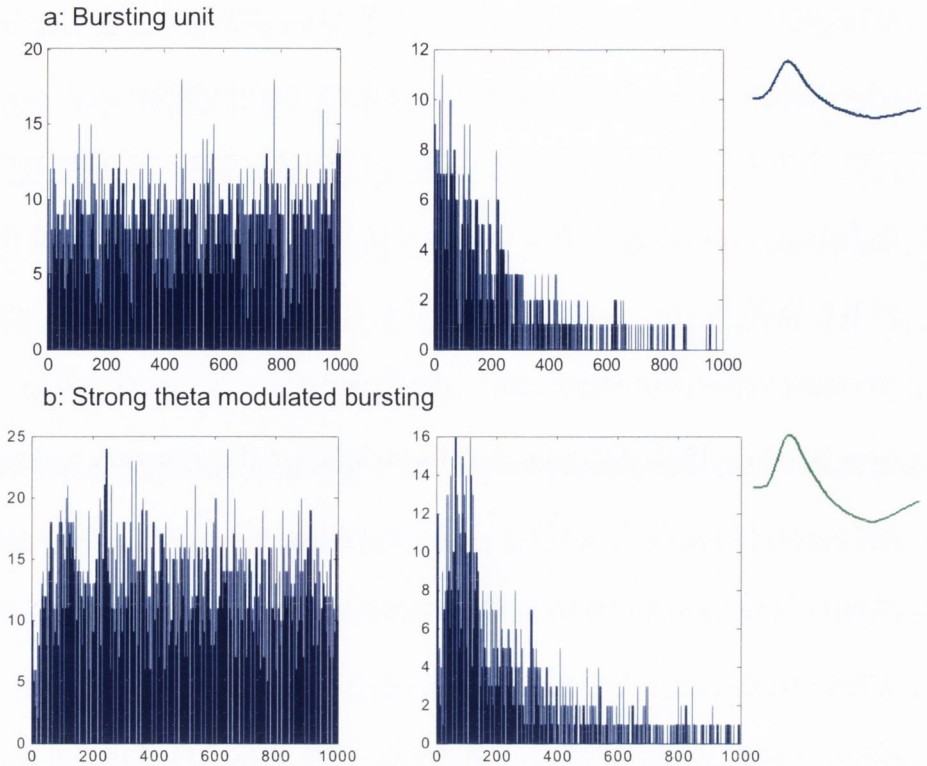
Non-Bursting Theta Modulated: cells included in this category had no clear peaks in the sort range of the ISIH finding their maximum values after the 30 msec bins. Also this group of cells presented theta modulation in their ISIH represented by peaks of activity in the range of theta modulation and a distribution sensibly biased to the right. This was also observed in the autocorrelograms histogram were modulation was clearly observed, see figure 2, unit b.

Regular Spiking Units: this type of unit presented ISIHs without dominants peaks. Similarly autocorrelograms were not theta modulated, see unit c in figure 2.

Table 1: Descriptive For Unit Type (Mean And S.E)

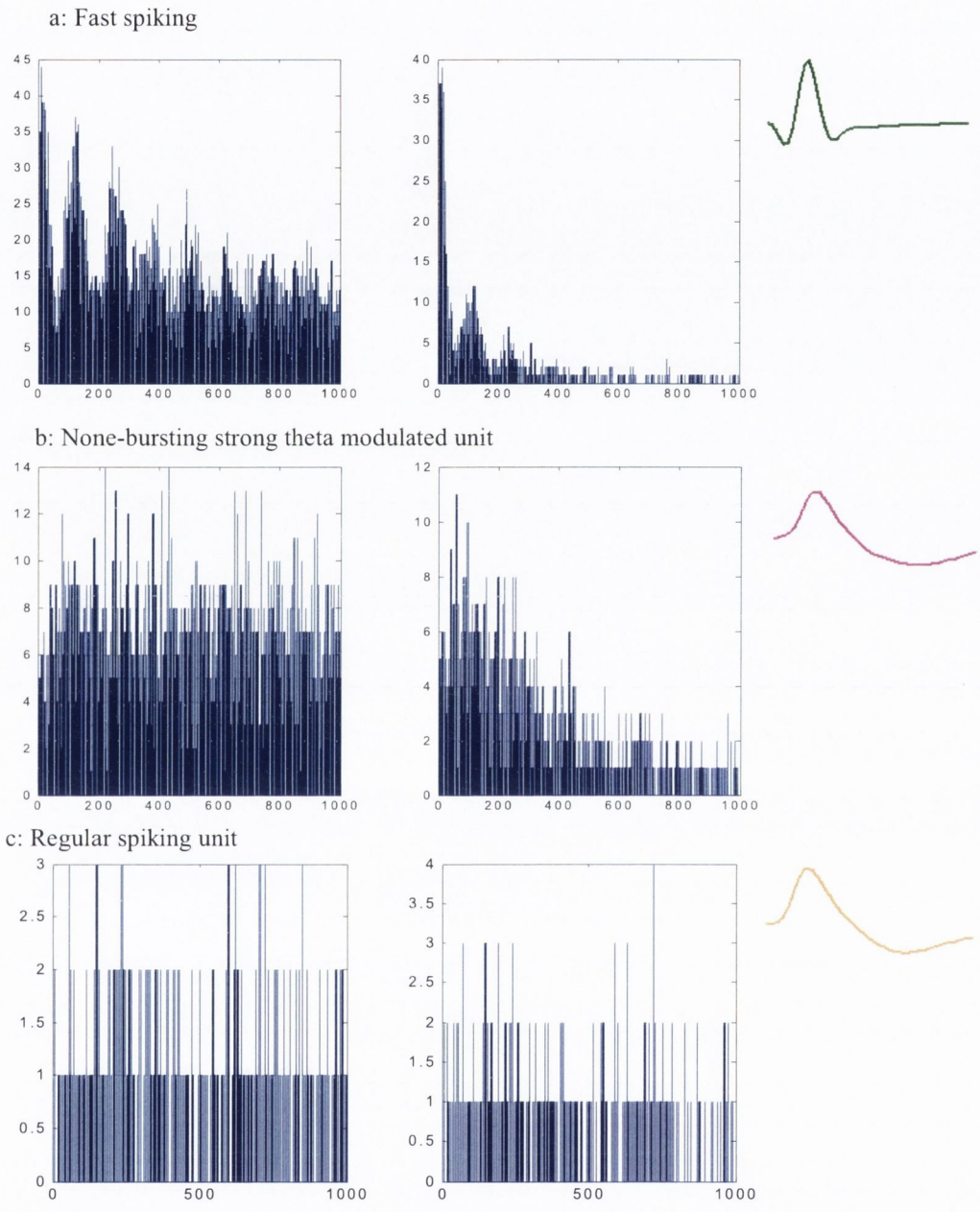
Unit Class	N	Height	Width	Frequency	POS value (Max value position in the ISIH)
Bursting units	57	205±8.29	470±9.28	3.75±0.32	14.07±2.94
Bursting strong theta modulated	16	253.08±17.76	490.63±8.86	4.45±0.72	37.7±7.21
Fast spiking	24	195±13.91	192.83±11.50	7.0±0.14	11.9±1.46
Non-bursting theta-modulated units	45	178.6±8.11	462.42±11.04	3.84±0.33	80.6±4.43
Regular spiking	22	176.6±10.00	486.10±18.28	2.21± 0.50	72.6±17.71

Figure 1: Autocorrelograms, ISIH And Spike Waveforms For Bursting Units



From right to left: autocorrelogram, inter-spike interval histograms and spike waveform. Unit a. and b. represents the typical bursting type, a. Bursting unit, b. Strong theta modulated bursting. Peaks in the autocorrelograms and in the ISIH in about 120 msec bins, indicate theta modulation. Peaks detected in the initial bins of the ISIH, proved tendency to burst. The second stronger peak in theta is clearly observable in unit b autocorrelogram.

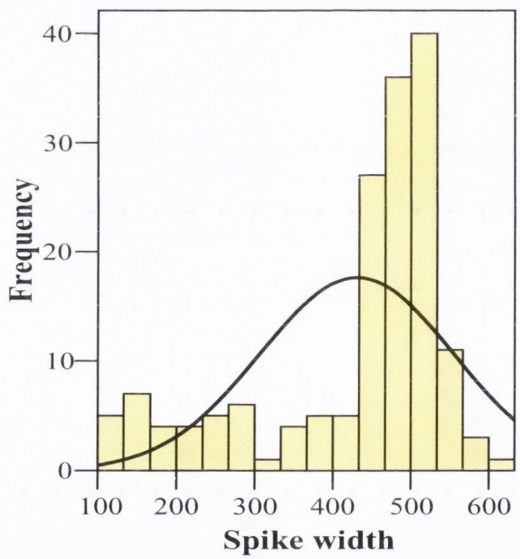
Figure 2: Autocorrelograms, ISIH And Spike Waveforms For Fast Spiking, None Bursting Theta Modulated And Regular Unit



From right to left: autocorrelogram, ISIH and waveform. Unit a: fast spiking unit with very strong theta modulation. Oscillations in the theta range are clearly observed in the autocorrelogram and in the ISIH. This unit was also characterised by having a narrow spike waveform. Unit b: represents a non bursting theta modulated cell. No peak in the short range of the ISIH was detected but peaks in the theta range were observed in the autocorrelogram and in the IS. Unit c: Regular spiking unit characterised in their autocorrelogram and ISIH by not having any clear peak or modulation.

Cell types were compared across width, height, frequency and maximum value position in the ISIH. Position of the maximum value of the ISIH (POS), was used as an indicative of cell tendency to burst. As explained above cells which tent to burst have clear peaks in the sort ISIH range, therefore maximum values are normally located in the first bins of the histogram. Spike width was not normally distributed, height, frequency and POS were logarithmically transformed to obtain normality and then ANOVA analysis implemented and complemented with Tukey's post-hoc test. Width, not being normally distributed was compared across cell types using non-parametric tests and Bonferroni corrections.

Figure 3: Spike Width Distribution

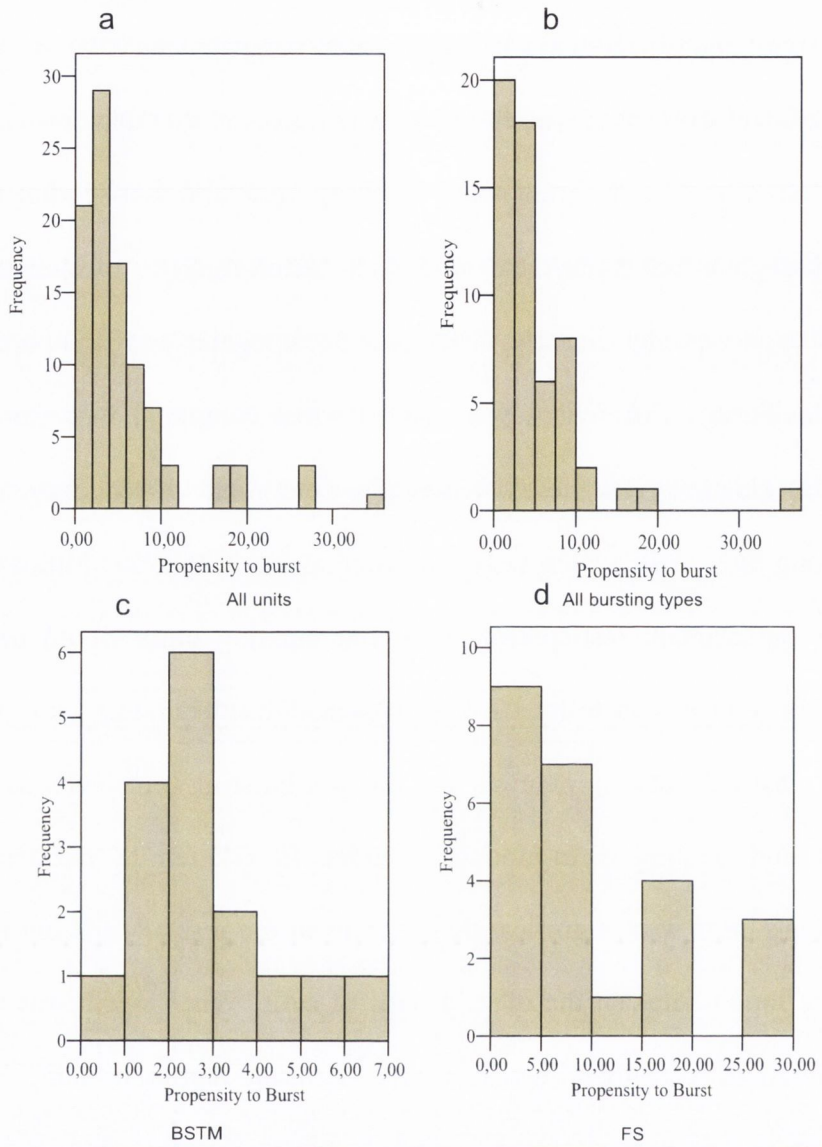


The normal curve is plotted against the sample spike width distribution. It can be observed how values are not normally distributed and how these tended to cluster around 500 μsec spike width.

Significant differences were found for height, $f_{(4,159)}=4.226$; $p<0.05$, width (Kruskal-Wallis, $p<0.05$) mean frequency, $f_{(4,159)}=59.037$; $p<0.05$, and position of the maximum value, (POS) in the ISIH $f_{(4,159)}=6.314$; $p<0.05$, across different cell types. Bursting strong-theta-modulated (BSTM) were significantly different in height when compared

with bursting units (B), non-bursting theta-modulated (NBTM) and regular units (R) but no significant difference was found when compared with fast-spiking (FS). No further significant difference was found between groups. Position for maximum values, POS, was significantly different when bursting units and fast-spiking units were compared with regular and none theta modulated. Interestingly strong-theta-modulated bursting cell type was significantly difference from regular and non-bursting theta-modulated units. Further differences were found when compared with fast-spiking and busting units. This suggests the existence of a continuum of tendency to burst of units even among those cells which were classified as bursting units. In this respect, a continuous was determined: fast-spiking units and bursting units would be the cell types with higher tendency to burst, Strong-theta-modulated bursting units would be in between the classical bursting neurons and the non-bursting units such as regular spiking units and non-bursting theta-modulated units. In relation to frequency of firing regular spiking units were significantly different to the rest of cells but no further differences were found between the other groups of cells. When width was compared, significant differences were found across groups (Kruskal-Wallis, $p<0.05$). Single test comparing group-by-group was applied using Bonferroni corrections. Significant values were found between fast-spiking and all the different groups but not between any of the other groups (Mann-Whitney, $p<0.01$).

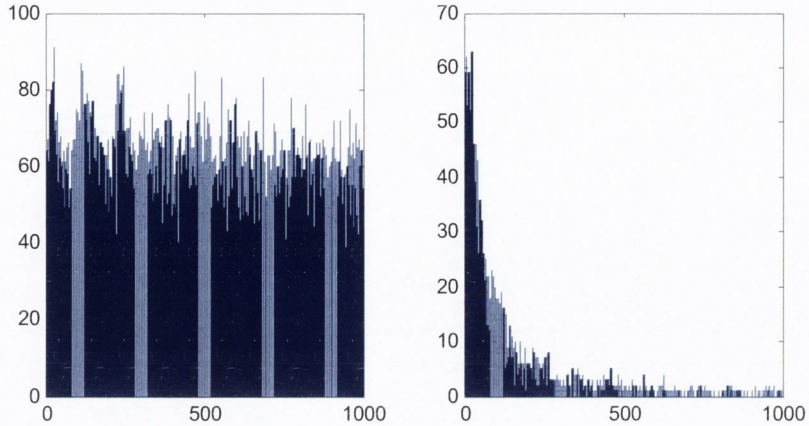
Figure 4: Propensity-To-Burst Histograms



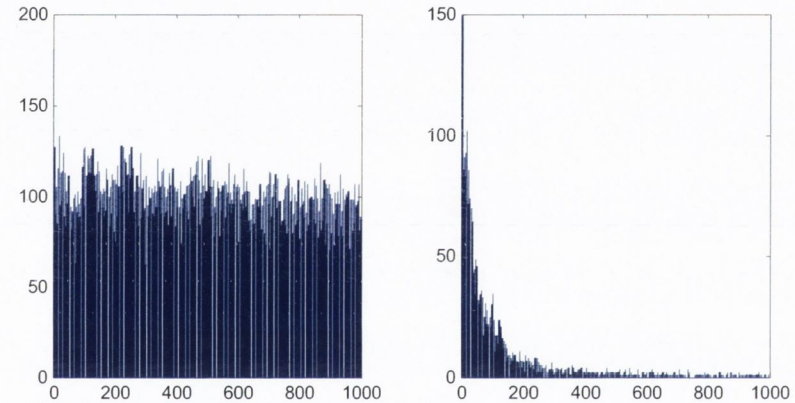
Histograms describe the number of units and the propensity-to-burst. **a**. represents propensity-to-burst for all bursting units together. Histogram **b** summarise data for bursting units including B and BSTM. Histogram **c** describes propensity-to-burst for Strong-theta-modulated bursting units and histogram **c** summarises data for fast-spiking units only. It can be seen how bursting units and fast-spiking units tended to burst more strongly that strong-theta-modulated bursting type.

Figure 5: Bursting Units, Autocorrelograms And ISIH

Cell A



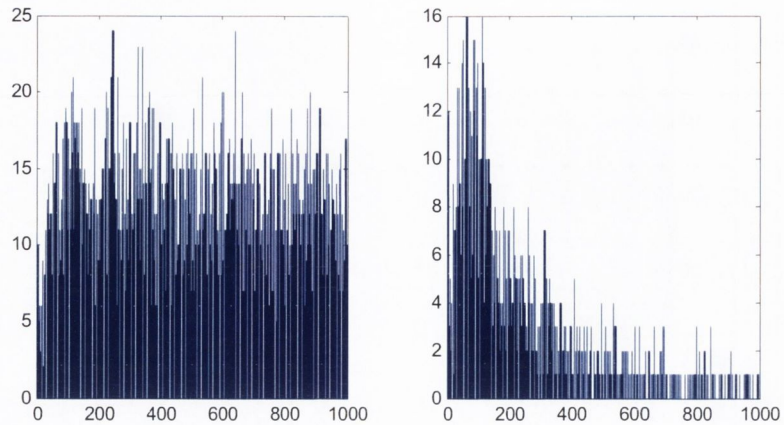
Cell B



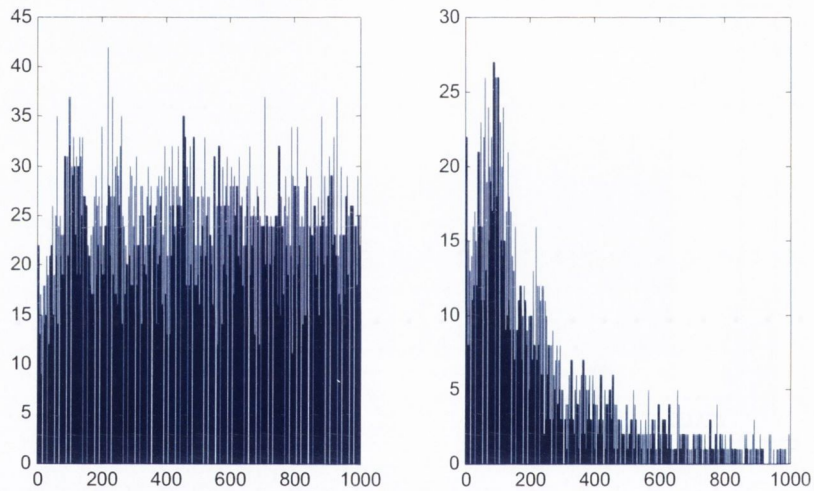
Examples of bursting units. Non-normalized autocorrelogram are displayed on the left while non normalized ISIH are located in the right. Both cells have clear peaks in the short range of the histograms. Also it can be observed how theta modulation is present in the autocorrelogram with peaks about 125 msec bins. Similarly. **Note:** No contamination of spikes was found for the first msec bin respecting physiological refractory period.

Figure 6: Strong Theta Modulation Bursting Units, Autocorrelograms And ISIH

Cell A



Cell B

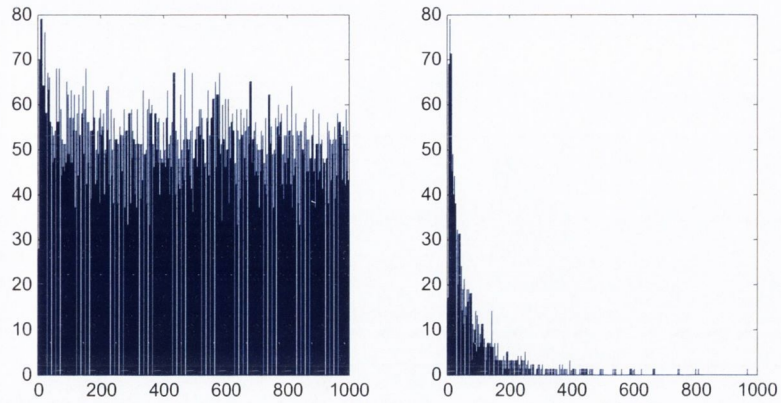


Non-normalised autocorrelograms and ISIH. Examples of strong theta modulated bursting units. It can be observed peaks in the sort range of the ISIH, histogram in the right, but also it can be appreciated a second clearly defined peak at about 125 msec, corresponding to theta modulation. This modulation is observed in the autocorrelogram on the left. The fact that there is a peak in the sort ISIH range made them different from non-bursting theta-modulated units. Finding a peak in the theta range, suggested that these cells are not the same physiological type as normal bursting units.

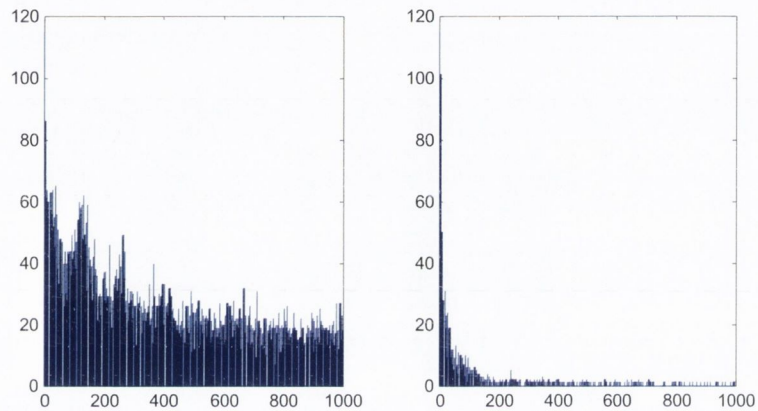
Note: Refractory period was respected.

Figure 7: Fast Spiking Units, Autocorrelograms And ISIH

Cell A



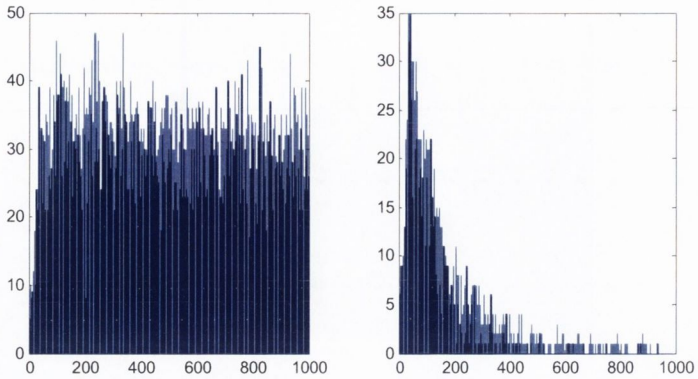
Cell B



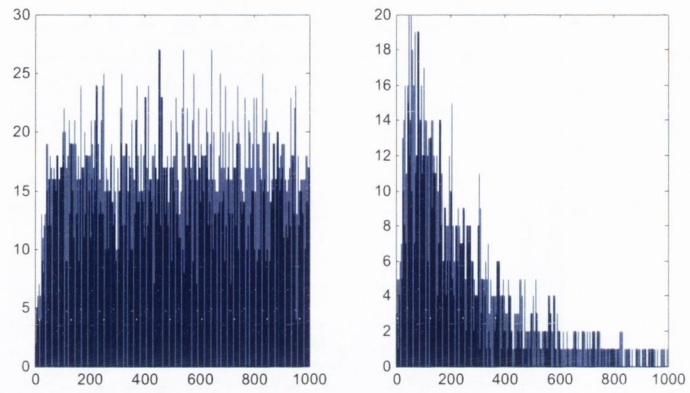
Fast-spiking unit. Non-normalised autocorrelogram and inter-spike interval histograms are displayed. On the left autocorrelograms is displayed and ISIH in the right. Similar to bursting units spikes are clearly located in the sort range of the ISIH. Again clear modulation is observed in the autocorrelograms were peaks appear clearly for cell B between 150 msec and 200 msec. Cell A presented also modulation in the autocorrelogram. **Note:** No contamination of spikes was found for the first msec bin respecting physiological refractory period.

Figure 8: Non-Bursting Theta-Modulated, Units Autocorrelograms And ISIH

Cell A



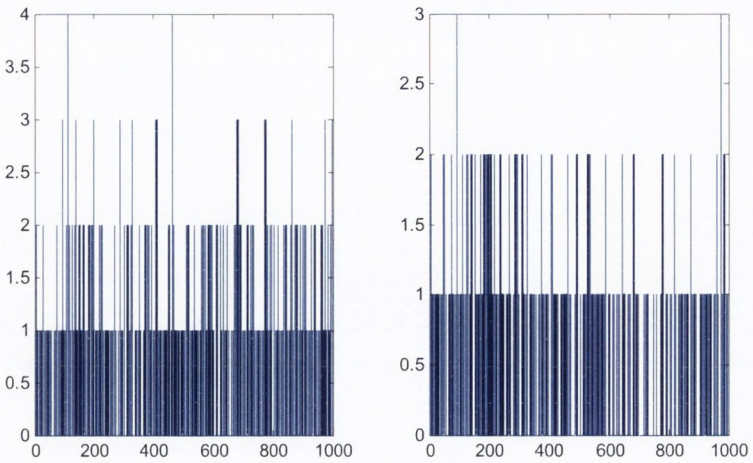
Cell B



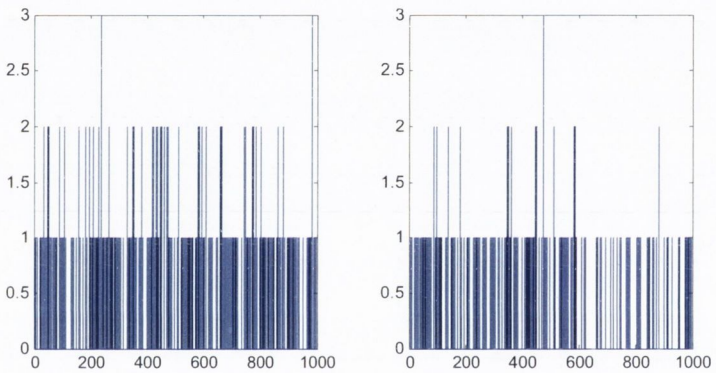
Non-normalised autocorrelograms and ISIH for non-bursting theta-modulated units. Peaks in the short ISIH range are not present in this type of unit, although there is still theta modulation, as revealed by the peaks about 125 msec in the autocorrelograms and ISIH. **Note:** No contamination of spikes was found for the first msec bin respecting physiological refractory period.

Figure 9: Regular Units, Autocorrelograms And ISIH

Cell A



Cell B



Non-normalised autocorrelograms and ISIH of two regular spiking units. There is a lack of structure in the histograms with no clear peaks at any interval in the ISIH and in the autocorrelogram and with no theta modulation **Note:** No contamination of spikes was found for the first msec bin respecting physiological refractory period.

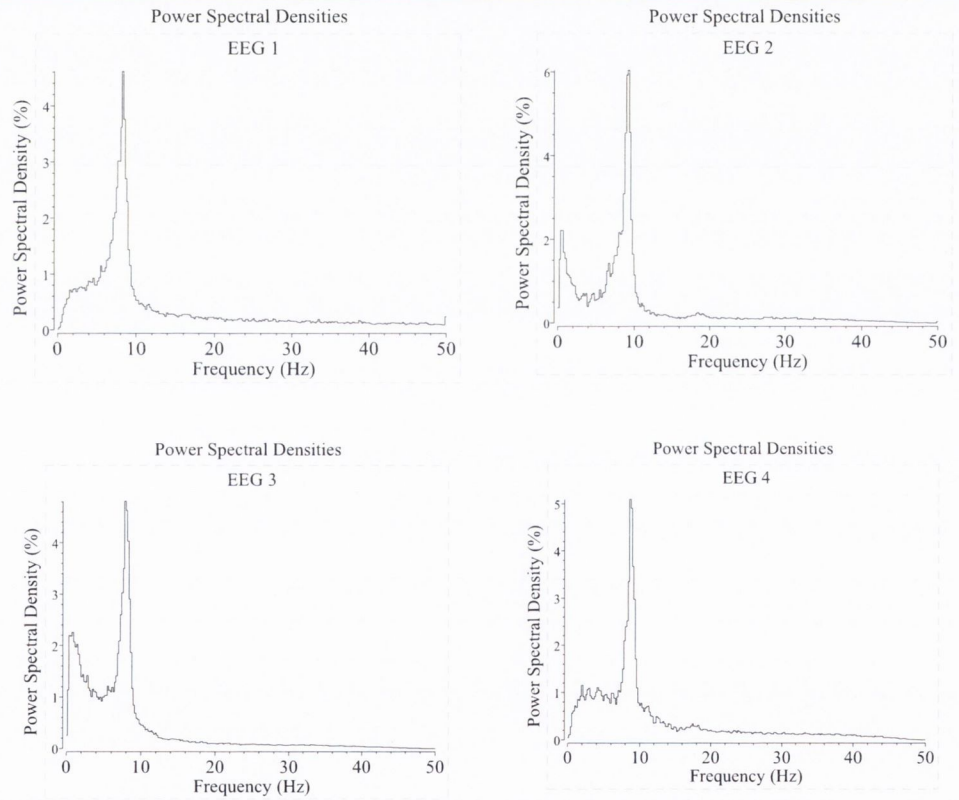
EEG Analysis

EEG simultaneously recorded was analysed using Fast Fourier transformation. EEG was dominated by theta modulation frequencies, ranging from 4-12 Hz although normally it could be observed bimodal distributions in the peridogram, see figure 10, in this cases power spectral analysis would have a peak in the slow frequency range 1-4 Hz, being therefore associated with LIA. The second peak was located in the theta range. These results were expected and were used as guide during recordings. As theta is the dominant frequency in the hippocampus and in the subiculum (O'Keefe & Nadel, 1978; Buzsaki, 1986; Anderson & O'Mara, 2003) it could be used to determine electrode location while recordings are performed. Theta activity would reflect periods of exploratory behaviour, rearing, locomotion and general voluntary complex behaviour. On the contrary LIA would be correlated with sniffing, grooming, staying still (or quiescence), and less complex behaviours (Bland & Oddie, 2001). In the figure below it can be observed an EEG trace of 1sec and a raster plot where blue lines signify spikes times. From trace 1 to 5 examples of different cell types waveform and raster plots are depicted.

Bursting units tend to fire more than 2 spikes in intervals of 1 to 7 msec. It can be seen how spikes happened in small trains with a minimum of 2 spikes. Spikes tended to happen at the trough of theta EEG, figure 11. As mention before bursting units tend to have theta modulation. This is confirmed by the relations displayed in the graph. Strong-theta-modulated bursting units also tend to fire in bursts but with a smaller propensity than bursting units, as shown in the second graph. Spikes happened at the trough of theta. It is clear that these units tend to fire less bursts as none was detected in this trace. Only single spikes were recorded for this 1 sec of activity. On the contrary fast-spiking units seem to fire strong burst related with theta activity. Actions potentials were more frequent that in any other unit type. Non-bursting theta-modulated units,

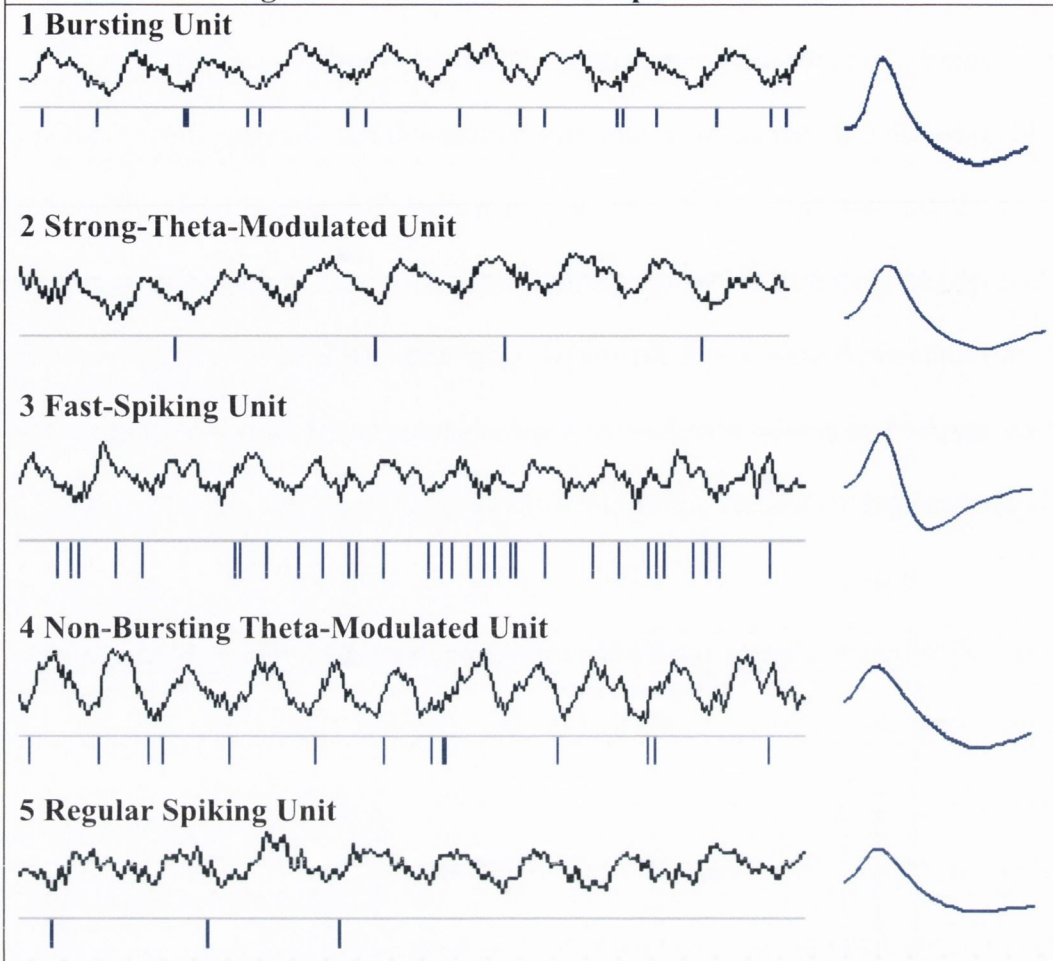
shown in fifth row, were less prompt to fire in burst but they were also able of firing small trains of action potential. Theta relationship was also detected. It could be possible that Strong-theta-modulated units and non-bursting theta-modulated units are a unique cell type and that those which were deemed to be bursting units were units which fire more burst. In the final trace a regular spiking unit raster plot shows not propensity-to-burst and no relationship with EEG activity. Unit waveforms are also graphed. It can be seen how fast-spiking units had a narrower spike width than the rest of groups.

Figure 10: EEG Power Spectra Of 4 Different Recordings



Dominant frequencies in the power spectral analysis. Peaks were found in the range of theta, peak between 4 Hz to 12 Hz and also for SIA, peaks in the low range frequency.

Figure 11: EEG Trace And Spike Raster Plot



EEG trace and raster plot of 5 different cell types. Also waveforms for each unit type are shown. Relationships between EEG and unit activity can be seen for all cell types except for regular spiking units in the bottom.

3.5 *Discussion*

Electrophysiological analysis of units recorded yielded 4 different cell types: Bursting units further divided in bursting units and strong-theta-modulated bursting cell. This subdivision was based in the smaller propensity-to-burst and stronger theta modulation of the BSTM. Fast spiking units, characterised by having a narrow spike, higher frequency of firing and theta modulation. Non-bursting theta-modulated units did not show propensity-to-burst but they showed theta modulation in the ISIH and autocorrelogram. Finally, regular spiking units, showed no theta modulation and no propensity-to-burst. Bursting units described here could be the equivalent to those described previously, (Sharp & Green, 1994; Anderson & O'Mara, 2003), although no such correspondence could be proposed between bursting strong-theta-modulated and any previous work in vivo. Anderson found large variability across bursting units ISIH, and therefore it could be possible that this group of cells was included in the same class. However the smaller propensity to burst in this unit type, could relate to weak bursting units described by others (Staff et al., 2000; Menendez de la Prida et al., 2003). It is not clear if physiological modulation by the behaviour of the animal, for example speed of movement has been clearly related with an increase in theta modulation (Bland & Oddie, 2001), could modify units activity making them change their activity pattern. In any case, unit electrophysiology can not be only explained by functional states. For example R units, almost never showed a burst, therefore intrinsic unit characteristics modulate the interaction between neurons and electrophysiological states behaviourally dependent. Fast-spiking units seem to correspond with fast-spiking described by Anderson & O'Mara (2003) and possibly with theta cell as described by Sharp & Green, (1994). Non-bursting theta-modulated cells are unrelated with previously described units. The fact that there was no correlation between unit's activity and EEG, limits the interpretation of the results. Regular spiking units were characterised by no clear peak

of activity in the autocorrelogram or ISIH and they seem to correspond to regular spiking units as described by Anderson & O'Mara (2003) and other in vitro work (Taube, 1993; Staff et al., 2000 ;Jung et al., 2001; Menendez de la Prida et al., 2003;). Data suggest a continuum of propensity-to-burst by different unit types, and although there is a strong tendency of neurons to be bursting, different cell types were recorded suggesting that the subiculum is a more complex structure as has been suggested before (Menendez de la Prida et al., 2003). Difference in methodology and recording techniques across all different work here described, including the present data, complicates results comparison and generalisation of the classification obtained.

Chapter 4 : Visual Modulation Of Subicular Place Cells

Chapter Content

1. Summary
2. Introduction.
3. Methodology
4. Results
5. Discussion

4.1 Summary

Preliminary studies demonstrated that hippocampal PCs are strongly modulated by visual information, firing fields, for example, rotated coherently after visual polarising cues positions were modified (Muller & Kubie, 1987). Moreover visual input help place cells to keep their firing field location stable while recordings in the darkness produced PC to remap (Quirk et al, 1990). In a similar way to hippocampal place cells, subicular firing fields followed visual cues rotation in a congruent fashion (Sharp , 1994),. No previous experiment has been aimed to study subicular units response during the dark. Subicular representation of space is less sensitive to environmental contextual changes than the hippocampal PCs (Sharp, 1997), and also it has been proposed as one of the brain structures involved in path integration (Redish & Touretzky, 1997; Sharp, 1999b). As proposed by Redish et al, (1997), if subicular PCs are involved in path integration, these units should tend to be stable during recordings in the dark. Recording of subicular neurons were performed on freely-behaving animals across light-dark transitions. It was found that 50% of cells were stable across all possible comparisons between 6 different conditions and 50% were plastic in at least 1 condition. Previous data suggested that plasticity could be the result of error accumulation in the path integration system across conditions. In this way and in spite of some units remapping across specific conditions, subicular PCs were mostly stable in the darkness with independence of the order of the trials, possibly as result of a path integration mechanism.

4.2 Introduction

Several experiments demonstrated that the rotation of a visual polarising cue produced congruent rotation of firing field locations by keeping the same angular relation to the visual card after this was rotated (Muller & Kubie, 1987). In later work, PCs were recorded under light-dark transitions, finding that hippocampal PCs kept the same firing pattern during the dark period if the animal had gone through a light period before. On the contrary, if the animal was placed in the arena in the dark, the firing pattern remapped in relation to both the previous light and dark trials (Quirk et al., 1990). Markus et al. (1994) trained rats to navigate in a radial arm maze under the light and while in the darkness. As in previous experiments, described above, units were more stable if the animal experienced the light trial before. Spatial information and spatial reliability was deteriorated when the lights were switched off. This reduction in the spatial signal correlated with an impaired performance in the navigational task. These results suggest that PCs need visual cues to set their firing fields in order to keep a stable representation during the dark. Recent data suggest that PCs integrate multiple sensory cues to keep their firing during dark trials (Save, Nerad, & Poucet, 2000). In despite of visual information being dominant in most conditions, early-blind rats have stable firing fields, probably by using object recognition and motor input (Save et al., 1998). Local cues such as odours seem to be of strong importance for the animal to keep stable firing, but also path integration processes seem to be involved in firing field stability during periods of darkness, this would allow animals to navigate efficiently in all possible conditions.

The view of the subiculum as an output structure of CA1 (Barnes et al., 1990), suggest that its representation would be affected by changes in CA1 representation of space. Results proved that subicular PCs represent the environment in a qualitative different way to that of the hippocampus. SPCs display similar firing fields across multiple

conditions, but also their activity predict firing field position in advance to CA1 units indicating idiosyncratic dynamics of spatial information processing (Sharp, 1997; Sharp, 1999c). Signals in the subiculum are directionally- and motor-modulated and therefore must be involved in path integration mechanism. Recent experiments reported that Grid Cells in the entorhinal cortex are capable of displaying the same basic firing structure across different arena size, shapes and in the absence of visual information (Hafting et al., 2005).

The aim of this study was to probe the role of the subiculum as part of the path integration system. If SPCs were capable of keeping their firing fields stable only during the light periods this would suggest that these cells are dominated by visual information involving the subiculum in allocentric processes. On the contrary stability across conditions would support a role of the subiculum in path integration. Finding differing types of cells in accordance with these two general hypotheses would suggest that the subiculum is involved in both process and could be acting as integrator of different flows of information.

4.3 Methods

For a complete description of methods refer to chapter 2. Variations in methods used are described below.

Subjects

Six Lister-hooded male rats weighing 300g on arrival were housed individually in a standard cage and kept on a 12-h light/dark schedule with food and water *at libitum*.

Apparatus

The recording apparatus consisted in an open field of 50 cm x 50 cm and 50 cm height, black painted and with a grey washable floor. The apparatus was cleaned between trials and between light-dark transitions to eliminate possible cues generated by the animal.

Behavioural Task

Animals were food-deprived up to 80% of their original weight after which recordings commenced. Grains of sweetened rice were thrown in every 20 sec to random locations within the open field, keeping the animal in continuous locomotion, thereby allowing a complete sampling of the environment and brain activity correlated with all different areas.

Electrode Implantation

After training animals were anesthetised with ketamine (90 mg/kg) and xylazine (10 mg/kg). Anaesthesia was maintained using Isuoflorane at 2%. Rats were implanted with two or 4 tetrodes consisting of four 25μm twisted coated platinum-iridium (California Fine Wire) wires mounted in a microdrive (Axona Ltd) aimed at the dorsal subiculum. The dura was removed and the electrodes lowered into the brain 1mm. which allowed the electrodes to be lowered in steps of 25-50μm. Animals were left to recover for 7 days.

Electrophysiological Recording

Signals were fed through a unity gain preamplifier, connected to the microdrive feeding the signal to the acquisition system (Axona, Ltd). Single-unit signals were amplified between 10-40000 times and band-pass filtered between 380 Hz and 6 KHz. One channel was used for EEG recording. Signals were filtered between 0Hz-500Hz and sampled at 250 Hz. A notch filter set to 50 Hz was used when necessary to avoid noise interference in the signal.

Data Analysis And Unit Isolation

Data were analysed with cluster cutting techniques (Tint, Axona Ltd). Units were classified by being assigned to different clusters using different parameters such as spike amplitude, voltage, time, width etc. Autocorrelogram histograms and ISIH were constructed verifying refractory periods were respected and that spike trains were free from artefacts.

Firing Field Stability

Distance Between Centres Of Mass

Centre of mass distribution for each firing map was obtained in two different ways:

Distance of map type 1, the centre of mass was obtained including all firing map bins and then the distance city block between different maps was obtained.

Distance of map 2 centre of mass of the distribution was calculated only for bins which firing exceeds 60% of maximum firing frequency. Then difference between firing maps was obtained. Cells were deemed to be plastic whenever the distance between two centres of mass 2 was above 9 bins.

Distance Between Maximum Firing Bins

Maximum bin location for each firing map was calculated and then the distance between them across condition obtained to evaluate firing field drift.

Firing Maps Correlations.

Also bin-by-bin correlation was used to evaluate firing field stability.

Firing Field Size

Size of firing field was calculated as the total percentage area of the arena in which firing field was above 20% of the maximum firing frequency, for what was called firing field size 1while firing field size 2 was calculated restricting the area to the total percentage of the arena in which firing field was above 60% of the maximum firing frequency.

Histological Analysis

Once no more units could be isolated animals were anesthetised and transcardially perfused with saline and paraformaldehyde at 4%. Brains were extracted and store for slicing using a vibratome and Nissl stained for electrode location.

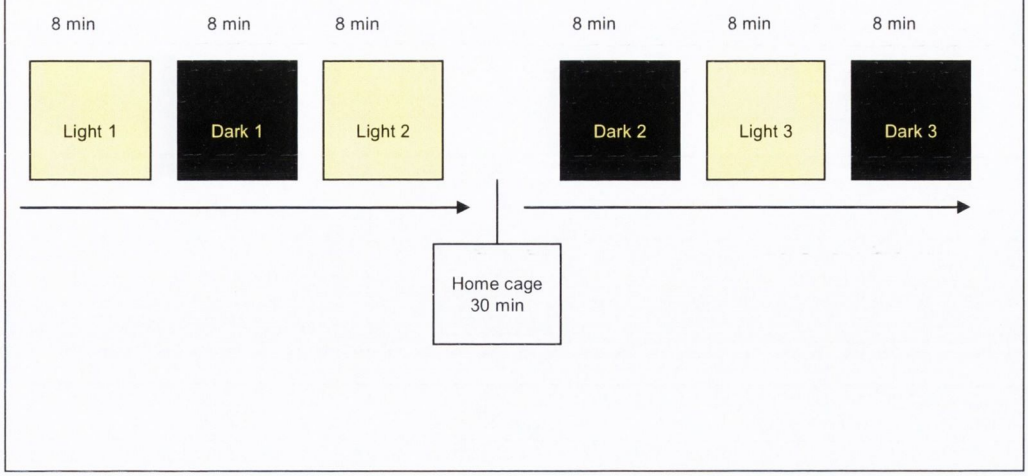
Experimental Protocol

The testing protocol consisted of two complementary parts named protocol 1 and protocol 2.

1. Protocol 1, Light-Dark-Light: After unit activity isolation, the animal was left to forage for food in the arena. The recording started with a period of 8 minutes of recording with the lights on, following 8 minutes of dark and 8 minutes of light. After the session was finished the electrode was lowered to search for more cells.

2. Protocol 2, Dark-Light-Dark: The same procedure as explained above was followed but this time the initial condition is dark, following Light and Dark transitions. Animals were only disconnected and left to rest in their home cage between protocols.

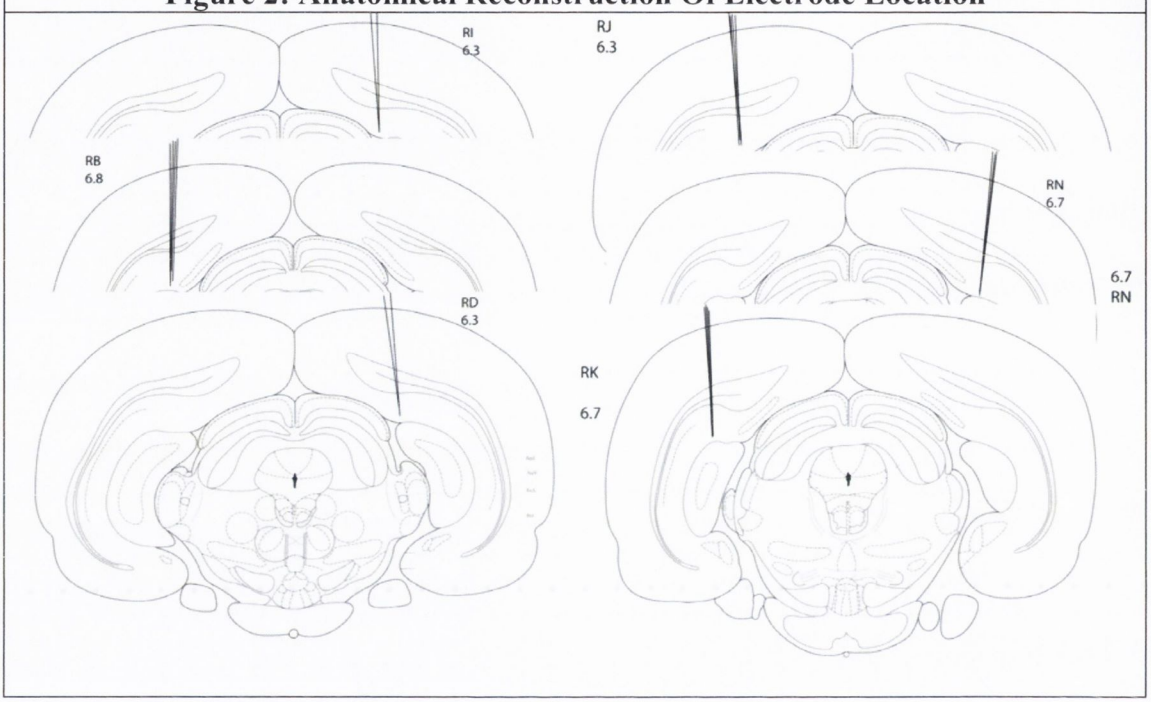
Figure 1: Protocol For Light-Dark Experiment



4.4 Results

After histological analysis confirming electrodes located in the dorsal subiculum, 64 cells from six different animals were included for data analysis. The mean amplitude for the whole set of data was 188.83±6.93 μv, mean spike width was 422.97±16.34 μsec and mean frequency of firing 2.84±0.34 Hz.

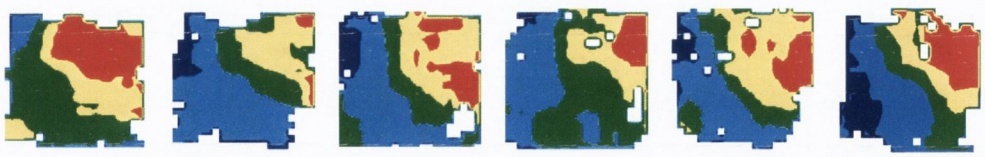
Figure 2: Anatomical Reconstruction Of Electrode Location



Spatial Characteristics

Subicular PCs display large firing fields, multiple peaks of activity, low spatial selectivity and their firing cover most of the arena regions and falling in a continuum of spatiality (Barnes et al., 1990; Sharp & Green, 1994). For these reasons it was decided to include all cells recorded for complete spatial analysis. In this section mean values for 64 subicular cells across 6 different experimental conditions are described. As observed during the recordings, subicular activity was dispersed throughout the whole arena but demonstrated clear location-related firing. The general spatial pattern revealed itself as similar to that described by others (Sharp et al, 1994).

Figure 3. Firing Map Of Subicular PCs



9.11Hz	10.86 Hz	7.94 Hz	8.67 Hz	6.04 Hz	8.12 Hz
Light	Dark	Light	Dark	Light	Dark

Firing field of a typical cell. Firing is represented as a scale of five intervals. Dark blue represents firing between 0% and 20% of the maximum firing field, blue 20% to 40%, green 40% to 60 %, yellow 60% to 80% and red 80% to 100%. White bins are bins not visited by the animal. Most of the arena area is above 20% of the maximum firing frequency. The core of the firing field is clearly located at the north east corner of the arena across conditions. Mean coherence= .82, Mean Skaggs= 0.25, Mean spatial selectivity= 2.26, mean firing field size (20%-100%)= 73.57% mean firing field size (60%-100%)=27.91%, mean firing frequency= 3.84 Hz, mean In-field maximum frequency= 8.46Hz.

Visual inspection of firing maps revealed spatial firing, see figure above. High spatial coherence values, indicated spatial related firing (r=0.80). To evaluate spatial information in bits per spike, the Skaggs coefficient was calculated (Markus et al., 1994), yielding lower mean values, Skaggs 0.24 bits of spatial information per spike, than those described for hippocampal PCs, 1.6 (Terrazas et al, 2005). In-field maximum frequency mean value was 5.95 Hz while the mean frequency of firing was 2.71Hz and non-zero rate was 2.93 Hz. Small differences between mean frequency and mean non-zero rate can be explained as a consequence of spread firing throughout the arena resulting in low spatial selectivity, 2.67. Firing field size is defined in different ways across different studies (Sharp & Green, 1994; Hollup, Kjelstrup, Hoff, Moser, & Moser, 2001; Anderson & O'Mara, 2004.). Since subicular firing is distributed throughout the whole arena two different measurements were made. First the percentage of the area in which the firing frequency was above 20% of the maximum firing rate

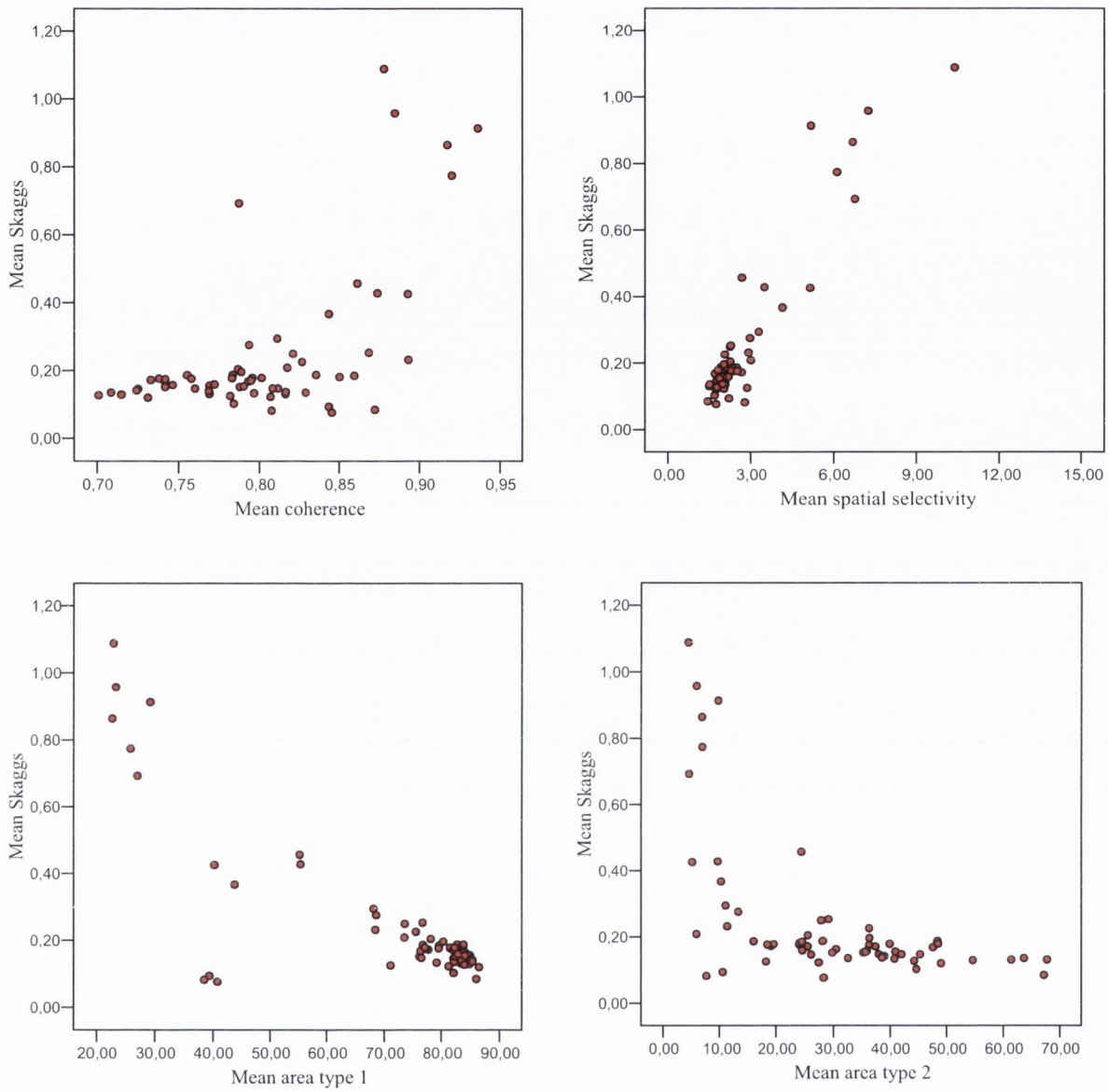
was calculated, secondly and more conservatively, size was computed as the percentage of the arena which was above 60% of the maximum firing frequency. In relation to the first measurement it was found that the mean firing field size type 1was 71.35 %, covering most of the arena size, while firing field size 2 was more restricted, 29.67 %.

Table 1. Spatial Parameters					
Spatial Coherence	**Skaggs**	**Spatial Selectivity**	**Firing field Size (area above 20%)**	**Firing field Size (area above 60%)**	**In-field maximum frequency**
0.80± 0.006	0.24± 0.22	2.67± 0.027	71.35 ±2.41	29.67± 2.04	5.95± 2.41
Values are expressed as mean and standard error					

Spatial Parameters Relationship

Since variables were not normally distributed, Spearman non-parametric correlation index was used. It was expected that Skaggs would have a negative relation with firing field size, and a positive relation with spatial selectivity and spatial coherence. Values for the correlation between Skaggs and the two arena size types yielded a clear negative correlation, ρ=-0.56 $p<0.000$ for FF size 1 and for FF size type 2 ρ=-0.66 $p<0.000$. As expected correlation values for spatial coherence and spatial selectivity were positive, ρ=0.45 $p<0.0001$, while similar trend was also found for Skaggs and Spatial selectivity ρ=0.76 $p<0.0001$, see figure 4.

Figure 4. Scatter Plots For Spatial Parameters

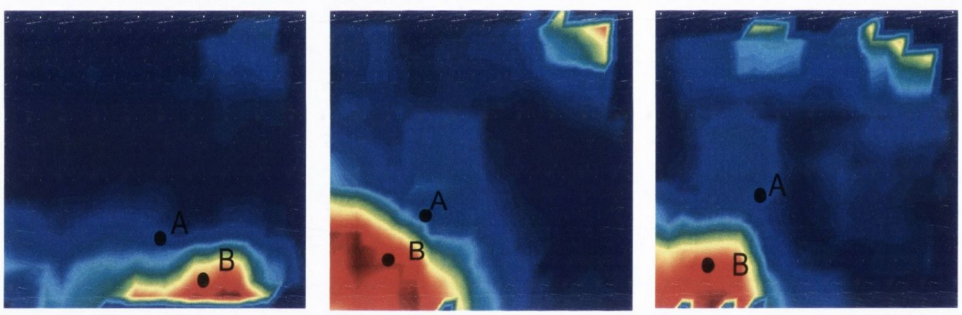


Top left graph, positive correlation between Skaggs and spatial coherence can be observed. Top right graph: linear relationship is observed between Skaggs and spatial selectivity. Similar result is found between Skaggs and firing field size 1, bottom left. Significant correlation was also found for Skaggs and firing field size 2.

Spatial Firing And Stability Measurement

Spatial firing of cells was assessed across different conditions to evaluate the plasticity Vs the stability of place cells. Bin-by-bin correlations were calculated across conditions. To understand better firing field changes three more complementary measurements were used. The distance to the maximum firing bin between conditions is used in the literature to evaluate firing field drift across conditions (Lever et al., 2002). The fact that firing field size is larger in the subiculum and that more scattered firing occurs throughout the whole arena made the distance to the maximum firing bin sensitive to spurious changes. Thus complementary parameters were used to study SPCs stability and plasticity.

Figure 5: Firing Field Centre Of Mass



6.2 Hz 6.7 Hz 7 Hz

Exact centre of mass is plotted for a cell which presented plasticity across light-dark-light transitions. It can be seen how firing field originally located in the southwest corner drift position to the southeast corner and came back to the original position once the light was turned on. Point A represents the centre of mass type 1 and point B centre of mass type 2.

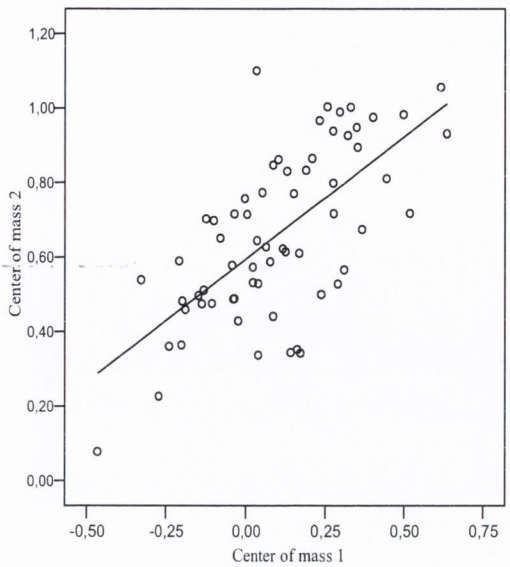
The centre of mass of the firing map distribution was thought to be more robust and less affected by spurious changes and it was calculated in two different ways. First the centre of mass for each firing map was calculated and then the distance (city block distance) between firing maps across condition was calculated. This was called distance to the centre of mass type 1. Second the centre of mass for each firing map distribution was calculated but only including the portion of the matrix in which the firing frequency was above 60% of the maximum firing frequency. The distance to the centre of mass 1 would be sensitive to change in the whole firing distribution whereas the distance of mass 2 would be more sensitive to a specific change in bins where firing frequency is higher and therefore closer to the core of the firing field. Centre of mass type 1 and type 2 can be observed in the firing fields above, figure 5.

To validate the distance of mass type 1 and type 2 as a measurement of firing field change, mean values were calculated and correlated with mean correlation values. Also the relationship between distance of mass and distance to the maximum firing bin was analysed. If high correlations revealed high similarity between two firing fields and high values for distance of mass and distance to the maximum firing bin were due to firing field change, then it was expected that the distance of map would have negative linear relationship with Pearson correlation and positive linear relationship with the distance to the maximum firing bin. To ensure normality distance of mass type 1 and distance of mass type 2 were logarithmic transformed and then Pearson correlation applied.

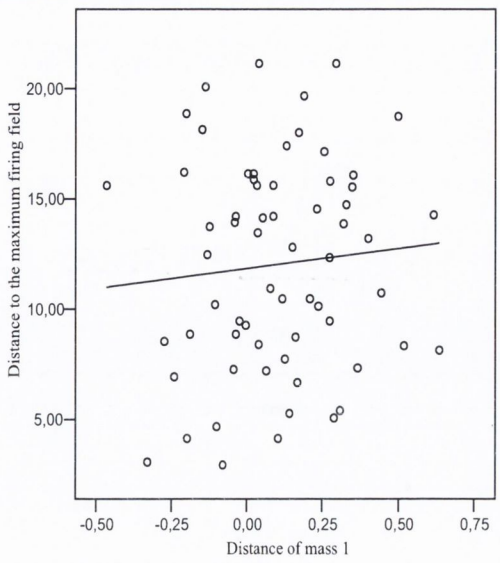
Table 2: Correlation Matrix For Stability Plasticity Measurements				
Distance of mass type 1	**Distance of mass type 1**	**Distance of mass type 2**	**Mean correlation**	**Mean distance to maximum firing bin**
Distance of mass type 1	1	0.667 p<0.000	-0.584 p<0.00	0.087 p>0.05
Distance of mass type 2	0.667 p<0.000	1	-0.730 p<0.00	0.247 p<0.05
Mean correlation	-.0584 p<0.000	-0.730 p<0.000	1	-0.317p<0.01
Mean distance to maximum firing bin	0.087 p>0.05	0.247 p<0.05	-0.317 p<0.0	1

As expected the distance of mass type 1 and type 2 correlated between themselves in a strong positive linear manner. A significant correlation between distance of mass type 1 and distance to the maximum firing field was not found but it was confirmed with distance of mass type 2. Negative linear relationship was confirmed also between mean correlation values and the 3 different distances. Results suggest that parameters propose to measure firing field change and stability are measuring what they were intended to. This can be clearly seen not only in the correlation index but also in the scatter plots below.

Figure 6: Scatter Plots For Distance Of Mass And Max Bin



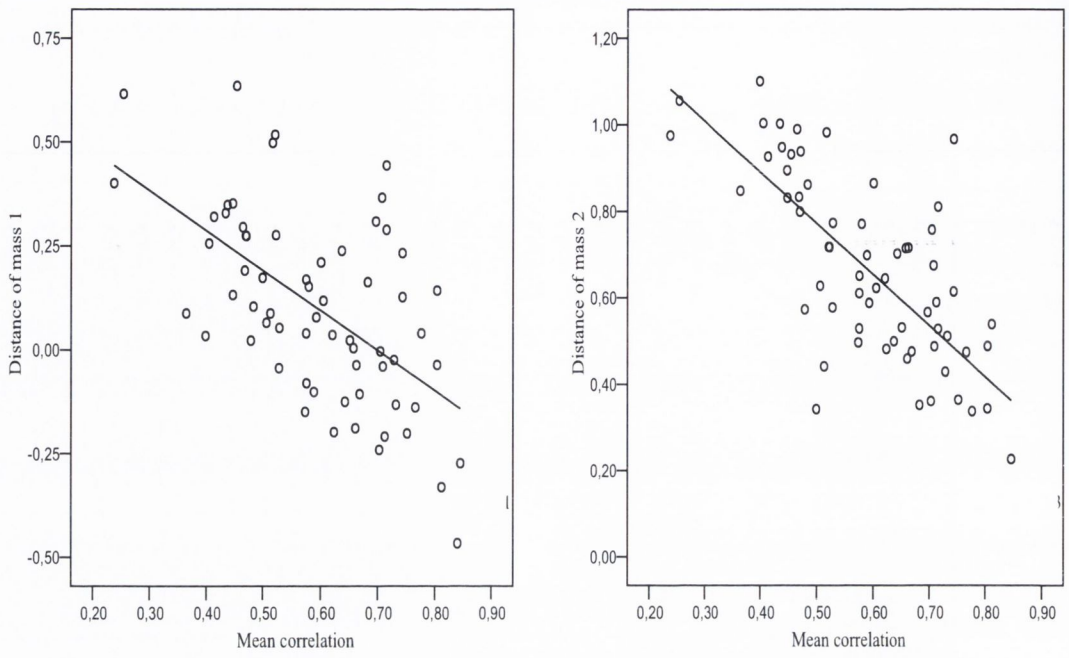
A. Distance to the centre of Mass 1 Vs Distance to the Centre of Mass 2



B. Distance to the centre of Mass 1 Vs Distance to the maximum frequency firing bin

Centre of mass 1 and 2 are plotted after logarithmic transformation. It can be seen how centre of mass 1 and centre of mass 2 presented strong relationship whereas distance to the maximum firing bin with distance of mass type 1 is not existent

Figure 7: Scatter Plots For Distance To The Centre Of Mass And Mean Correlation



Both distance of mass presented strong negative relation with mean correlation.

Cross-Correlation And Cells Plasticity

Initially a correlation-based approach was used to describe cells response to environmental manipulation. Similar to previous studies (Quirk et al., 1990; Anderson & Jeffery, 2003; Moita, Rosis, Zhou, LeDoux, & Blair, 2004;) quantitative criteria were used to classify cells as stable or plastic as mention in other studies. A user defined criteria was established to classify place cell as stable or plastic. If correlations were above r=0.3, cells were deemed as stable while stability criteria was not achieved, cells were regarded as plastic units. Using this approach of 64 cells recorded in the two halves of the protocol a total of 47 (73.4%) cells displayed cross-correlation above r=0.30 for all conditions comparisons while 17 (26.6%) cells were plastic at least across two different conditions. Mean correlations for the first group of cells was r=0.64 and for the second group r=0.45. Although the mean correlation is lower for plastic neurons this last group presented high correlation value which yielded high determination coefficient of r^2=0.20.

Table 3: Descriptive Statistics Plastic And Stable Units Classified Using Correlation Index

	Plastic neurons		Stable neurons	
	Mean	**Std. Deviation**	**Mean**	**Std. Deviation**
Firing field size 1	61.1151	21.21465	75.0587	17.33196
Firing field size 2	17.7286	10.92880	33.9981	15.87409
Mean frequency (Hz)	1.9404	1.71204	3.0008	2.22955
Mean non-zero rate (Hz)	2.0941	1.83720	3.2423	2.40283
Mean in field Frequency (Hz)	5.0693	3.57669	6.2786	4.41362
Mean coherence	.8159	.06068	.7975	.05266
Mean Skaggs	.2757	.25211	.2357	.21321
Mean spatial selectivity	3.0553	1.36870	2.5421	1.70445

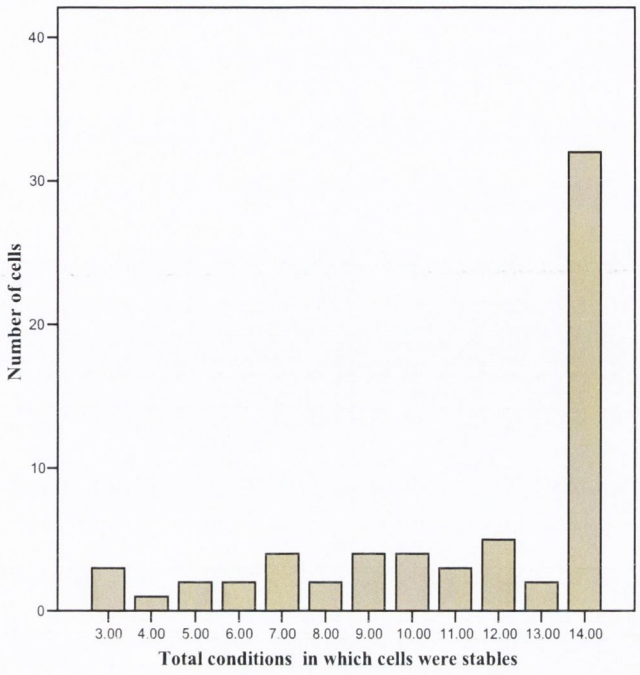
Stability-Plasticity: type of change based in distance of mass type 2

As suggested before, distance of mass could be more sensitive to firing field shifts that other proposed measurement of stability-plasticity and therefore should be used to determine how firing field location changes. It is clear that although bin-by-bin correlation is sensitive to firing changes it evaluates the whole distribution, possibly not being as much sensitive to possible changes in firing field core location. Again a user-defined criterion was used to evaluate firing plasticity. To deem a cell as a plastic unit the distance between two centres of mass had to be above 9 bins. This was thought to be sensitive enough to detect changes and determine if there was difference across two different firing maps. To study the response of SPCs to light-dark transitions data it is described from the most general possible classification to the most specific. Firstly SPCs behaviour for all conditions is studied; plasticity within each protocol, then condition-by-condition and finally comparison between light and dark conditions across protocols.

Stability across all conditions

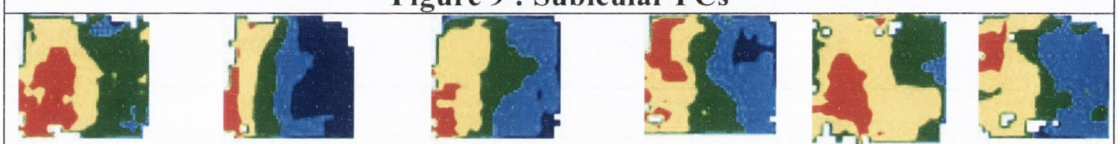
32 cells (50%) met the criteria for stability across all possible comparison between each conditions while 32 cells (50%), were plastic at least for one of the comparisons. This suggests strong stability across light and dark transitions with independence of the order of the trials. None of the recorded cells were plastic for all conditions keeping similar firing at leas in 3 conditions for the most plastic cell confirming subicular spatial modulation and cross condition stability. To study PCs cells response a stability-plasticity index was calculated. This index was obtained by giving one point for each cross-condition comparison in which stability criteria was met. There were a total of 14 possible comparisons across conditions, therefore the maximum score was 14 while the minimum 0. It can be seen how none of the recorded cells was below 3. Mean stability-plasticity index for SPCs was 11.28±3.47 were the minimum score was 3 and the maximum 14. See figure 8 below.

Figure 8: Stability-Plasticity index sample distribution

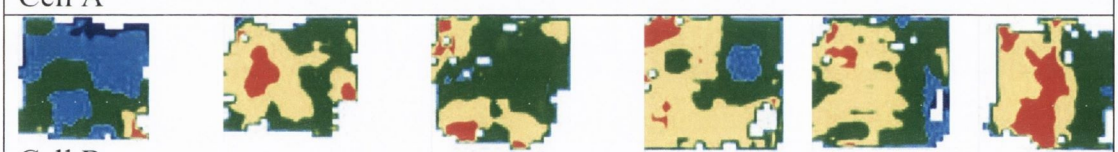


Number of cells stable across different conditions. Most of cells kept their firing field location stable. 32 cells were stable across all different conditions while none was plastic across less than 3 conditions. It can be said that PCs in the subiculum fall in a continuum of stability.

Figure 9 : Subicular PCs



Cell A



Cell B

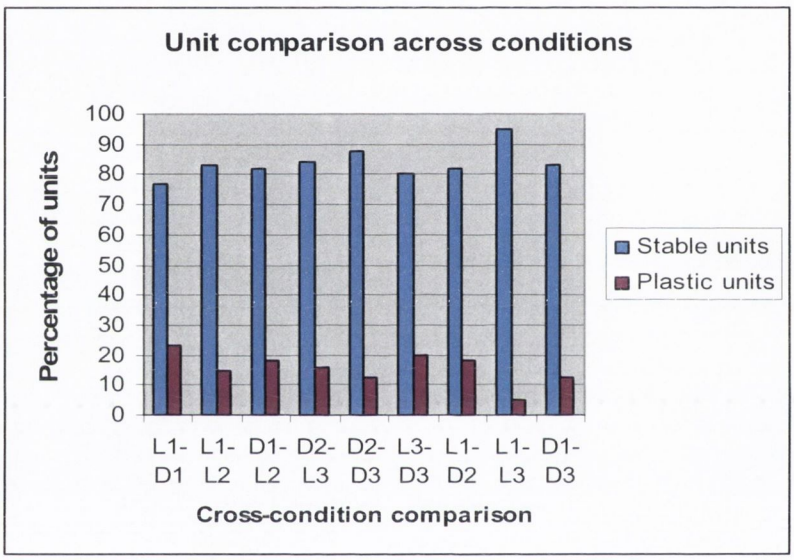
Cell A was deemed as a stable unit. It can be seen how this neuron presented very similar firing fields across all conditions. Firing field is located along the west barrier in condition 1 and very little firing field drift happens across all the different conditions. Mean correlation r=0.64 Mean distance of mass type, 1=1.173, Mean distance of mass type 2=3.160 and mean distance to the maximum firing bin= 10.13. Cell B on the contrary is an example of a very plastic neuron in which the firing field drifted across different conditions during the first protocol (3 first firing fields) but which was more stable in the 3 last conditions. It can be observed how firing field tend to be located close to the west and south barrier in the 3 last conditions. Mean correlation r=0.47 Mean distance of mass type, 1=1.55, Mean distance of mass type 2= 6.8 and mean distance to the maximum firing bin= 19.67.

Protocol 1

Complete Protocol 1

42 (68.1%) were stable across the complete protocol 1 while 19 cells (31.9%) were plastic at least in of the conditions indicating plasticity of SPCs but also strong stability across light-dark-light transitions. Of these 19 cells only 2 cells were plastic across the 3 different conditions. The total percentage of stable and plastic units per condition is described below.

Figure 10: Condition By Condition Stability-Plasticity



Cross-condition stability-plasticity. It can be observed how SPC tented to be stable across conditions with a dominant frequency of stable units Vs plastic units in all possible comparison. It is possible to observe a major tendency of units to be stable when comparisons are made within the same visual condition, light Vs light and dark Vs dark.

Light 1 Vs Dark 1

A total of 47 cells, (77%), presented smaller distance between the centres of mass across light Vs dark conditions and 14 cells (23%) of cells presented larger distance between centres of mass. See figure 11 for examples of stable and plastic units across different conditions.

Light 1 Vs Light 2

52 (83.2%) cells were stable when compared across light Vs light in the first protocol while 9 cells, (14.8%) were plastic.

Dark 1 Vs Light 2

A total of 50 (82%), cells were stable from dark to light transition and 11 cells, (18%) were plastic.

Protocol 2

Complete Protocol 2

50 cells (78.1%) were stable across all light-dark transition in protocol 2 while 14 cells (21.9%) showed plasticity at least in one of the transitions. Of this only 2 cells were plastic across all conditions.

Dark 2 Vs Light 3

54 (84%) of recorded cells were stable Vs 10 (15.6%) cells which were plastic in the first transition between dark and light.

Dark 2 Vs Dark 3

56 (87.5%) cells were stable across dark to dark condition. 8 cells (12.5%) were plastic across Dark to Dark comparisons.

Light 3 Vs Dark 3

55 (85.9%) of cells were stable while 9 cells (14.1%) were plastic.

Comparison Between Protocols

Light 1 Vs Dark 2

To understand the impact of visual information and PCs representation it is necessary to compare the representation in the first light condition against the representation of PCs when the animal has not experienced the lighted environment. After comparing the first light condition against dark starting trial in protocol 2, it was found that 49 cells were stable (80.3%) and 12 cells (19.7%) were plastic.

Light 1 Vs Light 3

To evaluate the impact of starting the protocol during the light condition, Light 1 Vs light 3 were compare finding that 50 cells (82%) were stable while 11 (18%) were plastic.

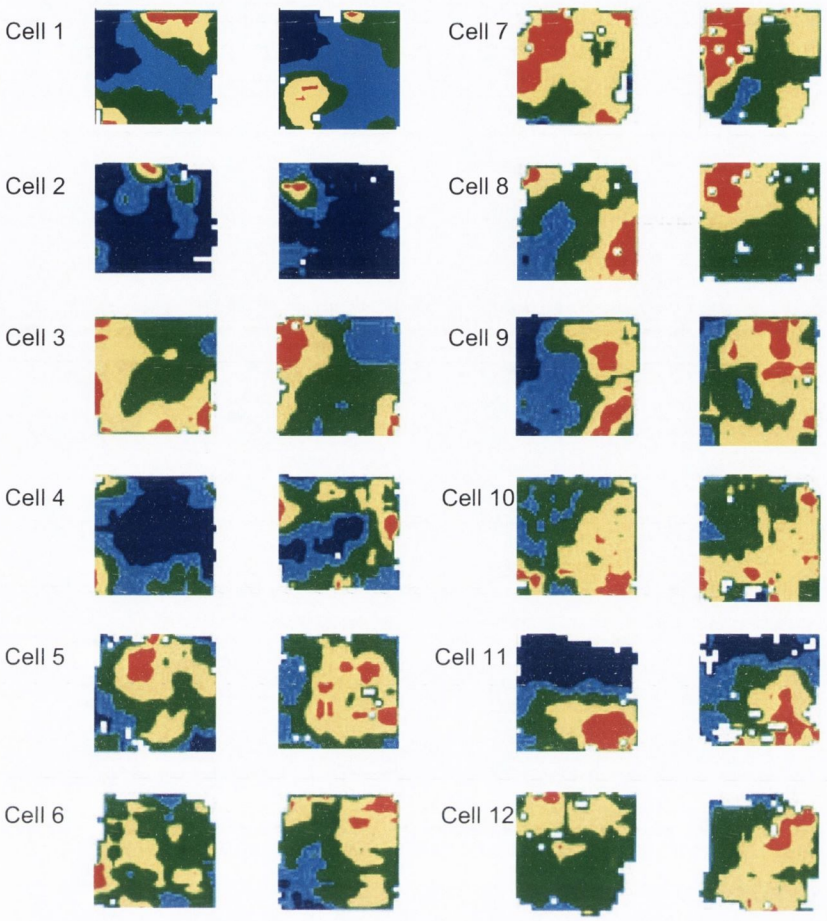
Dark 1 Vs Dark 2

When comparing cells stability across the first dark condition and the second dark condition it was found that most of cells were stable 58 (95.1%) while very few could be classified as plastic, only 3 (4.9%) were deemed to be.

Dark Vs Dark 3

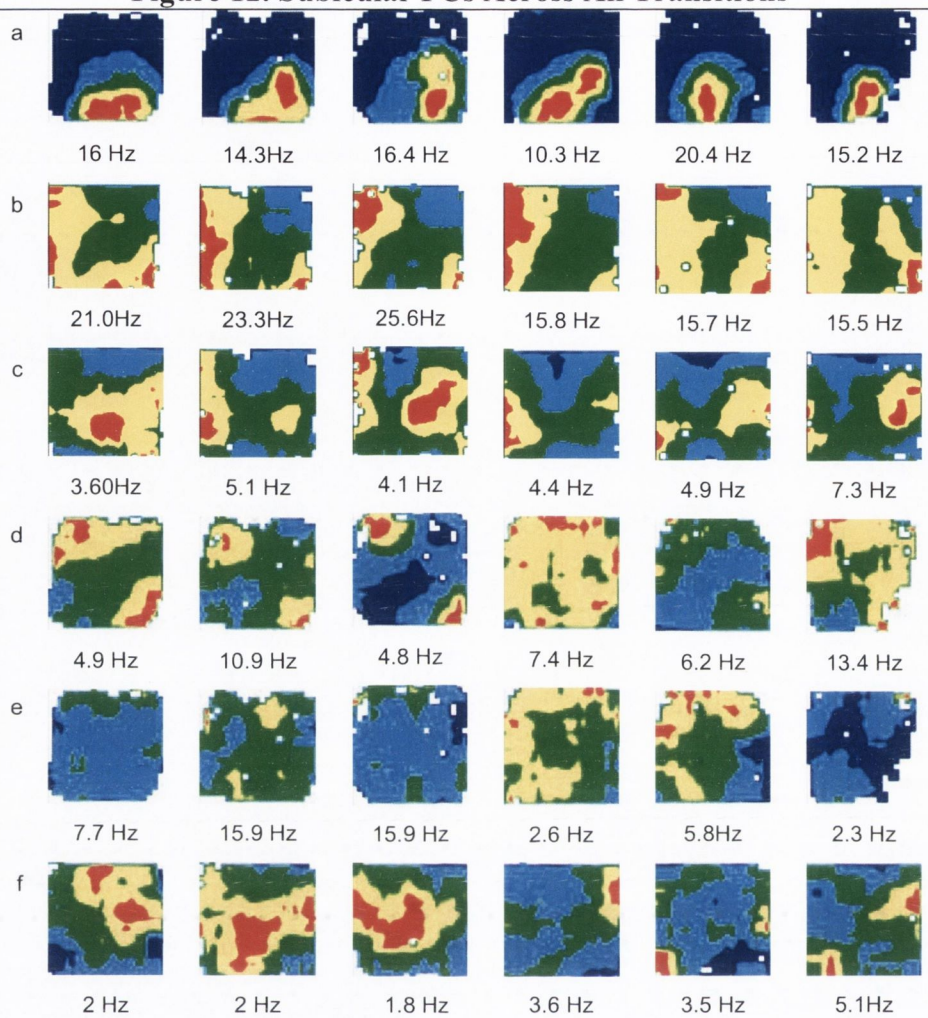
When comparing stability between dark condition in the first protocol and the second dark condition in the second protocol, a total of 53 (82.8%) were stable while 8 (12.5%) were plastic.

Figure 11. Example Of Plastic And Stable Units Across Different Conditions



Comparisons across light dark conditions in protocol 1	Comparisons across light dark conditions in protocol 2
C1 Light 1-Dark 1 stable	C7 Dark 2- light 3 stable
C2 Light 1-Dark 1 plastic	C8 Dark 2-Light 3 plastic
C3 Light 1-Light 2 stable	C9 Dark 2-Dark 3 stable
C4 Light 1-Light 1 plastic	C10 Dark 2-Dark 3 plastic
C5 Dark 1-Light 2 stable	C11 Light 3-Dark 3 stable
C6 Dark 1-Light 2 plastic	C12 Light 3-Dark 3 plastic

Figure 12. Subicular PCs Across All Transitions



Cells A, B, C and D are considered stable. It can be seen how firing fields are consistent across all conditions with very little shift of firing field location. Cell E, F and G show plasticity across different conditions. Cell E seems to keep the same representation in protocol 1. Firing is affected during initial dark trial in protocol 2 and then it seems that firing is reset during the light condition and then kept in the last dark condition. Cell F is the most plastic neuron. It can be seen how the most similar firing maps are condition 4 and condition 5. Cell G firing changed across condition but much clearly across protocol.

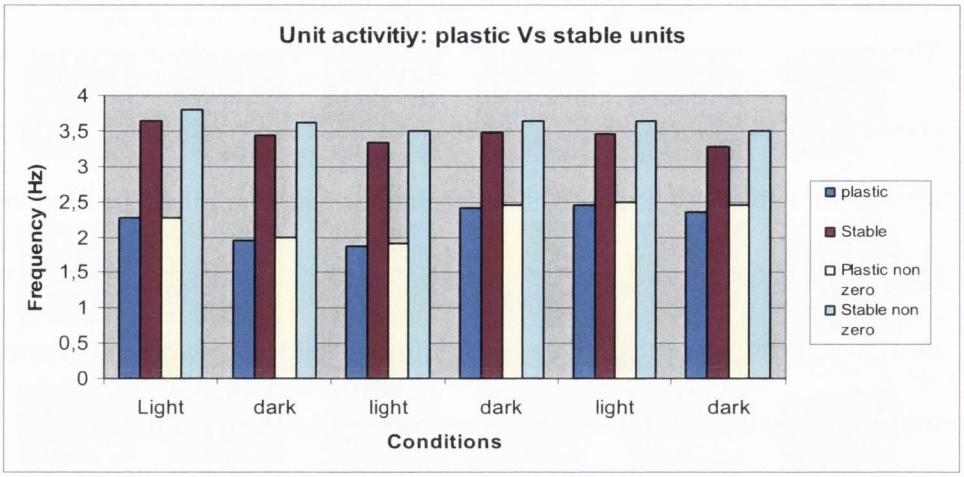
Spatial Characteristics And Cell Type Across Condition

The main spatial parameters were studied across condition and between plastic and stable units to determine the existence of difference in spatial coding between both groups of cells. Logarithmic transformations were applied: in-field frequency, mean frequency and nonzero rate frequency, ensuring normality and the mixed-ANOVA design was implemented. Skaggs and firing field size were not normally distributed under any circumstance therefore none parametric test were used.

Infield Frequency, Mean Frequency And Non Zero Rate, Spatial Coherence

No significant difference were found across condition for in field frequency $f_{(4,184)}=0.29$; $p>0.05$, mean frequency, $f_{(4,203)}=0.24$; $p>0.05$ and non zero rate frequency, $f_{(4,203)}=0.251$; $p>0.05$. Similarly when cell type, plastic and stable, were compare across condition no significant difference was determine for in field frequency, $f_{(1,48)}=0.44$; $p>0.05$. On the contrary units demonstrated significant difference for mean frequency, $f_{(1,56)}=5.193$; $p<0.05$, and non zero rate, $f_{(1,56)}=5.177$; $p<0.05$, both units were significantly different in the first dark, second light, second dark and fourth light condition as demonstrated by independent t-test ($p<0.05$). No significant difference was found for spatial coherence across condition or between cell types, $f_{(4,243)}=2.24$; $p>0.05$, $f_{(1,59)}=0.130$ $p>0.05$.

Figure 13: Plastic And Stable Units Activity Across Condition



Subicular place cells tend to fire in all areas of the open field as suggested by the small difference between mean frequency and none zero rate. Stable units kept stronger activity levels across all conditions. Although as suggested above these difference were only significant in the first dark, second light, second dark and last light condition.

Skaggs And Firing Field Size

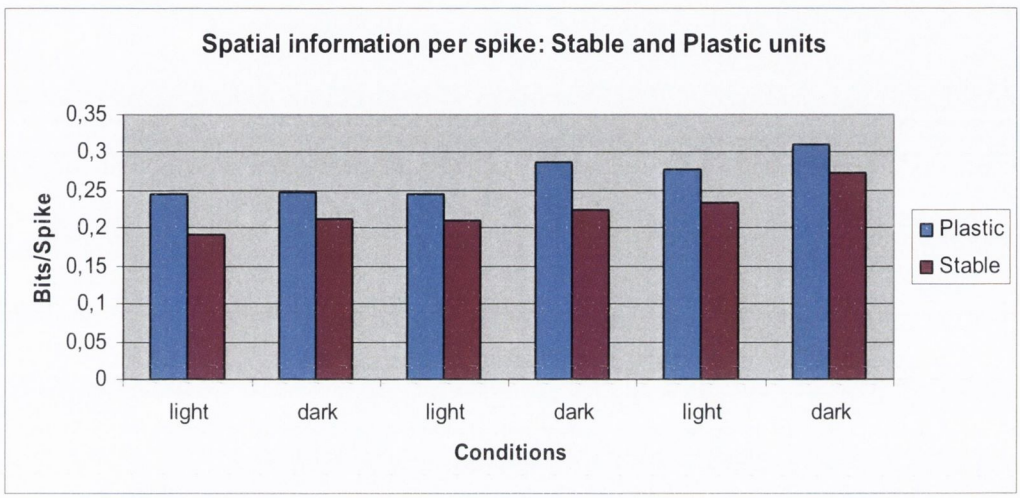
Significant difference were proved for Skaggs values within conditions (Friedman, $p<0.05$). Planned tests, light condition was taken as baseline, found significant difference between baseline condition and values for the three last conditions, second and third dark and third light recordings, (Wilcoxon, $p<0,02$, Bonferroni corrections were applied). Although stable units tended to have lower Skaggs values, no significant difference were found between plastic and stable units across conditions (Mann-Whitney; $p>0.01$; Bonferrony corrections were applied).

Firing field size 1, percentage of area above 20% of the maximum firing frequency, tended to be larger for stable units than for plastic neurons. This is coherent with the fact that stable units had lower Skaggs values. No significant difference were found between stable and plastic units across trials, (Mann-Whitney $p>0.01$, Bonferroni corrections were applied) but significant difference were found between baseline, initial

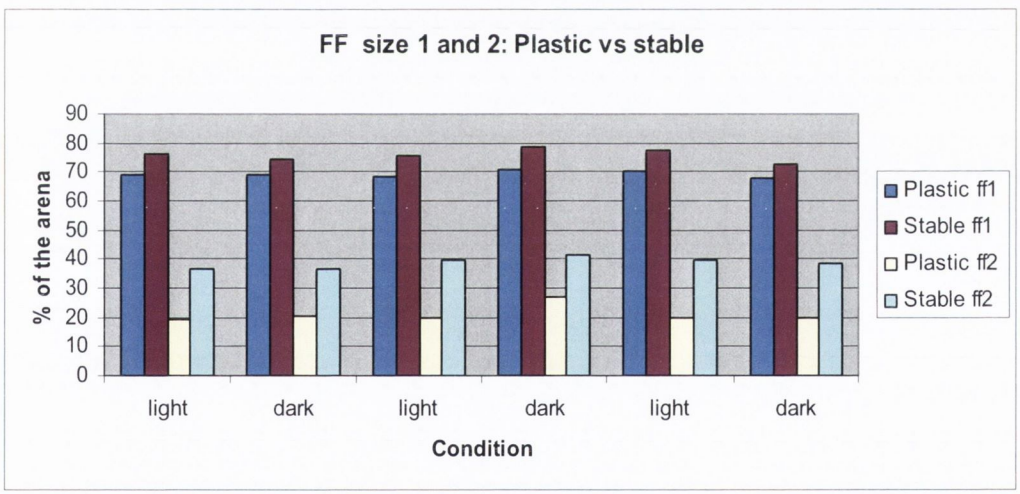
light condition, and the last recording session in the dark, (Wilcoxon, $p<0.01$; Bonferroni corrections were applied).

Figure 14: Stable And Plastic Units, Skaggs And Firing Field Size

a



b



a. Spatial information per spike across condition and between cell types. b. Firing field size one and two across condition and between cell types. Stable units tended to have lower Skaggs coefficient and larger firing field size type 1 and type 2. There was significant difference only across initial light condition and final dark trial for firing field size 1 but not between stable and plastic units. Skaggs were only different between the initial condition and the three last conditions, a result not predicted from any initial hypothesis since values are higher in the second part of the experiment when trials order starter in the dark.

4.5 Discussion

General Spatial Representation Of Subicular PCs

Results describe above mirror previous descriptions of spatial representation by subicular PCs (Anderson & O'Mara, 2004; Barnes et al., 1990; Sharp, 1997, 1999a; Sharp & Green, 1994). These data also proved that PCs in the subiculum displayed large firing field sizes. Firing field area was computed as area in which the firing was above 20% of maximum firing was 71.35 %. Place cell firing, was distributed across the whole arena presenting in this way low spatial specificity 2.67 and low Skaggs coefficient 0.24 bits of spatial information per spike. It has to be said that Skaggs coefficient mean value for the whole sample recorded in this experiment it is below the minimum value reported by others.

Correlation Vs Mean Distance Of Mass

Results indicated similar tendencies using either centre of mass type 2 or correlation to evaluate stability-plasticity, although as mentioned above results indicated that distance between the centres of mass of the firing distribution used as the plasticity criterion was more sensitive than mean correlation. The aim of this study was to evaluate change, and therefore all the results discussed here are based on distance of mass. Possibly by using a more conservative correlation criterion results would have been more similar to DCM2. In any case correlation results would be used during the discussion to complement results.

Stability-Plasticity Across Transitions

50% of cells were fully stable across all possible firing maps comparisons while the rest 50% were plastic at least in one of the 14 possible comparisons. 32 cells were fully stable across all different transitions probing strong generalisation of SPCs spatial representation across multiple light-dark transitions. Most of units were stable across the first light-dark transition 77% were stable while only, 23% were plastic. If

remapping is studied between the first light condition of protocol 1 Vs the first dark condition of protocol 2, it can be seen again a high level of stability, 80.3% of stable Vs 19.7% of plastic cells. As described above these results are enough to explain that previous experience on the arena was not enough to modify firing field changes. If only light Vs dark trials were compared it was found that 34 cells (55.7%) were stable and 27 cells (44.3%) were plastic across all different transitions. This indicates that although plasticity happens across light-dark conditions subicular cells kept their spatial representation from light to dark. Also confirmed by correlation index, light Vs dark conditions r=0.688.

Overall, it appears that units could be classified in two different groups: stable SPCs (50%) and plastic SPCs (50%). Interestingly plasticity did not follow a clear light-dark pattern. The fact that there was no preliminary cell selection suggests that this tendency for stability could be even stronger if a preliminary test-rest prior to experimental condition had carried out. In any case this would have left out neurons which could be more sensitive to changes in the environment.

Data described here indicates that subicular neurons could be using information which is available either in the dark or in the light condition. Different authors have proposed different roles for the subiculum. The most classic approach to the subiculum proposes that this works as an output structure which acts as a relay station between the hippocampus and cortical and subcortical structures. Multiple hippocampal PCs would send different afferents to a unique subicular pyramidal cells which would be driven by hippocampal output. This would explain why subicular neurons present larger firing fields and multiple peaks in the arena (Barnes et al., 1990). Results presented in this work suggest that subicular PC representation is more stable across light-dark transitions than that of hippocampal PCs thus suggesting that SPC are not an output structure of the hippocampal PCs.

It is known that the PCs in the hippocampus are driven by external visual cues, that when rotated produce similar rotation of the firing field, this has been also described in the subiculum (Muller & Kubie, 1987; Sharp & Green, 1994), therefore, although the subiculum is driven by visual external cues, it seems that it can keep its spatial representation without visual information. It can be hypothesized that the angular initial position in which the animal is set in the arena is enough for setting its head direction cell system which combined with motor input information would be enough to reduce firing field drifting. These two sources of information, motor input and angular initial position, would be available at the beginning of each trial. Animals always enter the room using the same route, and they were never disoriented before the trial started. The angular relation to the visual cues was always kept at the beginning of each protocol, although during initial dark trials they were always place in the arena in full dark conditions. Firing field drifting occurred randomly, not following a light or dark pattern. Most interestingly, none of the units was switched off during the dark conditions, except for 3 units only detected in the second protocol. The literature describes a general tendency of hippocampal PCs to depend on visual input for keeping their firing active across light dark transitions, (Markus et al., 1994; Save et al., 2000). These authors found a reduction of active cells during dark trials, and therefore it was suggested that cells needed visual input to keep their spatial representation stable. It appears that SPCs don't depend on visual information as much as hippocampal PCs. If the subiculum is part of a theoretical path integration system, (McNaughton et al, 1996; Redish & Touretzky, 1997; Sharp, 1999b), SPCs should be able to keep their firing during the dark, as demonstrated in this experiment.

Further research would be necessary to determine the influence of motor information in subicular firing. Terrazas et al., (2005) trained animals to navigate a maze under 3 different conditions. In the first condition the animal had to walk in a circular track, in

the second one the animal was put in a toy train immobilised while navigated in the same maze but on the train. In the last condition the animal was hold in the train while the environment moved and the rat stayed still. In this way motor input, vestibular input and optic flow were dissociated across conditions. This produced a progressive decrease from walk to the last experimental condition in theta modulation and spatial parameters such as Skaggs index while producing a field size expansion. This implies that by eliminating motor input and vestibular input PCs spatial information becomes poorer while relying only in optical flow. Similar experiments while recordings are performed in the subiculum would untangle the relevance of locomotion, visual flow and vestibular inputs in SPCs firing.

Spatial Parameters Across Light-Dark Transitions And Between Stable And Plastic Units

No light Vs dark pattern emerged from statistical analysis of the different spatial parameters. Units demonstrated significant difference for mean frequency of firing and none zero rate in the first dark, second light, second dark and third dark condition. This significant difference was not followed by significant changes in field maximum frequency, spatial coherence, Skaggs or firing field size 1. Data trend suggested smaller firing field size for plastic units, but no significant difference was demonstrated. Similarly Skaggs values were higher for plastic than for stable units. Firing frequency difference across cell type in specific conditions could explain smaller firing field size and higher Skaggs index for plastic units, detected in specific conditions and between cells. It seems that both types of units only demonstrate differential response to the light-dark transitions but similar spatial coding except for smaller levels of activity, and therefore smaller firing fields, for plastic units.

Conclusions

Subicular PCs tend to be stable across light dark transition not finding any difference between spatial parameters across these two conditions except for Skaggs index, this last being difficult to explain. Plasticity was found randomly when comparing firing maps between multiples conditions.

Although there were plastic and stable cells, the second group was dominant in number therefore the hypothesis of the subiculum as a part of the path integration system gains support. Since it is not clear the role of locomotion and vestibular input in SPCs firing, future research should be concern with the relation between these variables and SPCs.

The subiculum is located anatomically in an excellent location to provide the spatial processor with the basic information to represent space as a system of coordinates in which shape and size could the basic dimensions and locomotion and directional input the basic navigation parameters.

Chapter 5 : Scaling Properties Of Subicular Place Cells In Multiple Size Environments.

Chapter content

1. Summary

2. Introduction

3. Methods

4. Results

5. Discussion

5.1 Summary

Classic work, (Sharp, 1999a), demonstrated that hippocampal place cells tend to remap across different size arenas, while subicular units could scale under specific conditions. Recent work has demonstrated the existence of grid cells in EC, units which replicate the same firing structure across different arena shapes and environments (Fyhn, Molden, Witter, Moser, & Moser, 2004; Hafting et al., 2005; Sargolini et al., 2006). Both subicular units and grid cells, appear to apply a unique firing structure independently of the size of the environment, although subicular units have never really been recorded in very large arenas, with the sole exception of Anderson and O'Mara (2004).

The aim of this chapter was to better define the scaling properties of subicular PCs in large arenas and to determine if a grid-like structure could emerge under these circumstances. Units were recorded across 3 different size arenas size ranging from 50 cm x 50 cm to 150 cm x 150 cm. Four different units' types were found: perfect scaling units, imperfect scaling units, remapping scaling units and size-specific units. The results presented here partially supported previous work and different models of spatial information processing (Barry et al., 2006; Sharp, 1999b). Units which presented similar structure to EC grid cells were also recorded. Moreover head direction cells were also found. The existence of classical subicular units and grid-like cells which extrapolated the same firing structure in different situations suggests a strong implication of the subiculum in general mapping of the environment.

5.2 Introduction

Previous experiments have been aimed to understand which factors affect place cells firing. Variables such as arena size, arena geometry as well as barriers insertion have been manipulated to study how geometrical and physical factors are coded by PCs. Muller & Kubie (1987) in an intensive experiment investigated how different manipulations affected PCs firing, demonstrating that hippocampal PCs tend to remap with more frequency when animals were place in arenas differentiated in size or shape. To evaluate how physical boundaries affect PCs firing, they placed a barrier where the firing field (FF) was detected. Units which had their receptive field in the same area as the barrier remapped, while FFs distant to the barriers were no modified suggesting that although PCs were arena specific, physical limits could affect their spatial representation. Similarly, Sharp (1997, 1999b) studied how subicular PCs firing was affected by arena size and shape demonstrating that subicular PCs replicated spatial representation across different arena size while HPCs were prompted to remap. In the same way when recordings were performed in different arena shapes, firing fields were very similar across arenas. Similar results were described in medial entorhinal cortex PCs (Quirk et al. 1992). Later work proved that neurons recorded from superficial layers of the dMEC cortex showed the capability of representing the environment in a regular structure in which multiple firing peaks spaced in a constant distance would be the nodes of a grid-like structure composed in space (Hafting et al., 2005). It was found that "grid cells" kept the same firing pattern across different arena shapes and size multiplying the number of FF in the largest arenas but keeping the distance between them constant.

Hippocampal PCs seem to be affected by size and shape in different fashion. O'Keefe & Burgess (1996) studied the effects of geometry on PCs firing. Animals were placed in a rectangular arena the dimensions of which could be modified by enlarging the open

field in different directions. It was demonstrated that hippocampal PCs would tend to enlarge their firing fields in the same direction in which the arena was enlarged. To explain these results, Hartley et al. (2000) proposed the boundary vector cell model (BVC). This model explains PCs firing as result of cortical input, from boundary vector cells, to HPCs. This input sets units in the hippocampus to fire whenever the animal at determined distance and allocentric orientation to the boundaries of the arena, predicting firing similarities across different arena shapes. This trend would change during familiarisation in new environments (Lever et al., 2002). Subicular PCs have been proposed as BVCs: they follow barriers, FF are located adjacent to them, are predominantly locational and stable over time (Barry et al., 2006). The aim of this experiment was to study further the scaling properties of subicular PCs.

In previous experiment cells were recorded in two different size arenas the small being 40cm x 40cm and the largest 80 cm x 80 cm. To evaluate scaling properties 3 different arenas were used, see methods, in this way a scaling gradient could be set across smaller to largest open fields. If subicular PC firing was driven by arena limits, it was expected to find scaling neurons, at least across some of the arena size. Scaling neurons would tend not only to have similar firing field size, but also similar location and shape. Secondly, arena-specific firing should be not observed or it should be observed seldom in comparison to scaling neurons. Larger arenas were determinant in the detection of grid cells in entorhinal cortex.

Previous studies of EC place cells demonstrated similar characteristics to those of subicular PCs due to this and the clear anatomical relationship between both structures it was though possible the existence of grid-like cells in the subiculum.

5.3 Methodology

For complete description of methods refer to chapter 2. Variations in methods used are described below.

Subjects

Six Lister-hooded male rats weighing 300g on arrival were housed individually in a standard cage and kept on a 12-h light/dark schedule with food and water *at libitum*. Animals were handled so that they would be familiar with the experimenter and to eliminate possible stressors during experimental procedure.

Apparatus

The recording apparatus consisted of different sized open fields: a small open field arena of 50 cm x 50 cm, an intermediate arena of 100cm x 100cm and a large arena of 150cm x150 cm. All were of 50 cm height and black painted and with a grey washable floor. The apparatus was cleaned between trials to eliminate the existence of proximal cues and a white card was used as visual cue being always attached to the north barrier of the maze.

Behavioural Task

Animals were food-deprived up to 80% of their original weight after which recordings started. Grains of sweetened rice were thrown in every 20 sec to random locations within the open field, keeping the animal in continuous locomotion, thereby allowing a complete sampling of the environment.

Electrode Implantation

After training animals were anesthetised with ketamine (90 mg/kg) and xylazine (10 mg/kg). Anaesthesia was maintained using isoflourane at 2%. Rats were implanted with two or 4 tetrodes consisting of four 25μm twisted coated platinum-iridium (California Fine Wire) wires mounted in a microdrive (Axona Ltd) aimed at the dorsal subiculum.

The dura was removed and the electrodes lowered into the brain to 1mm deep.. Animals were left to recover for 7 days.

Electrophysiological Recording

Signals were fed through a unity gain preamplifier which was, connected to the microdrive feeding the signal to the acquisition system (Axona, Ltd). Single-unit signals were amplified between 10-40000 times and band-pass filtered between 380 Hz and 6 KHz. One channel was used for EEG recording. Signals were filtered between 0Hz-500Hz and sampled at 250 Hz. A notch filter set to 50 Hz was used when necessary to avoid noise interference in the signal.

Unit Isolation

Data were analysed with the cluster cutting technique (Tint, Axona Ltd). Units were classified by being assigned to different clusters.

Spike trains were analysed plotting ISIH and autocorrelogram histogram ensuring clean refractory periods and that there was no artefact in spike trains.

Firing Maps

Animal tracking was performed using an overhead tracking camera which sampled animal position. Arena was divided in 322 pixels per meter. Position of the animal store in X, Y coordinates and binned to obtain firing map matrix. Frequency of firing at each position was then obtained by dividing the total number of spikes by the total time that the animal spent in each location.

Firing Field Stability

Centre of mass distribution for each firing map was obtained in two different ways. Distance of mass type 1, was obtained including all firing map bins. Distance of mass 2 only included bins which firing was above 60% of the peak rate. The distance between firing maps was then compared. Cells were deem to be plastic whenever the distance between two centre of mass 2 was above 9 bins, about 3 SD. Also bin-by-bin correlation

was used to evaluate firing field stability. To compare different arena size, the large and the intermediate arenas, (see below for a description of the dimensions), were scaled down to an equivalent size to the smaller arena. Then centre of mass and cross-correlation were calculated between conditions. This scaling was thought to be the most convenient to eliminate difference of dwelling time between the small and the large and intermediate arenas.

Firing Field Size

Size of firing field was calculated as the total percentage area of the arena in which firing field was above 20% of the maximum firing frequency, for what was called firing field size 1while firing field size 2 was calculated restricting the area to the total percentage of the arena in which firing field was above 60% of the maximum firing frequency. Finally to determine if firing fields scaled across conditions firing field size type 1 had to be within 2 SD of the total sample.

Histological Analysis

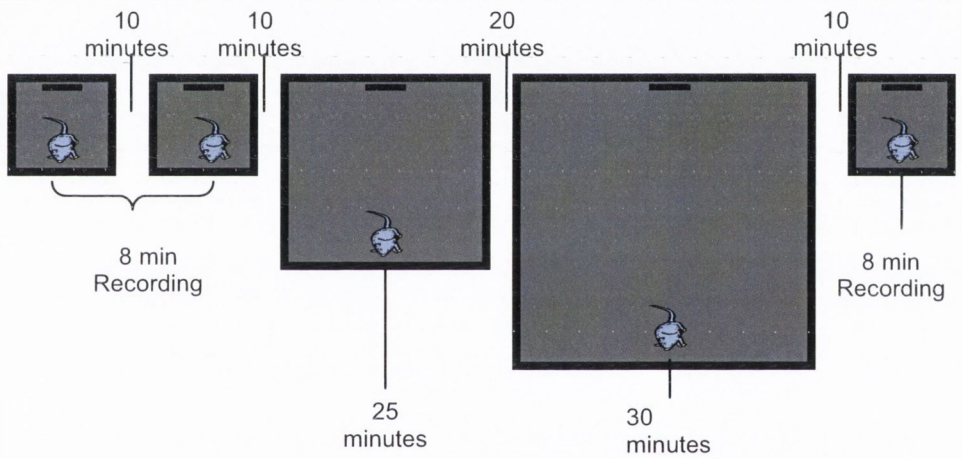
Once no more units could be isolated animals were anesthetised and transcardially perfused with saline and paraformaldehyde at 4%. Brains were extracted and store for slicing in a cryostat and stained for electrode location.

Experimental Protocol

To determine unit response to different size arenas recording started by placing the subject in the small area open field where recordings was performed for at least 8 minutes. Then the animal was left to rest in its home cage for 10 minutes and then returned to the small arena. Again recordings were implemented for at least 8 minutes. After these two first recordings the animal was left to rest again for 10 minutes and then it was placed in the intermediate arena size where recordings were performed for at least 25 minutes. Then the animal was left to rest in its home cage for 20 minutes and then placed in the large open-field arena where recordings were made for at least 30 minutes,

and to a maximum of 40 minutes. Time recording was extended in the largest arenas allowing the animal to explore the whole environment to obtain the best spatial sampling possible, although recordings were long-lasting, in some cases it was necessary to interrupt the recording session. A final recording was implemented in the small arena as a final control recording although the animal was left to rest for 10 minutes before this last recording session started. Since the arena size protocol changed from small to larger arenas and from large to small it was thought that order effects would be accounted for.

Figure 1: Behavioural Protocol

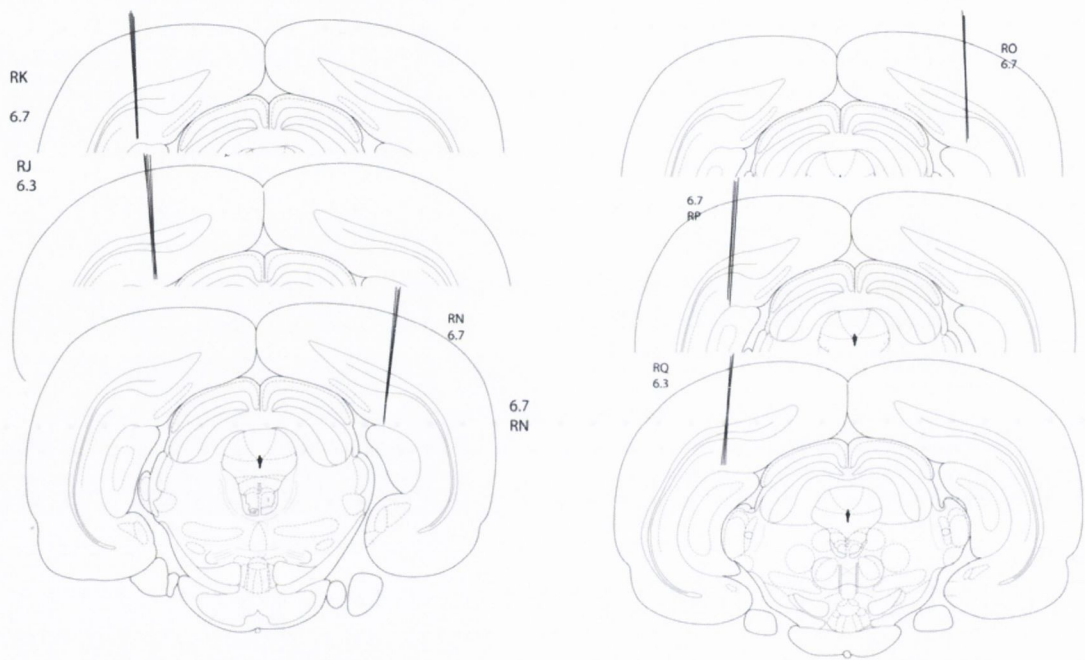


The drawing of the rat represents the initial position and orientation of the animal in all trials and its relation to the visual cues. Recordings times were increased in the largest arenas to obtain complete sampling of the arenas. Resting times were respected to keep the animal motivated in all conditions.

5.4 Results

In order of units to be included had to meet different criteria: they had to present firing in more than one condition, respect the refractory period and electrode location in the dorsal subiculum had to be verify. From an initial pool of 135 recorded during the experiment, 133 were selected for complete spatial analysis. Mean spike amplitude for the whole sample was 209 μv ±6.00 and spike width was 452 μsec±9.20.

Figure 2: Anatomical Reconstruction Of Electrode Location



General Spatial Characteristics

As described in the previous chapter and in previous experiments (Anderson & O'Mara, 2004; Sharp & Green, 1994), subicular PCs are characterised by large firing fields, high spatial coherence, low spatial selectivity and low Skaggs index. In the present work, firing field size was computed as the percentage of area above 20% maximum firing, arena size 1, and arena size 2 as the percentage of area in which firing was above 60% of the maximum firing rate. Initial recordings in the small arenas were no statistically different, and therefore values were averaged and descriptive statistics obtained. As

expected firing field size 1 was quite large and its mean value demonstrated the firing field size was above 50% of the arena size, 72.69± 1.19 %, evidencing quite distributed firing across the arena. Firing field size 2 was more restricted with an average value of 18.72%, coherence mean value was 0.81± 0.00, Skaggs and spatial selectivity mean values yielded 0.19± 0.01 and 2.43± 0.04 respectively. Since firing seems to happen throughout most of the arena area it was predictable to obtain low values in both parameters. Mean frequency firing Hz and since firing happens in most of the arena locations, the non-zero rate, 2.95±0.24 Hz, was not very different to the mean firing frequency, 2.58 Hz mean value.

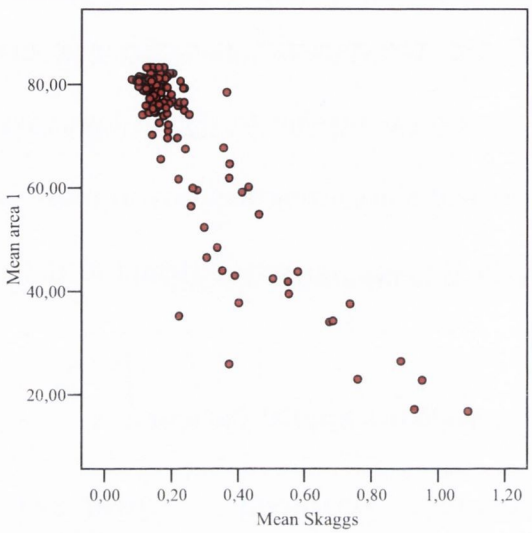
Table 1: Descriptive Statistics Spatial Parameters

	Minimum	Maximum	Mean and S.E
Mean in field maximum frequency (Hz)	0.89	21	6.03± 0.46
Mean frequency (Hz)	0.22	11.27	2.46 ±0.18
Mean non-zero rate frequency (Hz)	0.24	11.97	2.95±0.24
Mean spatial coherence	0.74	0.94	0.81± 0.00
Mean Skaggs (bits/spike)	0.07	1.09	0.19± 0.01
Mean spatial selectivity	1.57	12.38	2.43± 0.04
Mean area (above 20%)	15.12	81.68	72.69± 1.19
Mean area (above 60%)	2.63	58.99	18.72±1.08

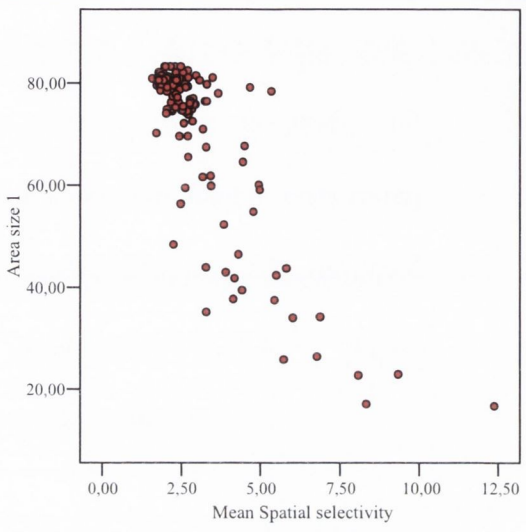
A negative relationship between Skaggs index and firing field size was found. This was also observed between spatial selectivity and firing field size, see figure 3.a and 3.b. On the contrary no such a relationship was found between firing field size, mean frequency and spatial coherence, see figure 3.c and 3.d. This indicates that it is not only the amount of spikes what produces small Skaggs index, but that firing happens across the whole arena producing a spatial gradient which generates strong spatial coherence and therefore spatial firing consistency. Firing field size 1 and firing field size 2 did not present a linear relationship, see figure 3.e. This could be due to differential change in

different portions of the firing fields. On the contrary, there was a clear linear relationship between mean frequency and mean field maximum frequency, see figure 3.

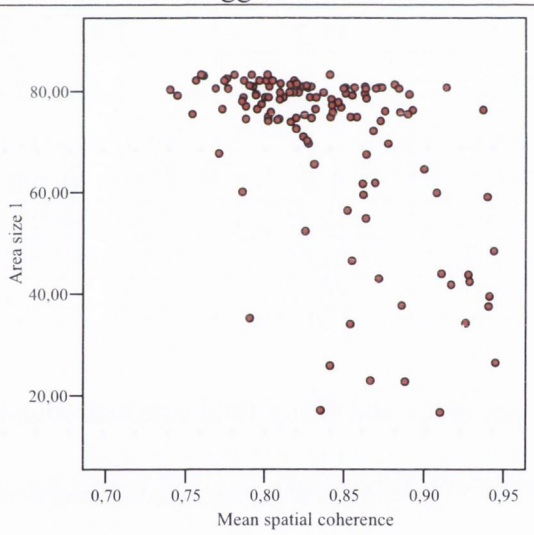
Figure 3: Scatter Plots For Spatial Parameters



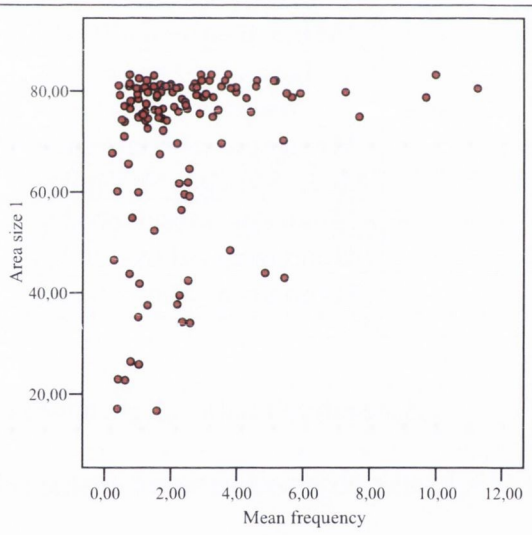
a. Area 1 and Skaggs index



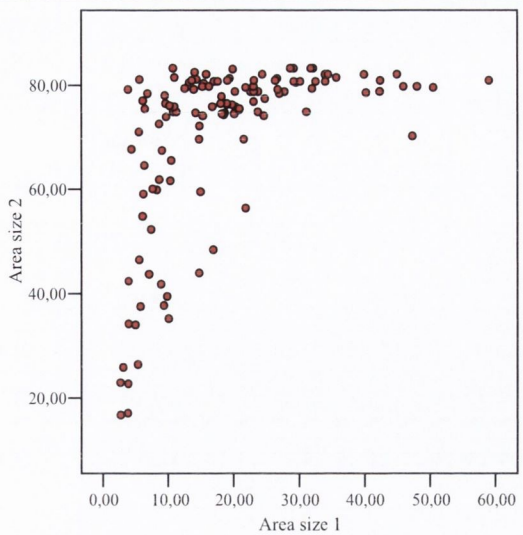
b. Area 1 and mean spatial selectivity



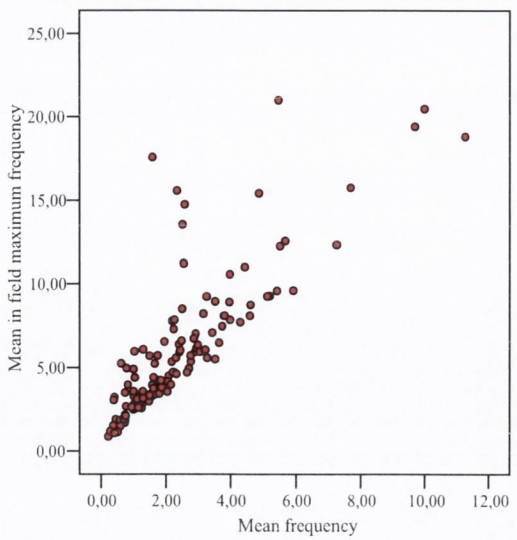
c. Area size 1 and spatial coherence



d. Area size 1 and mean frequency



e. Area size 2 and area size 1



f. In-field maximum frequency and mean frequency

Electrophysiological Type And Spatial Characteristics

Units recorded in this experiment were classified following the same criteria as in chapter 3. Bursting units had clear peaks in the short time intervals of the ISIH with higher values of the ISIH below 15 ms bins and not later than 30 ms bins. A total of 39 units (32%) were deemed as bursting units. Strong-theta-modulated bursting units were similar to the first group described above but showed a second dominant peak in the range of theta rhythm a total of 24 units (19.7%) were deemed as strong-theta-modulated bursting units. Fast-spiking units had to present a spike width below 0.300 msec. It was observed that this group of units had strong tendency to burst and they were theta modulated. A total of 11 units (9%) were classified as FS.

Non-bursting theta-modulated cells had no clear peaks in the short range of the ISIH; and presented theta modulation in their ISIH. A total of 26 units (21.3%) were classified as STM units. Regular spiking units presented ISIH without dominant peaks and no theta modulation. A total of 22 (18%) were deemed as regular spiking units. Mean values for frequency parameters as well as for spatial parameters are show in the table below.

Table 2: Frequency And Spatial Parameters Per Unit Class					
	Bursting Units	**Strong Theta Modulation Bursting Units**	**Fast Spiking**	**Non-Bursting Theta-Modulated Units**	**Regular Spiking**
	39	24	11	26	22
Spike Height (μv)	214.0± 12.58	246.41±17.45	193.73±17.47	203.86±10.46	182.42±8.99
Spike Width (μsec)	479.71±11.93	483.65±8.31	195.69±15.15	472.68±8.51	496.54±18.68
In-Field Maximum Frequency (Hz)	7.11±0.88	6.28±0.86	9.83±2.74	6.20±0.72	2.00±0.18
Mean Frequency (Hz)	2.86±0.31	3.30±0.44	4.41±1.65	2.84±0.42	0.87±0.08
Non-Zero Rate Frequency (Hz)	3.06±0.33	3.53±0.46	4.73±1.75	3.05±0.44	0.93±0.08
Spatial Coherence	0.84±0.01	0.82±0.01	0.85±0.02	0.79±0.016	0.78±0.01
Skaggs (Bits/Spike)	0.25±0.031	0.14±0.01	0.36±0.11	0.20±0.043	0.15±0.00
Spatial Selectivity	2.67±0.21	1.92±0.09	3.26±0.69	2.55±0.29	2.35±0.09
% Of Area Which Firing Was Above 20% Of Maximum	66.76±3.03	71.81±4.63	60.92±6.77	71.21±3.93	75.63±0.93
% Of Area Which Firing Was Above 60% Of Maximum	27.34±3.36	37.79±4.65	21.50±4.66	24.73±4.03	17.07±2.79
Values Are Expressed As Mean And S.E					

In-field maximum frequency, non-zero rate frequency and spike amplitude were logarithmically transformed to meet normality criteria. ANOVA analysis determines significant difference for in-field maximum frequency, mean frequency, non-zero rate frequency and spatial coherence, ($f_{(4,114)} = 11.921$; $p<0.05$, $f_{(4,114)} = 9.65$; $p<0.05$, $f_{(4,114)} = 9, 87$; $p<0.05$ $f_{(4,114)} = 3.021$ $p<0.05$). Tukey's post hoc HSD revealed significant difference between regular spiking units and the rest of unit types but no further differences between the other groups for in-field maximum frequency, mean frequency, and non-zero rate frequency. No significant differences were detected by ANOVA for amplitude and cell type, $f_{(4,114)} = 2.188$; $p>0.05$. Since apparent difference were present between STMB units and RS units further t-test was applied confirming significant differences between both groups $t_{(40)} = 3.015$ $p<0.05$. Spatial coherence significant difference was found between NBTM units and FS, B, BSTM and also between bursting units and regular spiking. Skaggs, spatial selectivity, spike width and arena size did not meet normality test even after logarithmic transformation were tested using non-parametric test. Kruskal-Wallis non-parametric test yielded no significant difference between groups for spatial selectivity, Skaggs, area size type 1 and 2 but yielded significant difference between units for spike width ($p>0.05$).

Unit Stability And Plasticity Across Conditions

To evaluate unit responses to size manipulation firing maps obtained in the intermediate arena size and in the large arena size were scaled down to the smallest arena size (50cm x 50cm), and then correlations and distance to the centre of mass type 2 were obtained. For a cell to be included in the data set it had to show stability in at least two different conditions. Of the 135 recorded cells 129 met these criteria for inclusion in data analysis.

Correlations were used to provide information about similarity between two firing maps while DCM2 described how much the core of a firing field had drifted between conditions. As discussed in chapter 4, the centre of mass seems to be more precise measurement of core firing field location for subicular place cells than maximum firing bin location. In table 3 values demonstrated that there was a stronger relationship between the centre of mass type one and correlation values.

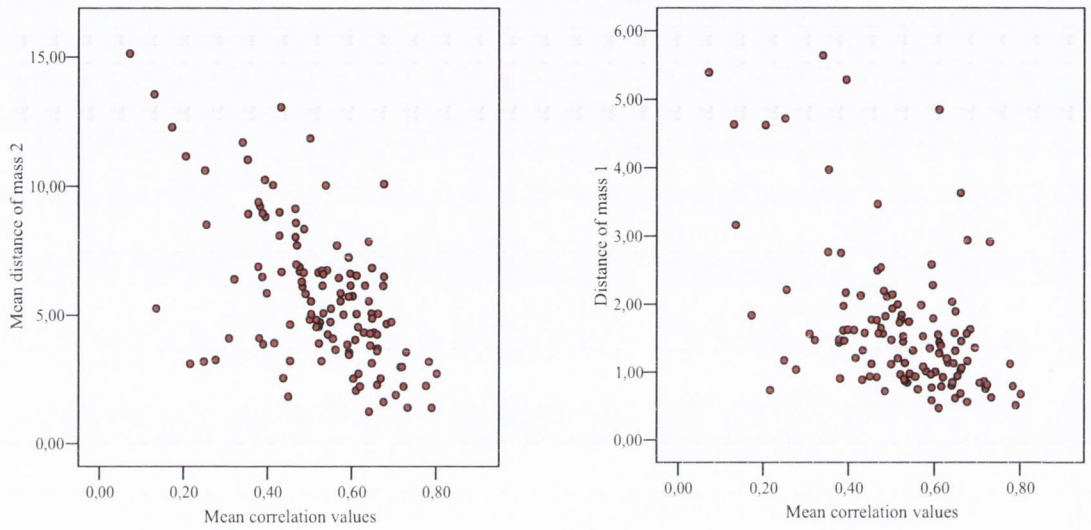
Table 3: Correlation Matrix For Plasticity-Stability Parameters

	Mean Correlation	**Mean Distance Of Mass 2**	**Mean Distance Of Mass 1**
Mean Correlation	1.000	-0.53 ($p<0.05$)	-0.44 ($p<0.05$)
Mean Distance Of Mass 2	-0.53 ($p<0.05$)	1.000	0.59 ($p<0.05$)
Mean Distance Of Mass 1	-00.44 ($p<0,05$)	0.59 ($p<0.05$)	1.000

It can be seen in the scatter plots, see figure 4, how a negative relationship between distance of mass 1 and distance of mass 2 with correlation values is confirmed as expected. It was clear that DCM1 was correlated in a significant way with correlation values but not as much as DCM2. Mean values for DCM2 and Pearson correlation

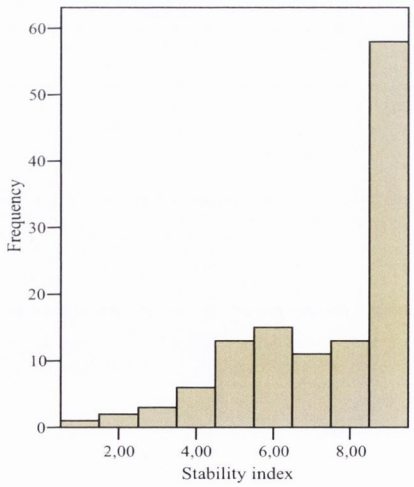
across condition suggested stability across conditions. As proposed in the previous chapter, a mean stability index for the whole set of cells was calculated by summing all the conditions in which cells were stable. Mean DCM2 value for the whole set of cells indicate strong stability 7.34, see figure 5.

Figure 4: Scatter Plots For Stability Parameters



A strong correlation was detected between DCM2 and mean correlation, (scatter plot on the left). The relationship between DCM1 and mean correlation was not as clear.

Figure 5: Stability Index



Unit stability across comparisons. There is a high tendency of units to be stable across conditions.

Unit Response To Arena Size Change

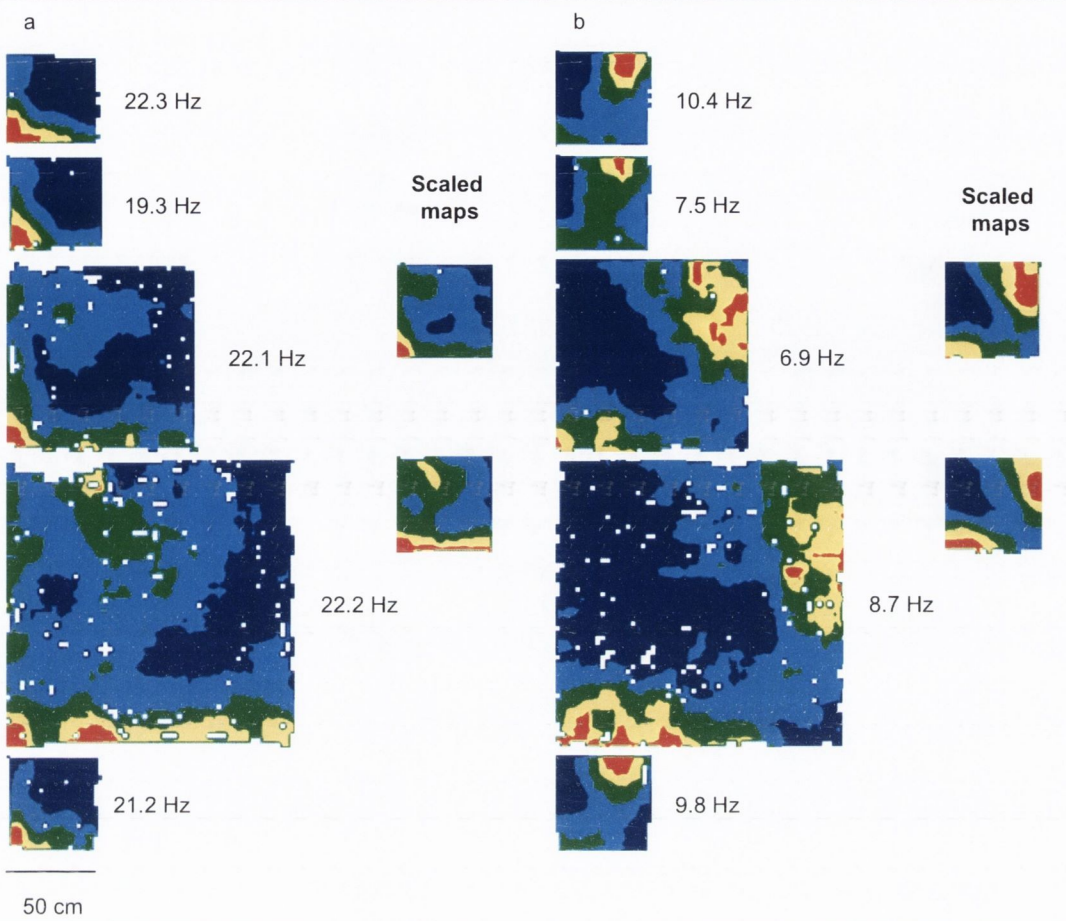
After visual evaluation of neurons, quantitative criteria were defined so that cells were objectively assigned to different groups. Centre of mass calculated for bins above 60% of the maximum firing rate and firing field size were compute as the percentage of area above 20% of the maximum firing field were used as classification parameters. To verify that neurons firing field was located in similar coordinated between conditions the DCM2 had to be lower than 9 bins across all conditions, just about 3 standard deviation from the mean distance of mass. To verify that firing field size was stable across conditions they had to be within 2 SD range of the mean arena size calculated only for those cells which fired in all conditions mean=65.6, S.D=16.49.

Criteria were combined so all possible outcomes were expressed.

Of the 129 cells 4 were not possible to classify and 9 cells were left out for posterior analysis due to extraordinary response, leaving a total of 116 cells which were active and stable at least in two leaving out from analysis those cells which could produce extreme scores.

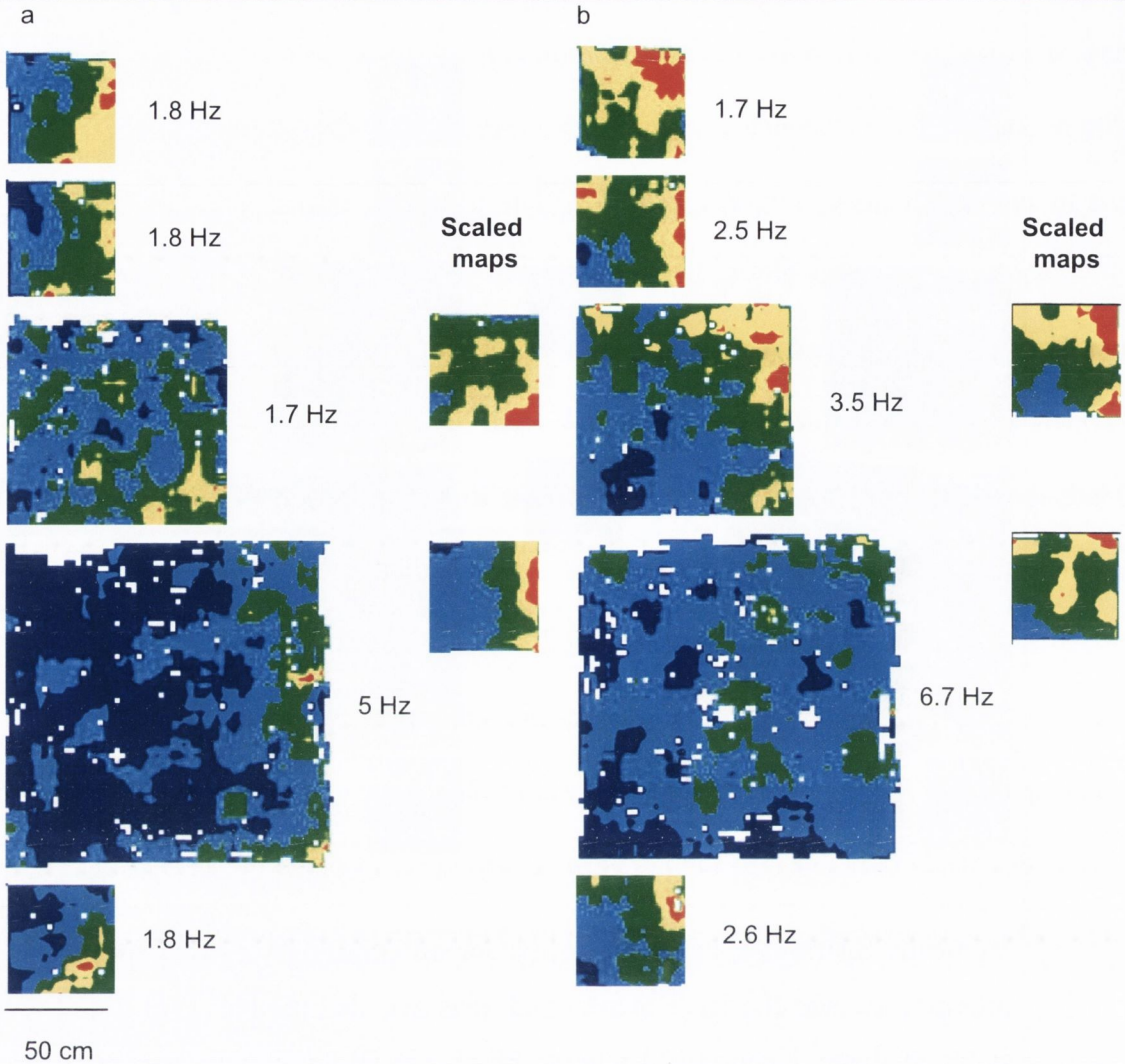
A total of 63 (54.3%) meet stability criteria for DCM2 and also firing field size stability, *good scaling neurons,* see figure 6 for examples. 5 cells (4.3%) meet DCM2 stability criteria but not arena size scaling, *imperfect scaling neurons,* see figure 7. 30 cells (25.9%) were stable in the two first condition but presented DCM2 above stability criteria but their firing field size was constant across all different conditions *remapping scaling neurons,* figure 8. Six cells, (5.1%) were stable across the two first conditions but they failed to meet DCM2 criteria across different size arenas. Finally 12 cells (10.3%) were not stable in the two first conditions but presented stability when compared between intermediate and large arena size. These two last groups were combined with a total of 18 cells (15.5%) and named *size sensitive units*, figure 9, due to their tendency of firing in the large and intermediate arena or in the small arena.

Figure 6: Good Scaling Units



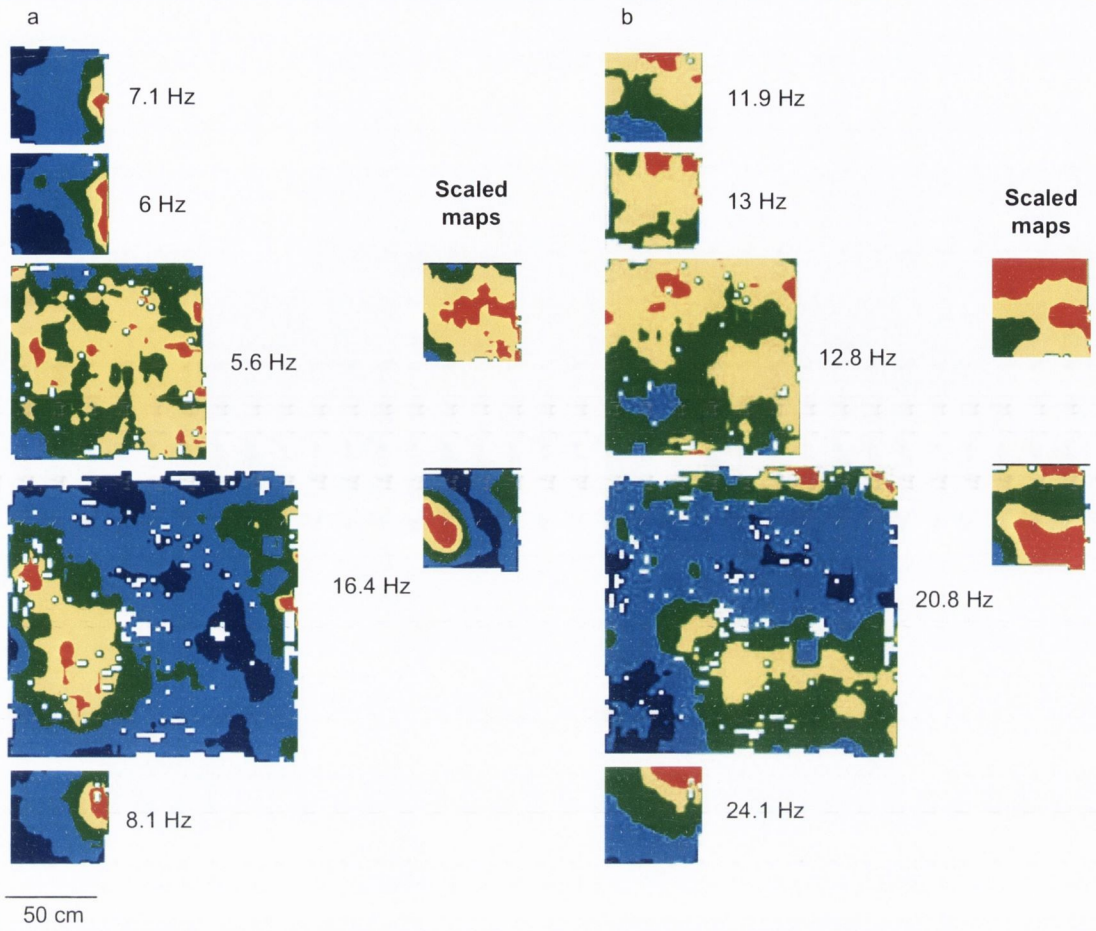
Vertically distributed firing rate maps for all conditions. Scaled firing maps for the intermediate and the large arena conditions are also plotted. Unit a and unit b were stable in shape, location and relative FF size in all conditions. Similarity between the enlarged arena conditions and the original base line is observed in the scaled firing maps.

33**Figure 7: Imperfect Scaling Units**



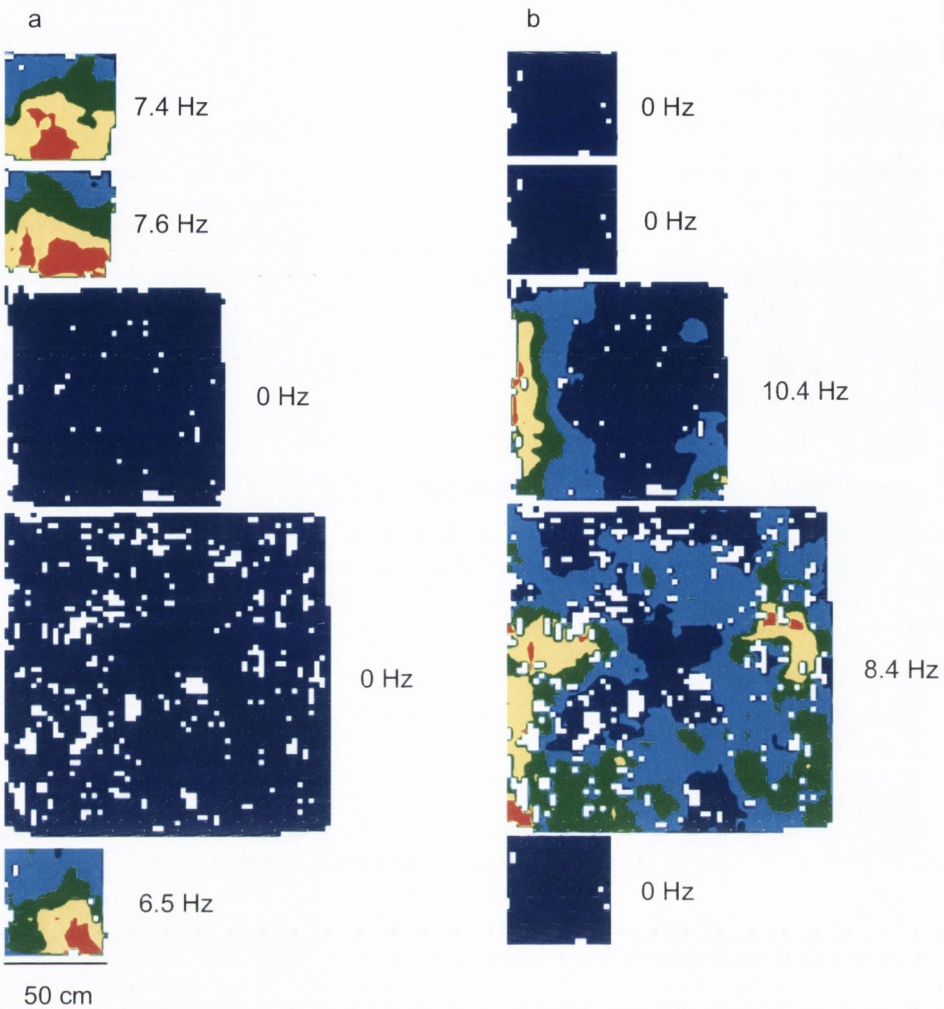
Vertically distributed firing rate maps for all conditions. Scaled firing maps for the intermediate and the large arena conditions are also plotted. Unit a and unit b were stable in shape, but not in relative FF size. Similarity between the enlarged arena conditions and the original base line is observed in the scaled firing maps.

Figure 8: Scaling Remapping Units



Vertically distributed firing rate maps for all conditions. Also, scaled firing maps for the intermediate and the large arena conditions are plotted. Unit a and unit b kept relatively similar FF sizes across different arena size but their location and shape was modified.

Figure 9: Arena-Specific Units.



Unit A had a clear stable firing field in the small arena but no firing was detected in the larger arena. Similarly unit B was silenced in the small arena but it fired in the two larger arena generating similar firing fields.

Cell Type And Condition Interaction

After cells were classified, interactions between cell group and condition were studied. Mixed ANOVA design and one way ANOVA to study simple effects between cell groups in each condition, were applied for normally distributed variables in order to determine cell group differences across conditions, see table 4 for descriptive values for the whole sample across conditions. Since no significant difference was found between the two initial conditions values were combined and mean values obtained.

Table 4: Mean Spatial Parameters Per Condition (Mean And S.E)

	Small Arenas	**Intermediate Arena**	**Large Arena**	**Control Small Arena**
In-Field Frequency	6.03± 0.46	6.10± 0.44	6.32± 0.49	5.20± 0.40
Mean Frequency	2.76± 0.23	2.40± 0.20	2.19± 0.22	2.43± 0.20
Non-Zero Rate	2.95±0.24	2.52± 0.21	2.35± 0.24	2.60± 0.21
Spatial Coherence	0.81± 0.00	0.87± 0.00	0.83±0.00	0.81± 0.00
Area 1 Size	72.69± 1.19	66.41±1.66	59.09±5.52	70.38±1.43
Skaggs	0.19± 0.01	0.19± 0.01	0.24± 0.01	0.18± 0.01
Spatial Selectivity	2.43± 0.08	3.00± 0.13	3.26± 0.22	2.42± 0.01
Mean And S.E.				

When variables were not normally distributed, even after logarithmic transformations, non-parametric tests were applied. Paired simple t-test was used to determine significant difference between conditions. Bonferroni corrections were used and 3 comparisons were made between the small arenas-intermediate arena, small arenas-large arena and intermediate-large arenas, significant $p<0.02$

Spatial Coherence

Significant differences were found between cell types ($f_{(3,110)}$=2.965; $p<0.05$) and across conditions, ($f_{(3.277)} = 9.361$; $p<0.05$). Fisher's least significant distance (LSD) post hoc determined significant difference between size-specific units and good scaling and between size-specific units and remapping scaling cells. No significant differences were found for others groups. Size-specific cells were those neurons which were stable either in the small arenas or the large arena and which scaled following the same pattern of arena specificity explaining difference between cell types and spatial coherence values. Simple effects were demonstrated, ($f_{(3,105)}$ =3.603 $p<0.016$), significant differences between size-specific neurons and good scaling neurons and between size-specific cells and remapping scaling neurons in the intermediate arena. Differences between the small arenas and the intermediate arena, small arena and large arena and intermediate and large arena were proved, $t_{(114)}$ =-8.9991; $p<0.02$, $t_{(114)}$ = -2.462; $p<0.02$, $t_{(114)}$ =-8.286; $p<0.02$.

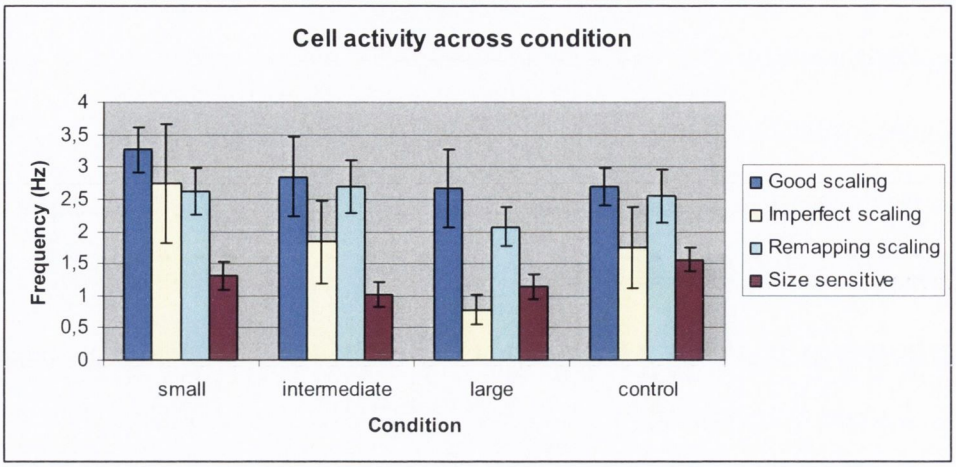
In-Field Frequency

Significant differences were found across conditions, $f_{(3,281)}$ = 54.616 $p<0.05$, and between groups of cells, $f_{(3,\ 110)}$ =3.757; $p<0.05$. LSD post hoc found significant difference between scaling and size-specific and remapping scaling and size specific. Simple effects analysis found significant difference in the small arenas, $f_{(3,106)}$ = 4.049; $p<0.05$, and in the intermediate arena, $f_{(3,106)}$ = 5.533; $p<0.05$. LSD post-hoc indicated significant differences between good scaling and size-specific neurons in the small arenas and in the intermediate arena, a result also obtained between size-specific and scaling remapping units. Across conditions comparisons yielded significant difference between the small arenas and the intermediate arena and the small arenas and the large arena, but not between the intermediate and large arena $t_{(114)}$ =-16.06; $p<0.016$, $t_{(114)}$ =-14.90; $p<0.016$, $t_{(114)}$ =-1.288 $p>0.016$ respectively.

Mean Frequency

Significant results were found within units across conditions, $f_{(3,272)} = 6.215$; $p<0.05$ and between groups of cells $f_{(3,110)} = 4.410$; $p<0.05$. LSD post hoc tests yielded significant differences between good scaling units and remapping scaling units when compared with size specific units. A simple effects study yielded significant difference between cell groups in the small arena, $f_{(3,106)} = 4.049$; $p<0.05$ intermediate size, $f_{(3,105)} = 4.569$; $p<0.05$ condition and large arena $f_{(3,107)} = 4.283$; $p<0.05$, except for the last condition $f_{(3,105)} = 1.867$; $p<0.05$. LSD post hoc tests proved significant differences between scaling remapping and size-specific and good scaling units and size-specific units finally, significant differences were also found between good scaling neurons and imperfect scaling neurons which were also different from scaling remapping neurons in the large arena. Differences between the small arenas and the intermediate arena, small arena and large arena and intermediate and large arena, $t_{(114)} = 2.854$; $p<0.016$, $t_{(114)} = 3.943$; $p<0.016$, $t_{(114)} = 1.698$; $p<0.016$.

Figure 10: Firing Frequency Across Condition



Size sensitive units were significantly less active that good scaling and remapping scaling units across conditions. Imperfect scaling units showed statistically reduced firing in the large arena when compared with units capable of scaling.

Non-Zero Rate

Significant differences were found within cells across conditions $f_{(3, 271)} = 5.443$; $p<0.05$ and between subjects, $f_{(3,110)} = 4.484$; $p<0.05$. LSD Post-Hoc tests yielded significant differences between good and remapping scaling neurons when compared with size specific units. No significant differences were found between imperfect scaling neurons and the rest of the cells. Simple effects yielded significant differences for small arenas $f_{(3,106)} = 4.154$; $p<0.05$, intermediate arena, $f_{(3,105)} = 4.616$; $p<0.05$ and large arena, $f_{(3,104)} = 4.362$; $p<0.05$ but not for the last condition, $f_{(3,105)} = 4.049$; $p>0.05$. LSD post hoc yielded significant differences between size-specific and perfect scaling and remapping scaling neurons. For condition 3 and 4 this same pattern was repeated although in condition 4 significant differences were also found between imperfect scaling and good scaling neurons. This outcome reproduces same results as those obtained for frequency of firing suggesting that imperfect scaling neurons were not able to keep their firing frequency at the same level while in the large arena. It could be suggested that different artefacts such as electrode movement or diminished speed of movement could explain differences across conditions, but no significant difference is

found in the rest of conditions, therefore it could be argued that differences could be result of some sort of physiological mechanism. It seems that arena size act as a gain factor for most of subicular neurons which adapting their firing to size are able to complete the same firing field area.

Significant differences were repeatedly obtained between size-specific neurons and scaling groups, (perfect scaling and remapping scaling), for most of the parameters. The fact that means frequency of firing and non-zero rate follow similar patterns of change is expected due to high correlation of both variables in subicular neurons.

Differences between the small arena and the intermediate arena, small arena and large arena and intermediate and large arena, $t_{(114)} = 3.098$; $p < 0.016$, $t_{(114)} = 3.649$; $p < 0.016$, $t_{(114)} = 1.172$; $p < 0.016$ were also found. It seems clear that size-specific neurons are different to the rest of groups, having a more specific response across different conditions. On the other hand imperfect scaling neurons seem to be only quantitatively different to the good scaling neurons, the frequency of firing being the only significantly different parameter. The fact that spatial coherence, is not significantly different, suggests that is qualitative similar to the scaling neurons as and that their firing only decreased across increasing arena size.

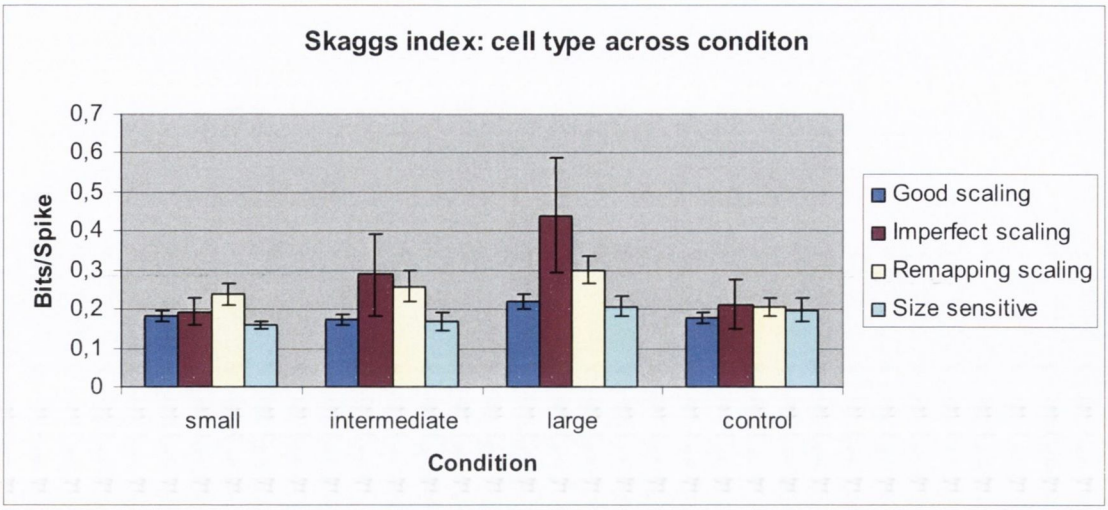
Skaggs, spatial selectivity and firing field size were not normally distributed. Thus non-parametric analysis could be performed without incurring in violation of model requirements. Non-parametric tests were used at expenses of loosing the possibility of studying interactions between factors.

Skaggs

The Friedman non-parametric test yielded significant differences within cells across different conditions. To study further difference across conditions groups were compared between them in all different conditions. Bonferroni correction was applied to reduce the probability of incurring in error type I. Condition by condition comparisons

were used to determine specific differences. Wilcoxon non-parametric test yielded significant differences between small arena Vs large arena. Also significant differences was found between intermediate and large arena ($p<0.02$). When comparing cell types across condition (Mann-Whitney), significant difference were found: good scaling and imperfect scaling in the large arena, possibly as result of the diminished frequency of firing described above. Significant difference were also found between good scaling and remapping scaling neurons in the small arena condition, the intermediate arena and the large arena but not in the control condition ($p<0.02$). When good scaling neurons were compared with size-specific units no significant differences were found. This suggest that spatial information capabilities across conditions are not different between this group of cells and that the major difference between these two groups are qualitative in the sense that size-specific units either fire or not in the different arenas but they convey as much spatial information as scaling neurons. On the contrary significant difference between good scaling and remapping units are difficult to explain. Mean Skaggs value in the first condition seems to revealed higher values for imperfect scaling that perfect scaling units. Imperfect scaling neurons were not different from scaling remapping group across different conditions and the same was found when the first group was compared against size-specific units. Again no significant differences were found between size-specific and scaling remapping neurons across conditions.

Figure 11: Skaggs Index Across Condition



Imperfect scaling units presented a smaller potential to scale in the large arena producing higher Skaggs index that other units types, and being significantly different when compare with good scaling units in the large arena, (150 cm x 150 cm).

Spatial Selectivity

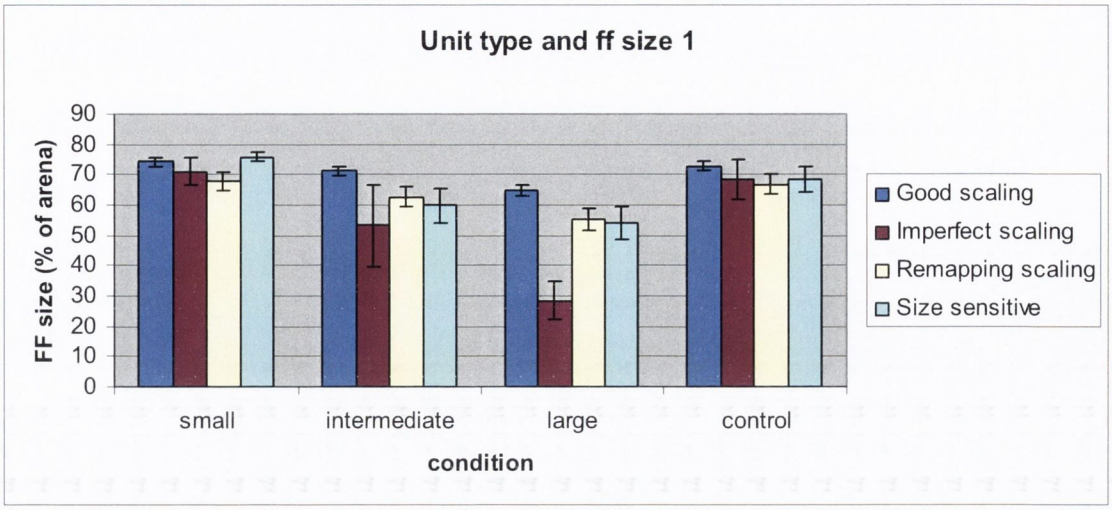
The Friedman non-parametric test yielded significant difference within subjects across conditions ($p<0.05$). Wilcoxon test found significant difference between the small and intermediate arena, small and large arena and between the intermediate and large arena but not between the small and control condition ($p<0.0125$). When groups were compared across conditions data revealed similar trend to that of Skaggs. Good scaling units only differed significantly from imperfect scaling neurons in the large arena condition. Good scaling units were different in spatial selectivity to scaling remapping units in the small arena and control condition. Difference was found between good scaling and arena-specific units in condition 1. When comparing spatial selectivity across imperfect scaling neurons and remapping scaling neurons, significant difference were only found for the large arena condition. This follows the same data trend described above for mean frequency and non-zero rate frequency, suggesting similarity between scaling neurons and difference between this type of units and imperfect scaling units. No significant difference was found between imperfect scaling and arena-specific

units. Finally when remapping scaling neurons are compared with size-specific no significant difference is found.

Firing Field Size

Friedman test revealed significant difference within units across different arena size, while Wilcoxon test demonstrated differences between the small and intermediate arena, the small and large arena, but no significant value was observed between small arena and control conditions, while again significant difference was found between intermediate and large arena. Statistic difference were found between good scaling and imperfect scaling neurons in the large arena lending support to the differentiation of both groups. No significant difference were found between good scaling and remapping scaling for the large arena condition were more extreme changes could be detected. Interestingly statistical difference was found in the small arenas and intermediate arenas. This could imply that these two groups of cells are similar in the way that they scale, and that under large scaling they have similar firing field size but that their initial firing fields are different in dimensions. Again, no significant differences were found between arena-specific and perfect scaling. When imperfect scaling neurons were compared with scaling remapping neurons no significant difference were found for any condition although p value for the large arena condition was close to significance value ($p<0.02$). No significant differences were found for any condition between arena size and size-specific and the same result was obtained when comparing scaling remapping neurons with size-specific neurons.

Figure 12: Firing Field Size And Unit Type Across Condition



Firing field size differed between cell groups as it could be predicted by their own classification. Imperfect scaling units were clearly the unit type with smaller FF size in the large arena but also in the intermediate. Statistical significance was found between good and imperfect scaling units.

Data trend suggests that cells tend to differ across two different groups of variables. Those clearly related with frequency of firing and those more directly related with spatial information processing. Although both elements are clearly related it seems that they could be grouped in two different factors.

Results indicate difference between good scaling neurons and scaling remapping neurons, in relationship to size-specific cells for those variables which were directly related with frequency of firing. Similarly, no significant difference between perfect scaling neurons and imperfect scaling neurons was detected for these variables except for mean frequency of firing in the large arena. Capabilities of neurons to keep their firing frequency across different conditions could explain also the potentiality to scale up the size of their firing fields. The fact that in-field maximum frequency was not different between this type of cells suggest that location is similarly coded by frequency of firing but the total amount of area to be cover in the larger arena would affect the total firing field size explaining difference in Skaggs values in the large arena a result

which is replicated for spatial selectivity. Interestingly Skaggs values were lower for perfect scaling neurons when compared with scaling remapping neurons. This could indicate that scaling remapping neurons could be more strongly modulated by location producing remapping across conditions. Thus while scaling remapping neurons could be more involved in spatial coding good scaling neurons could be using similar source of information to fix their firing across different conditions. The fact that significant difference was not found between remapping and size-specific could indicate that both groups of cells are coding space in a similar way being more location specific. It also could be argue that these two groups of cells failed in using the same input to establish their firing in the new arenas.

Extraordinary Subicular Neurons

As mention above a group of neurons were discarded from analysis due to a differential global response. A total of 9 neurons were included in this group. As described below 2 of them were clearly head direction cells and the other groups behave similar to entorhinal grid cells (Hafting et al., 2005). Most of these 7 cells presented either one or two clear firing peaks in the small arena. Then when the animal was located in the intermediate arena and large arena, firing fields were multiplied demonstrating tessellating properties.

This group of neurons presented strong similitude with grid cells and clear qualitative difference with all subicular cells described above, see figure 13.1. Firing tended to happened in different areas of the arena but keeping a clear organised structure which presented a clear grid-like structure which was defined by multiple firing fields spaced in an apparent equal distance which although not quantified seem similar for all cells except for one, see cell A in the table below. Mean area size 1 of firing field was 29.93 %. This value was lower than firing field size 1 for the whole sample of cells recorded

(67.14%). Also size differences were evident when looking to firing field size type 2 being 12.59% for grid-like cells and 18.11% for the whole sample of units recorded. Similar trend followed Skaggs index, which was also higher for grid-like cells, 0.68 and 0.23 respectively. Also spatial selectivity showed the same tendency, 6.87 for grid-like cells Vs 3.05 for all cells included for analysis. Significant difference were found between grid-like cells and classic subicular PCs for spatial coherence (t_{111}=-4.295, p>0.05). On the contrary no significant difference was detected for mean in-field firing frequency, mean frequency and mean non-zero rate. Significant difference was obtained when comparing Skaggs, spatial selectivity and firing field size 1 and firing field size 2, (Mann-Whitney<0.05). These results strongly support differences between both groups of cells.

Head direction cells were clearly directionally modulated. They seemed quite stable across different conditions but since double led was not used for tracking head direction more precisely, it was difficult to determine exact directional modulation of cell firing. Head direction cell A (see polar plot below, figure 14) had a clear firing peak when the animal was facing the west barrier of the arena across all conditions. Similarly cell B had selective directional firing when facing the east barrier of the arenas. In the table below it can be seen how head direction cells kept their firing within a clear range of firing across conditions.

Table 5: Preferred Angle And Frequency Of Firing

	Condition 1		Condition 2		Condition 3		Condition 4		Condition 5	
	Angle	**Hz**	**Angle**	**Hz**	**Angle**	**Hz**	**Angle**	**Hz**	**Angle**	**Hz**
Cell A	151	1.34	159	5.57	170	5.57	174	1.19	174	3.51
Cell B	344	1.91	332	2.25	353	1.98	347	2.52	332	1.81

Figure 13.1: Grid-Like Unit

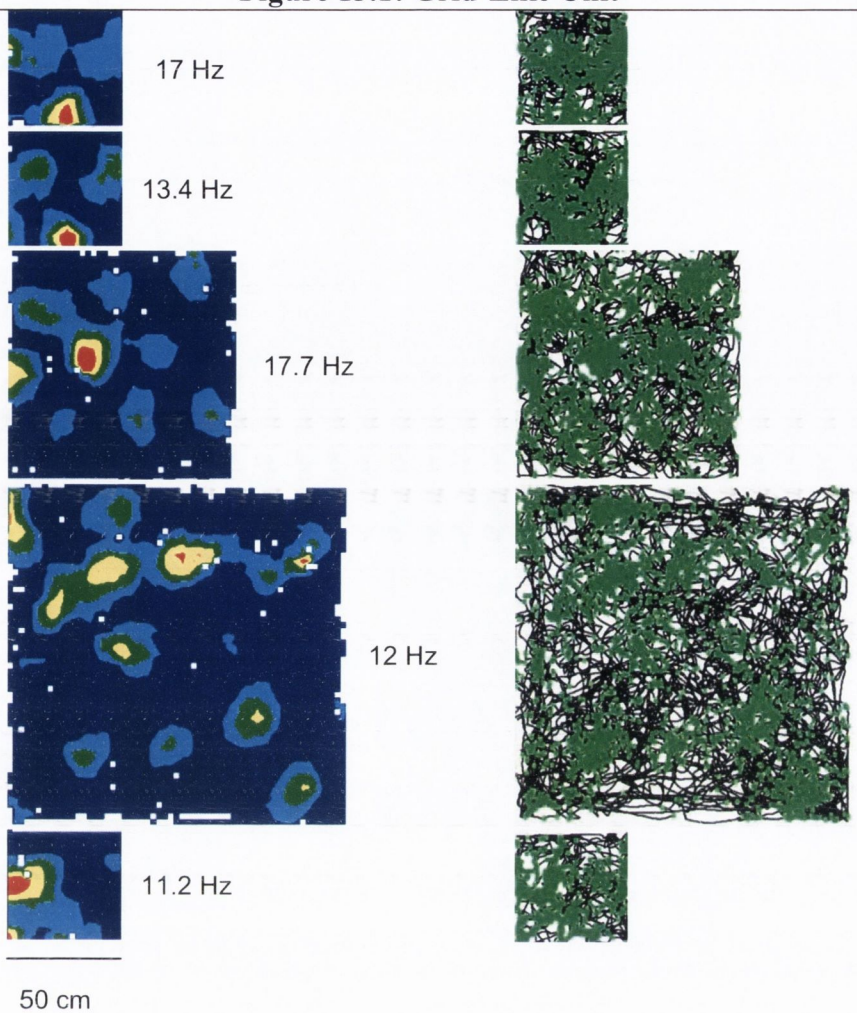
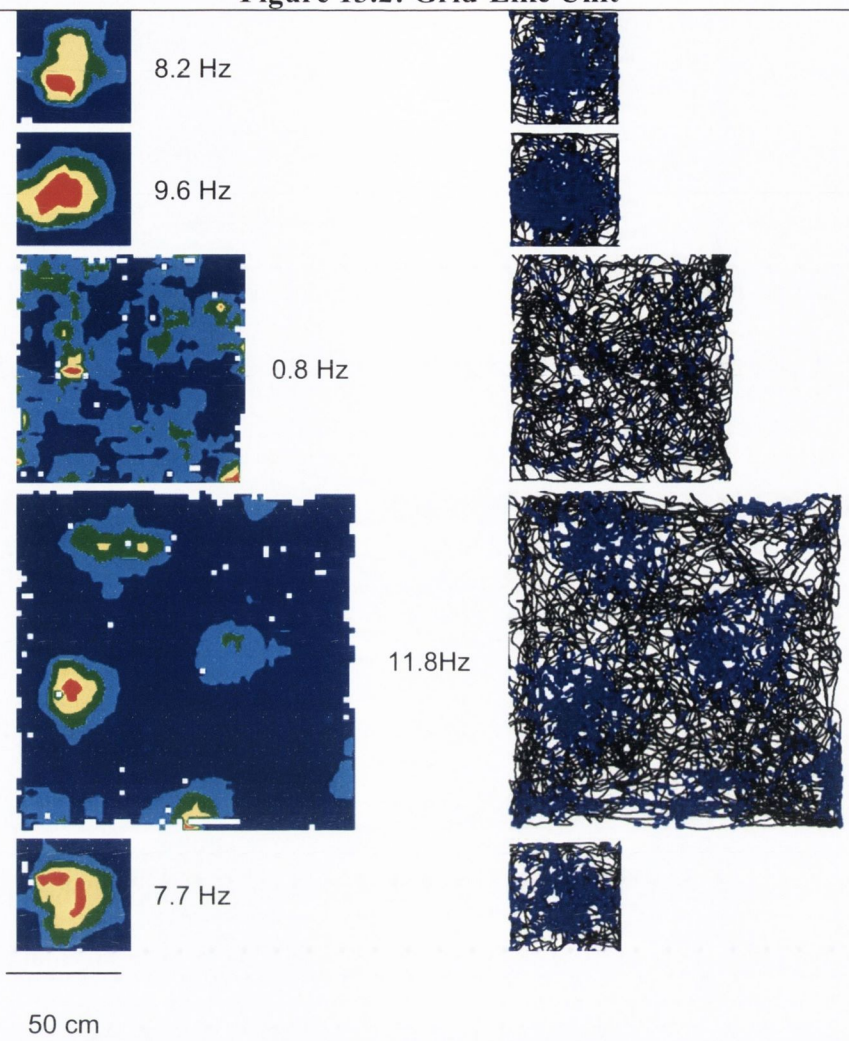


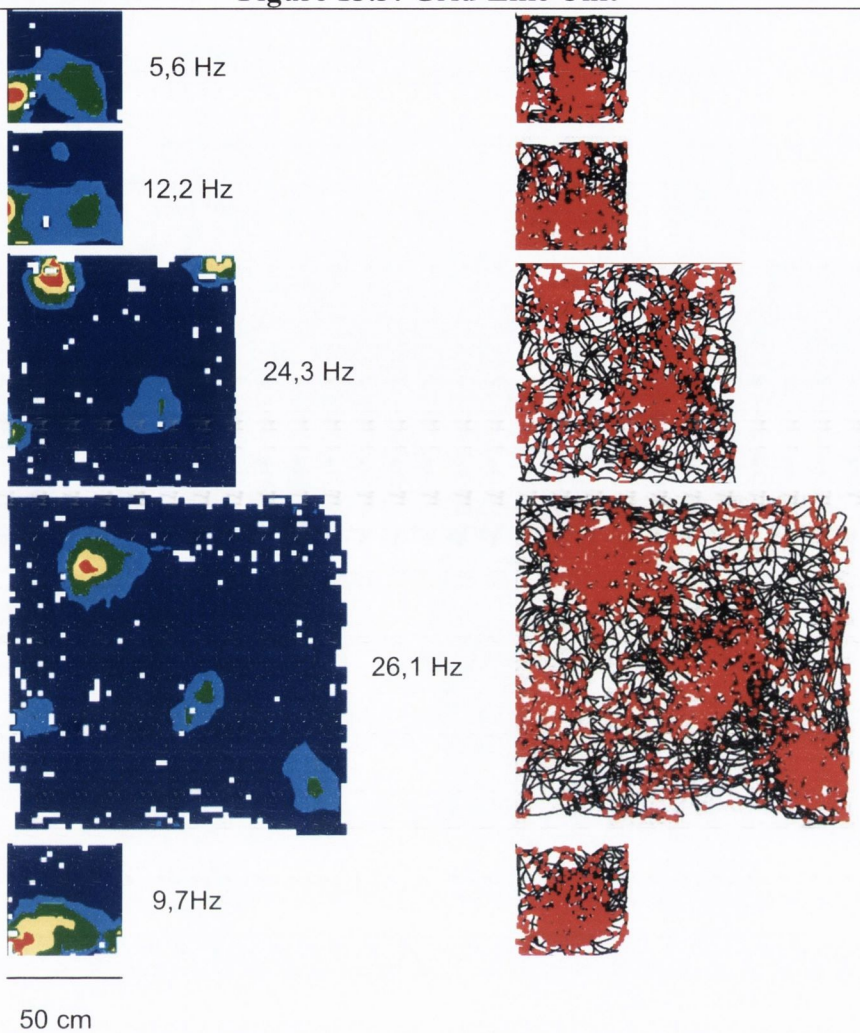
Figure 13.1: Grid-like presented multiple firing peak activity. Similar to those units described in the EC it can be seen how firing fields were organised in a very regular fashion. On the right, rat trajectory, black line, and spike positions, grey dots, reveal a grid like structure in which background firing was non existent. Not all grid cells showed the exact same spacing. This unit seems to have more density of firing fields that the rest and although they were recorded in similar ventral positions.

Figure 13.2: Grid-Like Unit



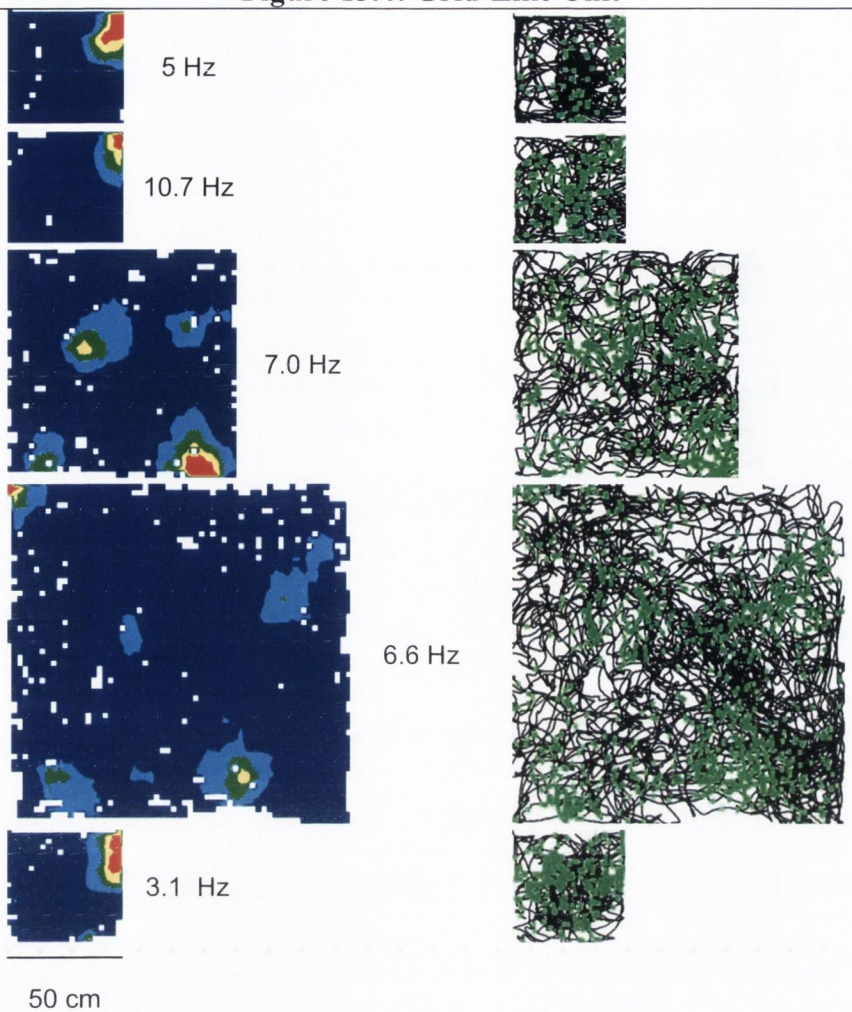
Similar to the unit in figure 13.1, this cell displayed a grid like structure although it was more silent during the intermediate arena condition. The fact that the unit was still recorded in the rest of the conditions rules out the possibility of diminished activity recorded due to electrode movement. It is possible that changes in the firing of these cells were due to transient adaptation to the arena change and the re-arrangement of spatial cues.

Figure 13.3: Grid-Like Unit



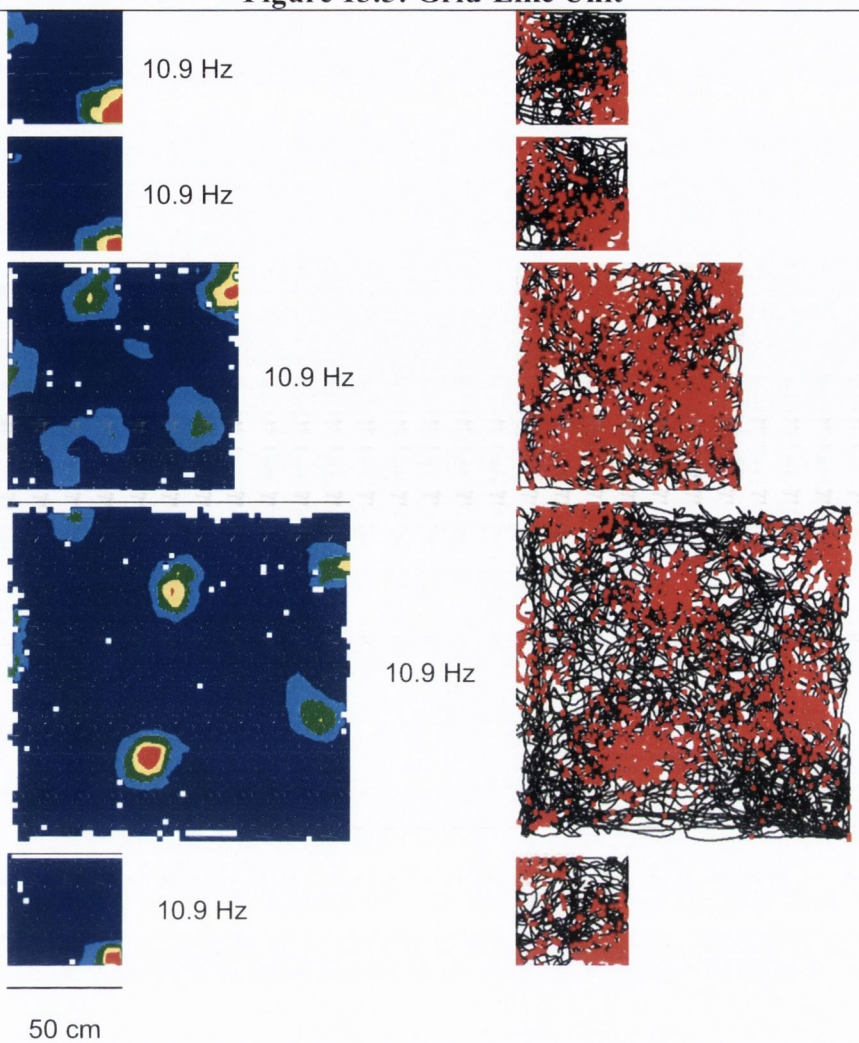
Unit firing was multiplied across different arena size environments. It seems that firing fields were more compact in the small and in the intermediate arena that in the large arena where it can be seen, map on the right how firing field is a bit more disperse in the outside areas of the firing field that in the other arenas.

Figure 13.4: Grid-Like Unit



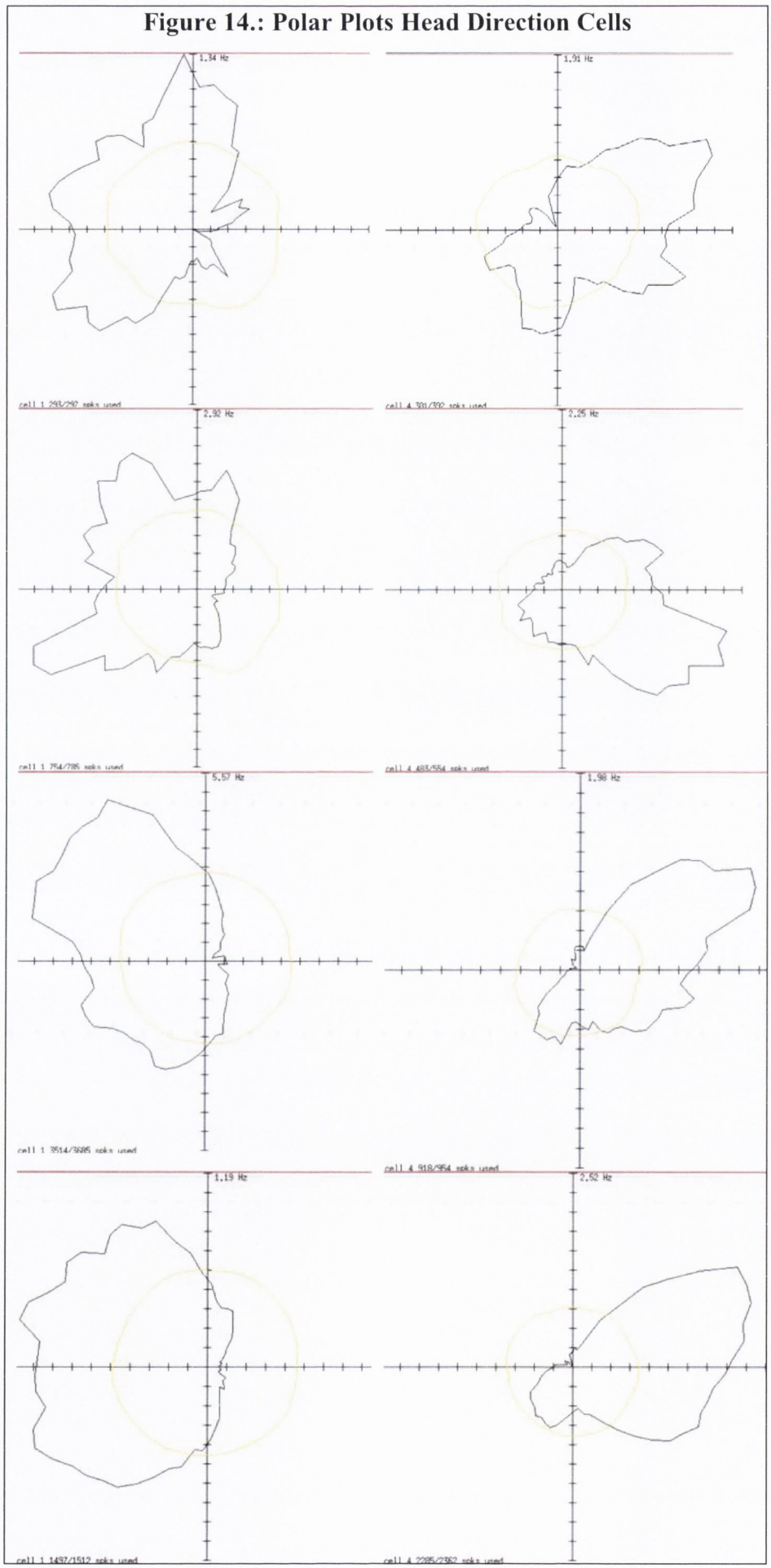
Grid-like cells show multiplication of firing fields across the largest arena size. Firing field regularity is not as striking as that observed in the EC grid cells

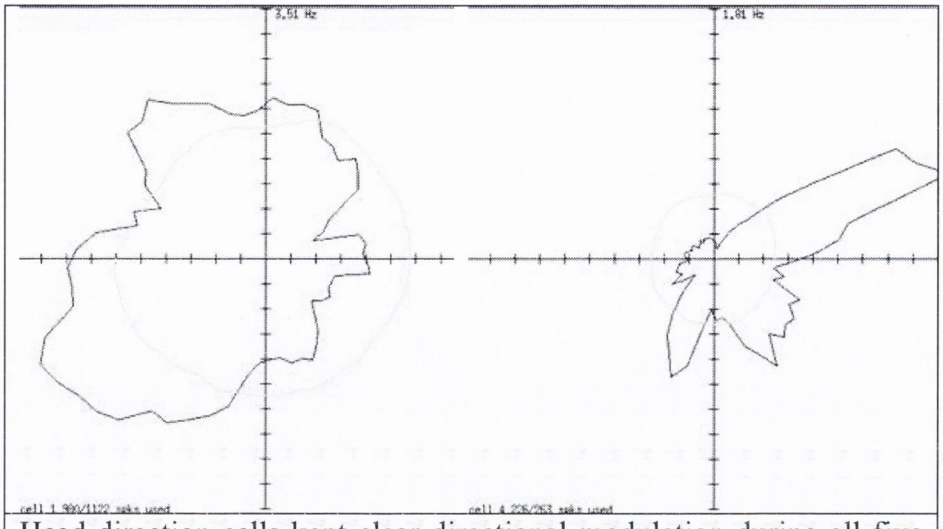
Figure 13.5: Grid-Like Unit



This unit presented quite sharp firing field in the small arena multiplying its firing in the larger arenas. Distance between firing field nodes seem to be smaller in the intermediate arena where it can be seen how two different firing fields are joined in the southwest quadrant while sharper firing fields appeared in the larger arena.

Figure 14.: Polar Plots Head Direction Cells





Head direction cells kept clear directional modulation during all five conditions, condition, first row, last condition in the fifth row. The polar plot in back represents the preferred firing angle. The yellow circle represents directional firing following the distributive hypothesis (Muller et al, 1994). This hypothesis assumes that firing is ideally location specific, and that all directional firing modulations are due to differences in the time that the rat spends in different portions of the firing field of the place cell in different head direction sectors. Yellow line is used to determine significant directional firing controlling for biased sampling

5.5 Discussion

Four different basic responses were found among subicular PCs: units which were stable across conditions and which expanded their firing fields in relation to the arena size, good scaling neurons being the largest group of cells, imperfect scaling, scaling remapping neurons and finally units which were arena specific. Most of the subicular neurons scaled across conditions keeping similar firing field shape and location, and as predicted a lower number of arena-specific neurons were present among the sample recorded. Scaling remapping neurons were similar to good scaling neurons as they kept similar firing field size but remapped from a positional point of view. Also, cells grid-like units in dMEC were recorded. The fact that recordings of these neurons were only reported in one animal, suggests that a specific area of the subiculum is either receiving monosynaptic direct input from EC grid cells, or that summation of multiple inputs would generate the same similar pattern observed in grid cells. Head direction cells were recorded in the same animal.

Good scaling neurons behave in a similar fashion as those described by (Sharp, 1999b), expanding their firing field across different arena size. It seems possible that this unit type is using arena barriers to set their firing and that arena size could act as a gaining factor. The increase in firing field size could be the direct result of EC input to SPCs. As stated above, grid cells seem to be able of replicating their firing fields across different arena size multiplying the number of firing fields (Hafting et al., 2005; Sargolini et al., 2006), if subicular cells were receiving multiple inputs from grid cells it could be expected to find sustained firing in larger environments as result of firing field multiplication of EC. This in return would increase firing filed size in subicular PCs. Subicular PCs could be using allocentric information, as suggested by Barry et al. (2006) and using grid cell information to increase firing field size. In this way expansion of subicular units would depend on metric information supplied by grid cells

and on allocentrical information obtained as result of distal cues and natural boundaries. The subiculum would be involved in the coordination and integration of idiothetic information as well as allocentric information. To this scheme subicular grid-like could be part of the path integration in subiculum. Future experiments should be aimed to understand further the role of the subiculum and the emergent characteristics of this structure. It share with the EC similar informational characteristic, both appear to code, direction, place, movement and metrics (Anderson & O'Mara, 2004; Sargolini et al., 2006) integrating the necessary inputs for an efficient navigation in new environments and in the darkness. Integration of these elements would allow the spatial processing system to access at least to a rough representation of space, before learning and familiarisation with a new environment is produced.

Although good scaling neurons was the more numerous group, remapping scaling units and size-specific units configured 50% of the sample studied, indicating that unit response to size it is more complex that so far described. Size-specific units seemed to be similar to hippocampal PCs as described by (Quirk et al., 1992; Sharp, 1997). It seems that some units could be set to fire in a range of distance larger than the provided by the small arena. This and the fact that in many cases units firing had much stepper distribution of their firing could indicate a limitation of the plasticity of units to respond to arena size. Moreover the fact that many units remapped but scaled perfectly across different arena size makes more complicate to explain the mechanism underlying unit response to environmental changes. On one side the fact that remapping units were capable of scaling across different size provides further support to the idea that environmental dimension activates a gaining mechanism which would increase firing field size in larger arenas but still is not clear why remapping happened. In some cases firing fields remapping seems to be the result of firing fields division and in other cases it seems that relative location of the FF to the barriers of the arena was different across

conditions. Mechanisms producing firing field division are not yet clarified. Previous results indicated that in some cases stretching firing field size could produce firing fields to become bimodal (O'Keefe & Burgess, 1996). Firing field division in units seems to follow a gradual process which generates different shapes, size and location in the largest arenas. This could indicate that the same unit can set its firing to two different preferred location driven by different scaling factors and different distance to barriers. It could be suggested that these units are flexible in the way they set their firing fields location rather that fire only in a specific direction or distance. Firing field division in the largest arenas could indicate the emergence of a grid-like structure although this point needs quantification. Sharp (1999b) suggested that is possible that subicular units could represent space in a fixate fashion, mapping the environment in a stable way. Pieces of this map would be displayed depending on the size of the arena. Although no support was found for this hypothesis, the fact that stepper firing distribution was found in the small arenas, and the fact that some units generate complex representation of the environment under some circumstances could suggest the need to reinterpreted the original idea in the light of EC grid cells and subicular grid-like cells, which seem to do what Sharp (1999b) described for subicular units.

Grid-like units response to arena size change followed similar pattern as described for EC (Hafting et al., 2005; Sargolini et al., 2006). EC grid cells seem not to be affected in their firing structure by changes in arena size, keeping distance between firing field constant. Further study of grid-like cells, here described is necessary to clarify the existence of different properties to those described for EC. Grid-like cells tended to multiply their firing across different arena size increasing the number of firing fields in the intermediate arena size arena and in the large arena. Interestingly, firing fields in the former arena seemed to have closer firing fields which are more difficult to discriminate from each other that when recordings were performed in the later.

Subicular PCs seems to be able to scale across different arena size, either keeping the same firing field shape across different arena size, possible using similar anchoring cues to set its firing, or by generating complex firing field which seem to response to the use of different external or internal cues. A portion of the population of units in the subiculum seems to code information in a similar way that grid cells, here named grid-like cells. Further studies are necessary to understand their characteristics. Also further studies should be aimed to understand the relationship between EC and the subiculum and their complementary role.

Chapter 6 : Effects Of Compartmentalisation Of A Familiar Environment In Subicular Place Cells.

Chapter content

1. Summary

2. Introduction.

3. Methodology

4. Results

5. Discussion

6.1 Summary

It has been proposed that subicular PCs could use barriers and directional information to extrapolate their representation to different arenas (Sharp, 1997, 1999; Sharp, 1999b). Later (Barry et al., 2006) suggested that boundary vector cells could be located in the subiculum. To verify if units in the subiculum are modulated by barriers and physical boundaries, recordings in open field were performed. This was later converted in to a complex environment by adding four barriers which divided the arena in four chambers and a transition central area between them. If barriers and directional input were modulating subicular place cells firing it was expected that firing field would be replicated in the different chambers. It was found that cells firing were modulated by barrier location and that in most cases they replicate firing in the different sub-chambers. This indicates that PCs firing in the subiculum were barrier modulated. Grid-like cells always remapped in the barrier condition, their firing field drifted position and in some cases the number of firing fields was modified.

6.2 Introduction

Sharp (1999b) proposed that firing of subicular PCs would be related to arena barriers but it relied on the path integration system which by monitoring continuous movement of the animal would provide SPCs with updating information. The idea that a path integration process was taking place in subicular PCs would fit with the concept of a transferable representation of space by the subiculum (Sharp, 1999b). Similarly, Hartley et al., (2000) proposed the BVCs model by which distance to a barrier and allocentric angular position information would drive PCs in the hippocampus underlying the relevance of physical boundaries in place cells firing. Different predictions can be made based in the BVC model: If a unit was modulated to fire in the north boundary and at close distance to the barrier insertion of a barrier in the centre of the arena would produced firing duplication by the appearance of a second firing field at short distance to the second barrier and when heading north. But also boundary insertion could eliminate firing of units tuned to longer distances. Firing fields would be more precisely tuned to short distance than long distances (Hartley et al., 2000). Different data confirms that hippocampal PCs are affected by geometrical aspects of the environment (Barry et al., 2006; Burgess & O'Keefe, 1996; O'Keefe & Burgess, 1996). Entorhinal grid cells replicate their firing structure in different arena shapes and to keep stable firing during dark and light conditions, reducing the number of firing fields in smaller arenas (Hafting et al., 2005). No experimental protocol has tried to explain the possible influences of barriers in grid cells firing. So far is clear that firing field number is delimited by the size of the arena and therefore by the barriers. If grid cells are coding for the metrics of the brain it can be expected almost no firing variation unless their firing is also affected by the general structure of the surrounding environment and in this way depending on external allocentric cues.

The aim of this study was to understand better the influence of barriers in SPCs firing. If as mention by Barry et al., (2006) subicular units are the best candidates to be BVC they should behave as it predicted by the model. The second aim of this work was to study the impact of barriers in grid-like cells. So far no modification of the grid seems to be possible after exposing the animal to different arena size. Similar behaviour was expected in those units with grid-like structure.

6.3 Methods

For complete description of methods refer to chapter 2. Variations in methods used are described below.

Subjects

5 Lister-hooded male rats weighing 300g on arrival were housed individually in a standard cage and kept on a 12-h light/dark schedule with food and water *at libitum*. Animals were handled to be familiarised with the experimenter and with general handling to eliminate possible stressors during experimental procedure.

Apparatus

The apparatus consisted in the same arena as the one used in the previous chapter, the intermediate arena 100cm x 100cm and 50 cm height. It was painted black and the floor was a grey washable vinyl, it was cleaned between trials to eliminate the existence of proximal cues. In this experiment two white cue cards were used. One, in the same way as previous experiments, was attached to the north barrier while the second was attached on the north curtain which surrounded the maze. Both cues wereidentical and no rotation was exerted in any of them. It was deemed necessary to use two cue cards due to the fact that in the second condition, four rooms conditions, one of the barriers would had have stayed on the cue location hampering its use by the animal. For the four-rooms condition, the same apparatus was divided in 4 different sub-arenas of the same size using 4 barrier extensions attached to the four different walls. Barrier extensions were of 35 cm length and 40 cm height. In this way the environment was divided in four equal size sub-arenas and a central area which the animal had to walk through whenever wanted to explore a different room.

Behavioural Task

Animals were food-deprived up to 80% of their original weight after which recordings commenced Grains of sweetened rice were thrown in every 20 sec to random locations

within the open field, keeping the animal in continuous locomotion, thereby allowing a complete sampling of the environment. Similar to previous chapter, experimental animals had to explore en search for food being the only difference in this experiment was that the arena was divided in 4 different rooms.

Electrode Implantation

Animals were anesthetised with ketamine (90 mg/kg) and xylazine (10 mg/kg). Anaesthesia was maintained using isoflourane at 2%. Rats were implanted with two or 4 tetrodes consisting of four 25μm twisted coated platinum-iridium (California Fine Wire) wires mounted in a microdrive (Axona Ltd) aimed at the dorsal subiculum. The dura was removed and the electrodes lowered into the brain 1mm. which allowed the electrodes to be lowered in steps of 25-50μm. Animals were left to recover for 7 days.

Electrophysiological Recording

Signals were fed through a unity gain pre-amplifier connected to the microdrive feeding the signal to the acquisition system (Axona, Ltd). Single-unit signals were amplified between 10-40000 times and band-pass filtered between 380 Hz and 6 KHz. One channel was used for EEG recording. Signals were filtered between 0Hz-500Hz and sampled at 250 Hz. A notch filter set to 50 Hz was used when necessary to avoid noise interference in the signal. *Data Analysis*

Unit Isolation

The data were analysed with cluster cutting techniques (Tint, Axona Ltd). Units were classified by being assigned to different clusters by showing clear refractory periods and following the same criteria defined in the main methods chapter.

Firing Maps

Animal tracking was performed using an overhead tracking camera which sampled animal position; the arena was divided in 322 pixels per meter. The position of the animal was stored in X,Y coordinates and binned to obtain a firing map matrix.

Frequency of firing at each position was then obtained by dividing the total number of spikes by the total time that the animal spent in each location.

Firing Field Stability

A centre of mass distribution for each firing map was obtained in two different ways. Distance of map type 1, was obtained including all firing map bins. Distance of map 2 only included bins which firing was above 60%. Then the difference between firing maps were compared. Cells were deemed to be plastic whenever the distance between two centres of mass 2 was above 9 bins, or about 3 SD. Also bin-by-bin correlations were used to evaluate firing field stability.

Firing Field Size

Size of firing field was calculated as the total percentage area of the arena in which firing field was above 20% of the maximum firing frequency, for what it was called firing field size 1while firing field size

Histological Analysis

Once no more units could be isolated animals were anesthetised and transcardially perfused with saline and paraformaldehyde at 4%. Brains were extracted and store for slicing in a cryostat and stained for electrode location.

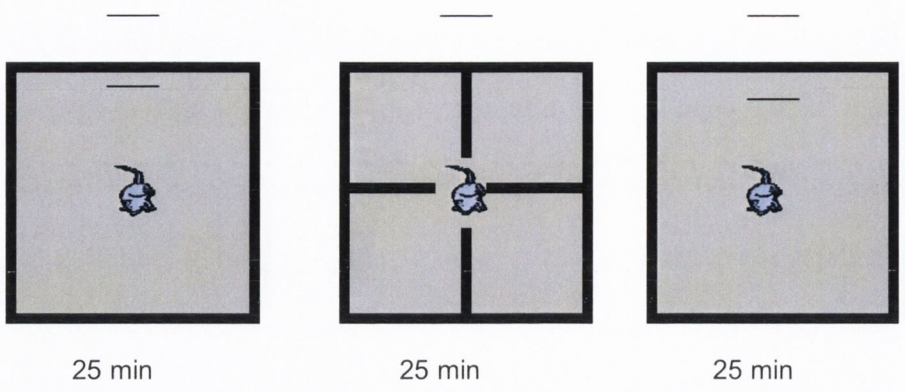
Experimental Protocol

Once the presence of unit activity in the oscilloscope was confirmed, the experiment started. First the animal was placed in the big open field without any barriers present. In this way animals were able to exploring and search for food in a familiar environment composed by a unique chamber with no barriers or boundaries except the external walls. Recordings took place for about 30 minutes except that any problem happened. Once the first recording was finished the animals were left to rest for 10 minutes in its home cage. The arena was then cleaned and extension barriers were attached so the

environment was divided in four different chambers, northwest, northeast, southwest and southeast and a central passing area.

Recordings were implemented for 30 minutes again and then animals were left to rest in their home cage for 10 minutes. The final condition was performed as a control recording to verify that whatever change observed in the recordings they were not due to electrode movement. Barriers were removed and the arena was cleaned. The intra maze white cue card was only present during the initial and final condition. Once the full protocol was implemented electrodes were lowered in the search of more units. Two animals underwent one baseline recording session and 4 recording sessions of 25 min each in the four-room arena with the objective to study possible changes during repeated exposure.

Figure 1: Standard Protocol For The Four Rooms



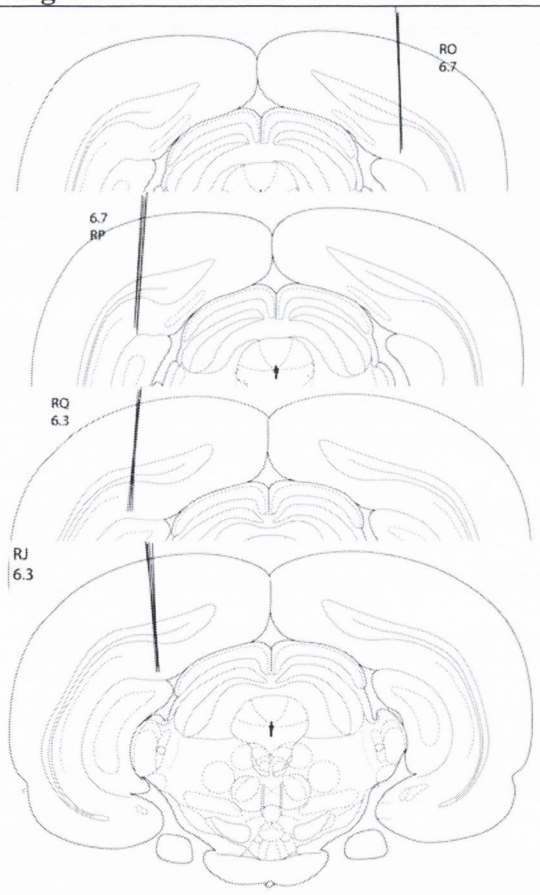
Two cue cards were available. One was attached to the north wall and the other on the north curtain aligned with the first. In this case during the insertion of barriers, animals had a polarising cue. During the standard protocol the animal was introduced in the open field and then in the four rooms and then in the open field again. Animals were always facing the south wall.

6.4 Results

Anatomical examination confirmed electrodes location in the dorsal subiculum. A total of 65 were recorded from 4 implanted animals. To be included in analysis, cells had to have clean refractory periods. Data from the first condition, big open field, was taken as baseline to describe cells electrophysiology and spatial parameters.

Mean spike amplitude for the whole set of cells recorded was 159.65±846, spike width 389.8±17.29, mean frequency 2.26±0.43 Hz

Figure 2: Anatomical Reconstruction



Spatial Characteristics

Spatial-related firing presented similar characteristics to those described in previous chapters and by other authors (Sharp, 1994). In addition to classic subicular cells and as described in the previous chapter, cells with similar firing structure to entorhinal grid cells were recorded. Mean values for all cells, grid-like cells and subicular PCs, are first described together, then similarities and differences are also studied and described. *Firing field size* adopted similar values as those described in previous chapters 64.41%. Mean frequency of firing was also similar to values obtained in previous chapters, 2.26 Hz. *Skaggs* values followed similar data trend to that observed in previous chapters, 0.27 bits/spike. Also, similar to previous chapters *spatial selectivity* was 3.75 Hz. *Mean frequency* and *non-zero rate frequency* were 2.26 Hz and 2.38 Hz respectively and *in field maximum frequency* was 5.88 Hz.

Grid-Like Cells Vs Classic Subicular Cells

A total of 8 cells (10.3%) were classified as "grid-like cells" due to their particular firing pattern being similar to that described by others (Hafting et al., 2005; Sargolini et al., 2006). Firing of this group of cells was characterised by having multiple firing fields which distance between them seemed to be constant in the open fields. Also sharpness and definition of produced firing field's was higher than average subicular place cells, which although presented multi-peak areas of activity firing fields tended to have a more distributed firing with activity peaks appearing in gradual fashion and therefore covering larger arena areas, see the tables 3.1 and 3.2 for descriptive statistics. Grid cells had smaller firing field size and tended to have also lower firing rates. Non-parametric test confirmed significant difference between grid-like cells and classic subicular places cells for firing field size (Mann-Whitney $p<0.05$). On the contrary no significant difference was observed between them for mean frequency, non-zero rate

values (Mann-Whitney $p<0.05$) and in-field maximum firing frequency ($t=_{(163)}$ 1.489 $p>0.05$). It seems that firing properties of both types of cells are not different to each other in relationship to their frequency of firing. On the contrary spatial information coding appeared to follow different pattern. First and as mention above grid-like cells have a clear grid-like structure in which firing peaks are the nodes of the grid-like structure, they tended to have similar distance between firing peaks, moreover Skaggs values and spatial selectivity were much higher than those obtained for subicular classic PCs. Subicular grid-like cells doubled spatial selectivity values with respect to classic subicular units. These differences were significantly different as suggested by t test,, $t=_{(61)}$ -3.416 ; $p<0.05$ for Skaggs values, $t=_{(61)}$ -3.523, $p<0.05$ for spatial selectivity. This difference was not found for spatial coherence which seem to be similar for both type of cells $t=_{(63)}$1.489 $p>0.05$.

Table 1.1: Spatial Parameters For Classic SPCs And Grid-Like Cells

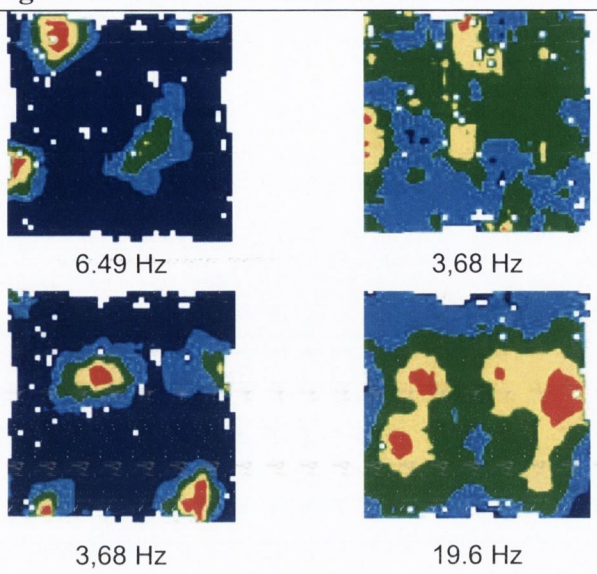
Spatial-Related Values For Grid-Like Cells And Classical Subicular Pc					
	N	**Spatial Coherence**	**Skaggs Bits/Spike**	**Spatial Selectivity**	**Arena Size (20%)**
Classic PC	57	0.87±0.01	0.22±0.02	3.32±0.25	68.92±2.93
Grid-Like Cells	8	0.89±0.01	0.68±0.12	6.79±1.05	33.93±9.37

Table 1.2: Spatial Parameters For Classic SPCs And Grid-Like Cells

Frequency Of Firing Values For Grid-Like Cells And Classical Subicular PC				
	N	**In Field Frequency (Hz)**	**Mean Frequency (Hz)**	**Non-Zero Rate (Hz)**
Classic PC	57	5.92±0.77	2.45±0.48	2.56±0.50
Grid-Like Cells	8	5.63±1.79	0.96±0.27	1.06±0.29
Values express mean and standard error				

Major differences between both cell types are observed for all spatial parameters except in spatial coherence. Although no significant difference was found for any parameter related with frequency of firing this could also be due to different sample size and large variability found in subicular place cells which firing frequency range from 0.12 Hz to a maximum of 23.40 Hz.).

Figure 3: Grid-Like Cells And Subicular PCs

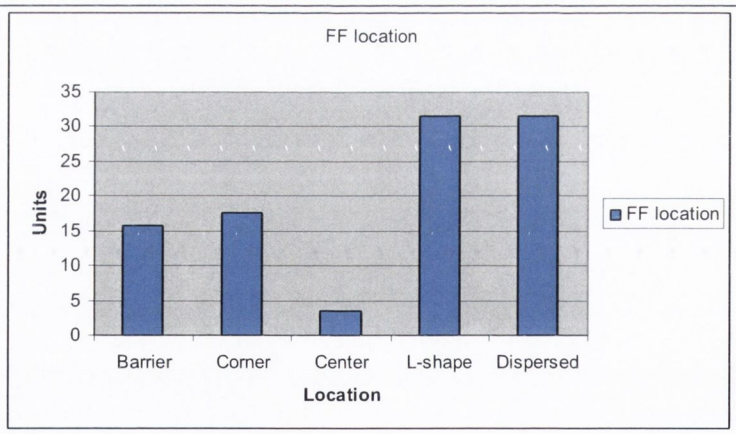


Grid-like cells can be observed in the left column, units A and C and classic subicular PCs in the right column, units B and D. It can be seen how both cell types presented multiple peaks activity although grid-like cells had less background firing that subicular cells, which firing fields are not as well differentiated from the background activity. Grid-like cells had sharper firing fields. Also firing field frequencies are higher for subicular PCs although no significant difference was found between them. Cells A, C and D were recorded from the same animal indicating that both cell types are found in a specific area. In field maximum firing frequency is expressed for each map.

Firing Field Location

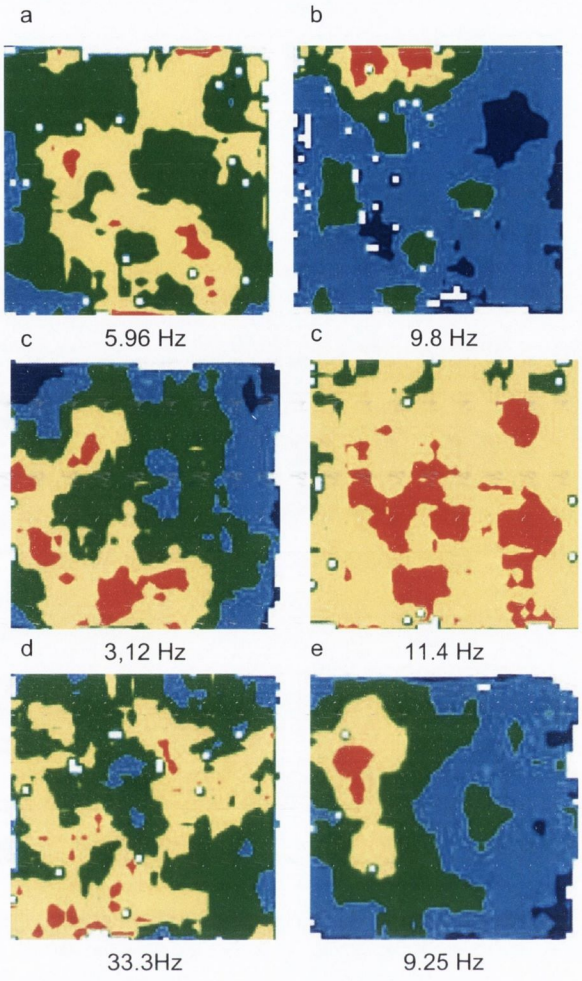
SPCs can present multiple peak activity. To simplify, firing field location codification, only the area with strongest or larger firing field was compute for each map. Grid-like cells were left out of the analysis. Units were grouped as belonging to those which firing close to one of the barriers, north, south, west and east, units which firing close to a corner, north-west, south-west, north-east and south-east, those which fired in the centre of the arena and those which had more dispersed firing. A total of 9 cells (15.7%) fired mainly close to one of the barriers. 10 cells (17.54%) presented dominant firing in one of the arena corners, 2 units (3.5%) fired mostly in the centre of the barrier, 18 units (31.57%) presented more complex firing, combining different peaks of activity or L shape firing fields alongside two barriers, since SPCs presented very dispersed activity 18 units (31.57 %) had a more distributed firing across different areas.

Figure 4: Firing Field Location



Firing field location distribution, most of unit firing field were located close to the barriers, corners or were L-Shape as expected if barriers were modulating subicular PCs

Figure 5: Different Firing Field Location



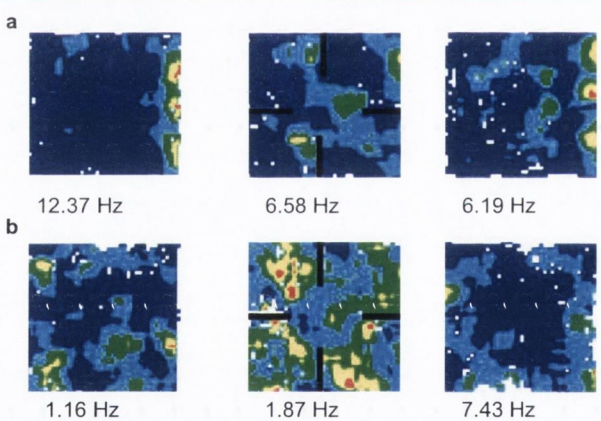
Units displayed different firing patterns. Unit **A** had strong firing in most of the arena only being less active in the north and shout barriers. of the arena. Unit **B** fired in the south and west barrier. Unit **C** and unit **F** had strong firing in all arena areas although unit E had higher frequency rate. Unit **D** had clear firing in the north barrier of the open field. Unit **F** fired the most in the north-west and along side the west barrier. In field maximum firing frequency is expressed for each map. In field maximum frequencies are expressed.

Unit Response To Environment Manipulation

Analysis of place cell stability was performed by comparing firing maps using quantitative criteria. Complete firing maps were compared in different conditions allowing for overall similitude analysis in the different trials. Further sub-quadrant analysis was implemented to obtain detailed information of firing field changes and characteristics in the different sub-quadrant.

To determine if a unit was plastic across two conditions distance between the centres of mass obtained in both conditions had to be above 1.96 S.D., being mean values 11.48±0.80 and SD= 6.13 distance between two centre of mass had to be above 12.26 bins, to calculated mean values 3 extreme values were eliminated from the data set.

Figure 6: Units Recorded In 3 Conditions



Units recorded in baseline, four rooms and final control condition. Unit **A** tended to fire in the west barrier. This firing pattern was extrapolated to the four rooms where it can be seen how although not as strong firing tend to clustered close the west boundaries in all chambers. Unit **B** presented quite disperse firing with different activity areas in the south west corner, north corner and east quadrant. Stronger firing appeared in the four room's condition and similar firing pattern to the original condition appeared in the last control recording. In field maximum firing frequency is expressed for each map

Results: Cells In 3 Conditions

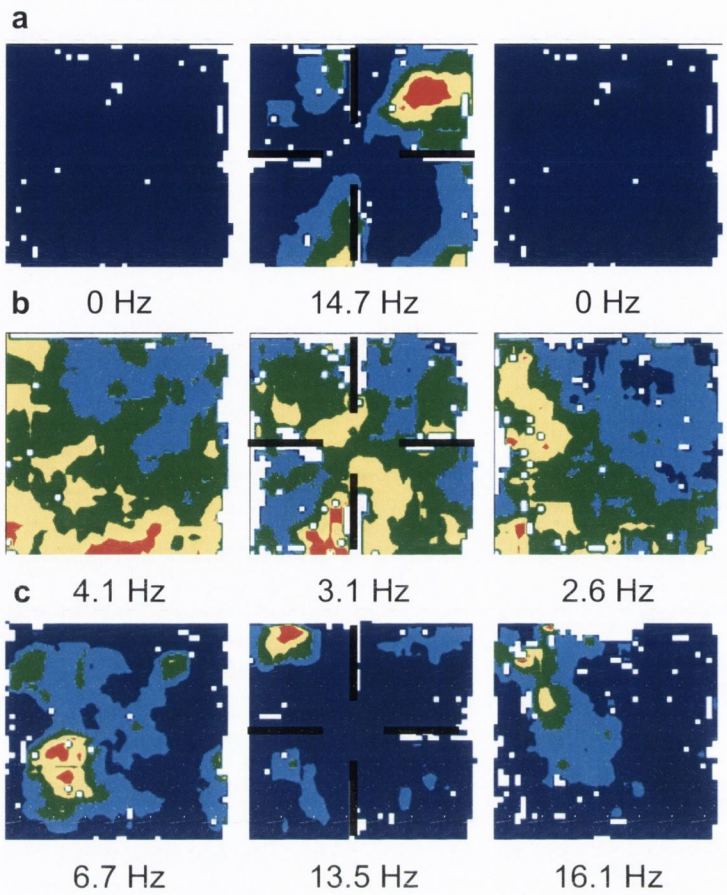
Cells only recorded in the 2 first conditions or only detected in the 2 last conditions were eliminated from the data set. In this way incomplete recording due to electrode movement or other artefacts were eliminated from the pool of cells. Units which underwent further recording were eliminated from the data set, leaving a final sample of 41 cells. Of these 18 cells (45%) meet stability criteria described above, while 22 cells (55%) showed plasticity at least in one of the conditions. A total of 15 cells (37.5.%) were deemed as plastic when condition 1 Vs condition 2 were compared, 12 cells (30 %) were plastic across condition 1 and condition 3 and finally 14 cells (35%) were deemed as unstable when the experimental condition was compared to baseline and final control condition. Moreover only one cell (2.38%) presented specific firing in the open field condition but not in the four-room condition.

After logarithmic transformation mean values for the distance of mass between the conditions and between groups were tested using repeated measurement ANOVA.

No significant difference between conditions was obtained for distance to the centre of mass, $f_{(2.62)} = 2.862$ $p > 0.05$. To further study significant difference between condition paired t-test were used, Bonferroni correction were applied, yielding no significant difference between values were obtained across conditions ($p > 0.02$).

Significant difference between plastic and stable units was found, $f_{(1, 34)} = 49.74$ $p < 0.05$. Main effects were further studied between groups in each condition. Significant values were obtained in all condition, (condition 12, $t_{(33)} = 5.337$ $p < 0.02$, condition 13 $t_{(33)} = 3.52$ $p < 0.02$ and condition 23 $t_{(33)} = 5.72$ $p < 0.02$).

Figure 7: Plastic And Stable Units



Examples of 3 different cell types: Unit a was only active during the barrier condition. Unit B although presented some apparent FF changes after barrier insertion, this unit was characterised by having not significantly different centre of mass across conditions. Unit C demonstrated high plasticity across all 3 conditions.

Correlation values were also significantly different when comparing plastic Vs stable cells in all conditions except for correlations between control baseline condition and control condition, (Mann-Whitney, $p<0.02$, Bonferroni corrections were applied, final significant value $p=0.02$). This result suggests that plastic and stable cells only differed in the response they had to experimental manipulation but that they were capable of keeping similar representation across different recordings under the same circumstances

therefore indicating a differential effect of arena modification between both groups of cells.

Table 2: Correlation And Distance Of Mass. Values For Plastic And Stable Cells Across Conditions		
	Stable	Plastic
Distance 1 Vs 2	6.48±0.72	17.54±1.83
Distance 1 Vs 3	5.30±0.74	13.71±1.95
Distance 2 Vs 3	6.48±0.70	17.77±1.76
Correlation 1 Vs 2	0.50±0.02	0.27±0.09
Correlation 1 Vs 3	0.53±0.02	0.50±0.03
Correlation 2 Vs 3	0.46±0.02	0.30±0.04
Mean And S.E.		

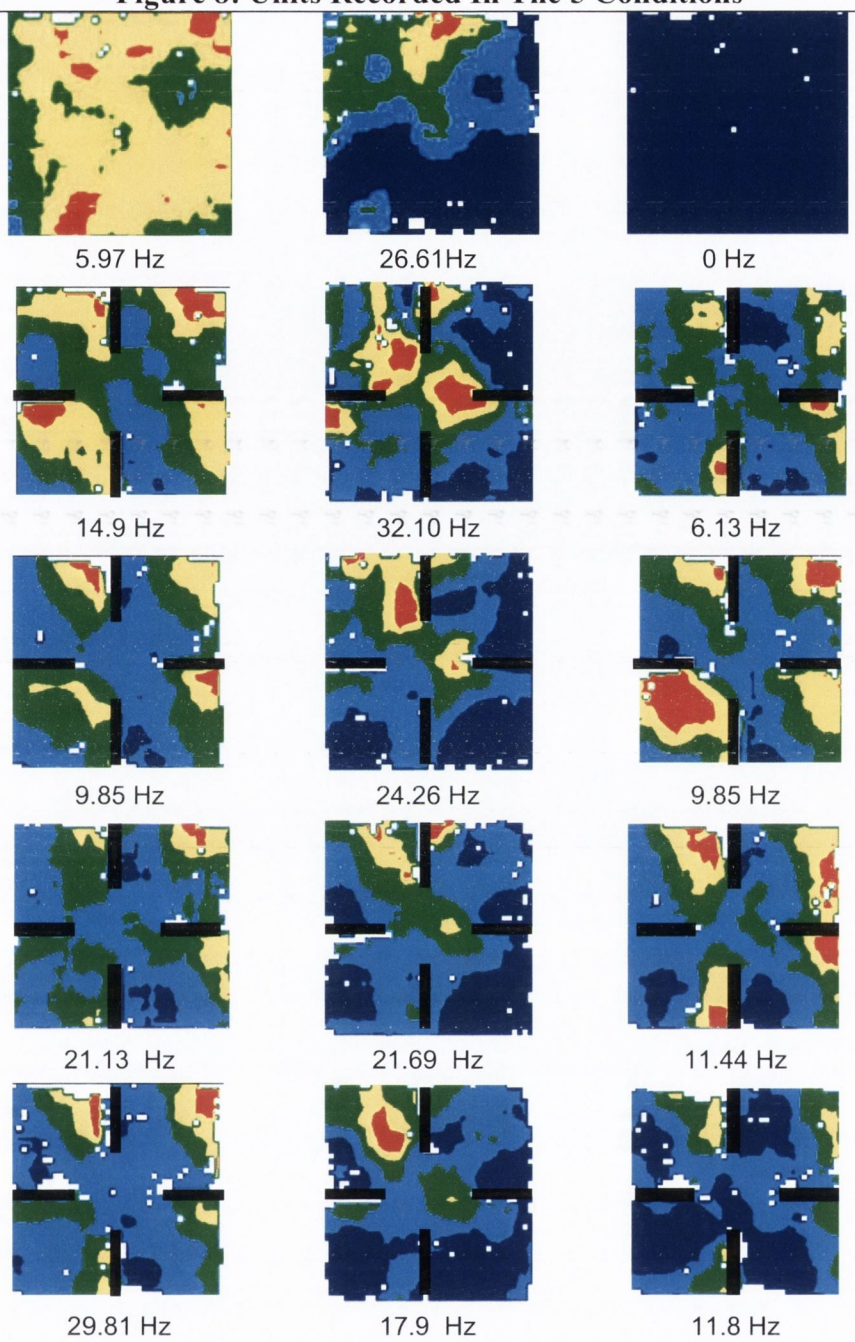
Cells Which Underwent Further Recording In The Four Room's Arena.

Nine units were recorded in the baseline arena and then underwent 4 recording sessions in the four rooms condition. Of these 9 units 2 were not included for analysis due inconsistency across the different conditions. All the rest were recorded during five sessions in a consistent way while only one unit was not detected in the baseline recording being present across all recordings in the experimental condition.

In the same way as described above units firing field stability across conditions was analysed correlating complete firing field matrixes and calculating the distance to the centre of mass between conditions. Mean correlation values for these units was r=0.59±0.02 and mean centre of mass was dm=8.016±0.97. Only one unit presented perfect stability between all 9 possible comparisons, see figure 9 unit c. 2 units were stable across 8 comparisons, 2 units across 2 and finally one unit was stable across 4 comparisons. It was observed a strong tendency of units to keep similar representation

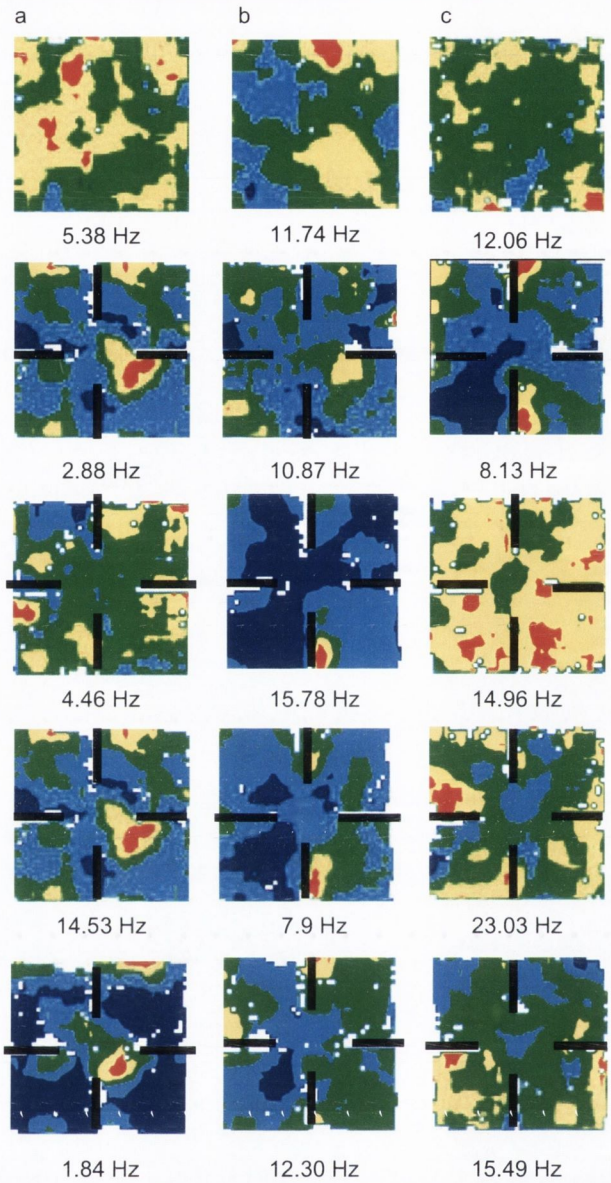
after repeated exposures to the experimental condition although firing fields tended to sharpen through the experiences in the same arena following similar results as those find by (Sharp, 1999a).

Figure 8: Units Recorded In The 5 Conditions



Units recorded in the experimental condition repeatedly tended to show high similarity across conditions with some tendency to sharpen their firing. It can be seen how units A and C had very similar structure in each sub-quadrant but also across repeated conditions. Unit B had less defined replication across sub-quadrants but its firing structure was constant across repeated exposures to the four rooms' arena.

Figure 9: Units Recorded In The 5 Conditions



Units tended to replicate their firing across multiple exposures to the four rooms condition. Although firing tended to be stable, it can be seen how unit **A** tended to reduce firing and displaying in the last condition sharper firing fields better differentiated form the background firing. Also although firing was quite dispersed in the open field it seems to adapt and to precise their firing in the small chambers. Unit **B** also showed similar firing in the four sub-quadrants. Again firing was quite dispersed in the open field arena modulating its firing down during the four rooms experiment. Unit **C** tended to present firing throughout the whole arena being not affected by the barrier conditions.

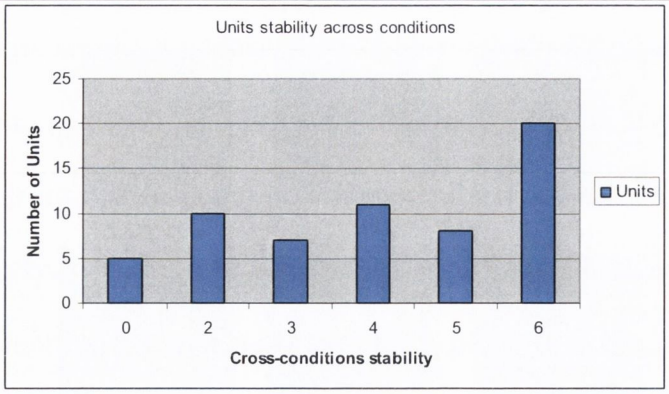
Specific Response To Experimental Manipulation

It was expected that units would display similar representation in the four room's arena. In this way if firing happened in the north-west corner in one of the sub-quadrants this would be replicated for all four different rooms except for that quadrant with no north-west corner. Also it was hypothesized that a relation between firing field location in the open arena and firing field location in the sub-quadrants could be found. For example if firing field location in the open field was found in the west barrier, it was expected to find that firing in the four different rooms would be also located in the west boundaries of each room. As in previous chapters, a quantitative criterion was used to determine if firing fields were equivalent in the different partitions of the arena. Distance of mass between two firing maps had to be below 9 bins, about 2.5 SD calculated for all conditions.

Of the 65 recorded units 3 (4.6%) displayed plasticity across all four sub-quadrants not replicating firing in any of the conditions while 2 cells (3.1%) stopped firing in the four-room arena, 10 units (15.4%) presented stability at least in two comparisons. 7 units (10.8%) were stable at least in 3 comparisons. 11 units (16.9%) were stable in four comparisons, 8 units (12.3%) were stable in 5 comparisons and 20 units were stable in 6 comparisons (30.8%), see figure 13 unit B.

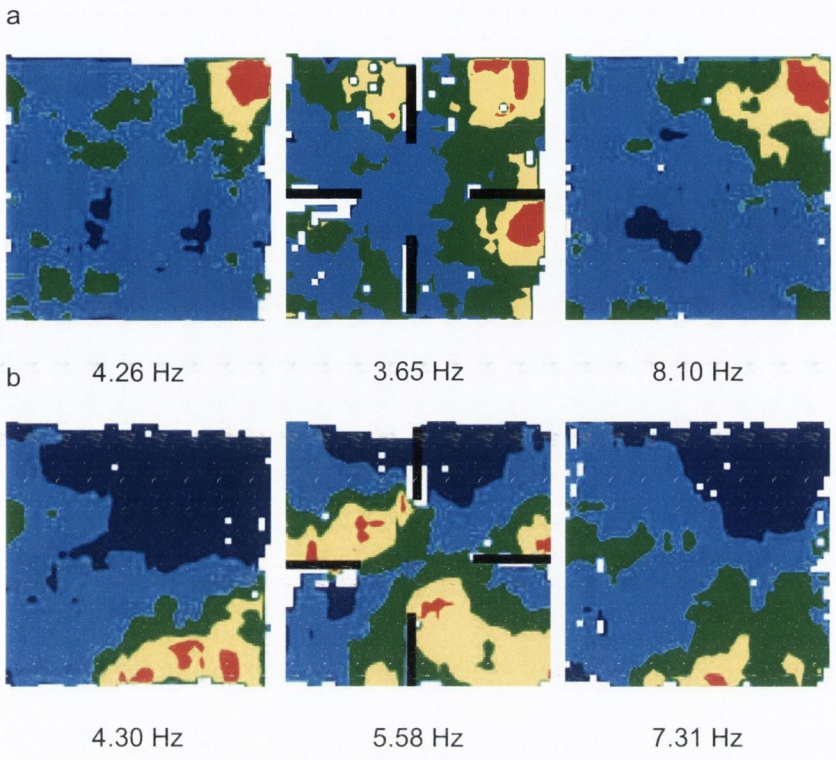
It was predicted that units would tend to extrapolate their firing across large and four room's arena. For example units firing were located in the west, east, and north or south were expected to fire in a similar way in the four sub-quadrants. In fact 21 units (32.3%) had different firing field locations, while 42 had (64.6%) equivalent firing in the four rooms and only two (3.1%) seemed not to be affected by barriers location.

Figure 10: Firing Field Replication



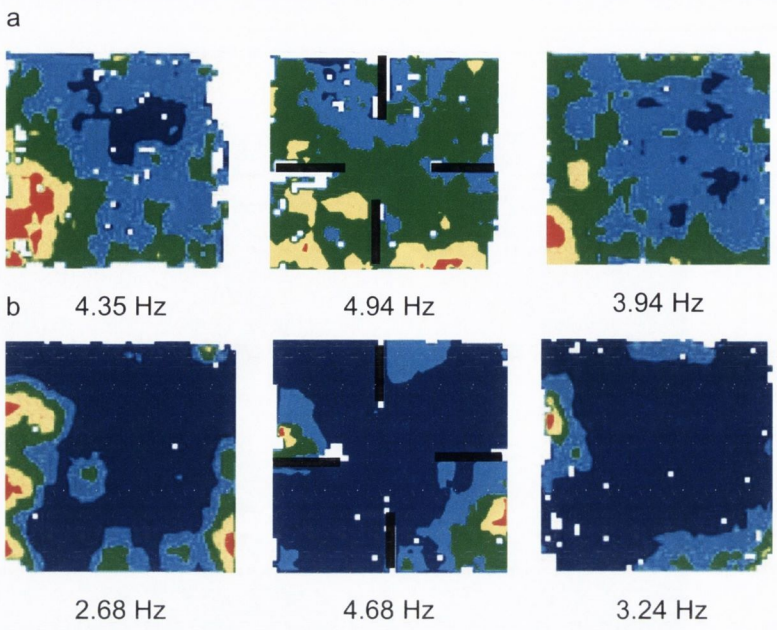
Units tended to replicate their firing across many conditions.

Figure 11: Firing Field Replication And Complex Response To Arena Division



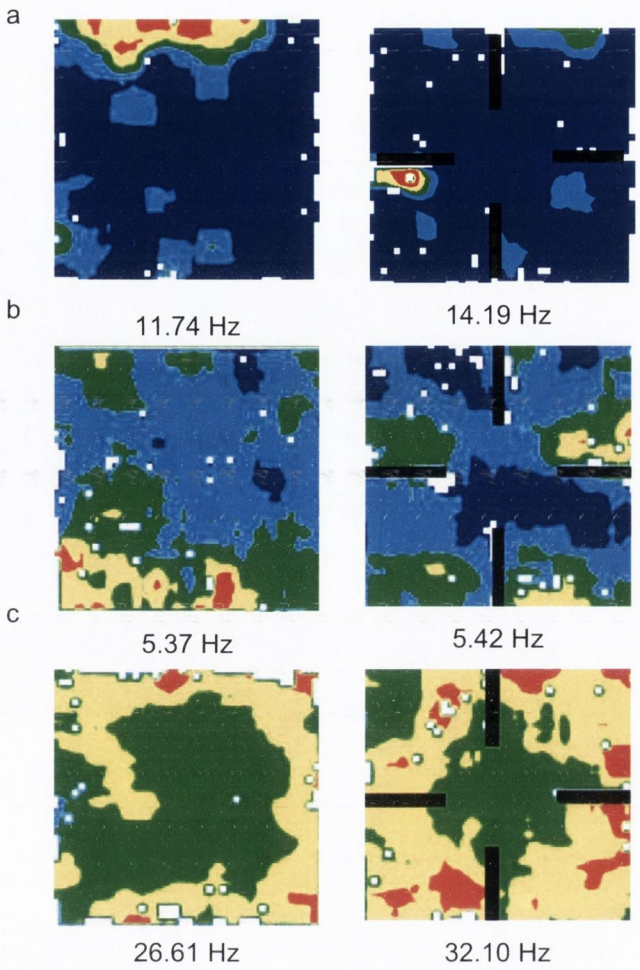
Unit a. Firing field location in the baseline condition was located in the north-east corner of the arena. This pattern of firing was replicated in the small arenas. It can be seen how all firing fields produced in the four-room chambers are located in the north-east corner. The only sub-chamber in which firing field location was not replicated was in the south west corner where no real north-east corner existed. Unit firing turned back to the original position in the open field arena. Distance of mass between north-west chamber, and north-east and south-east corner meet stability criteria. Unit b displayed a more complex behaviour. It replicated firing in all sub-chambers but also keeping strong stability with respect to the original firing pattern in the open field.

Figure 12: Units Recorded In The 3 Conditions



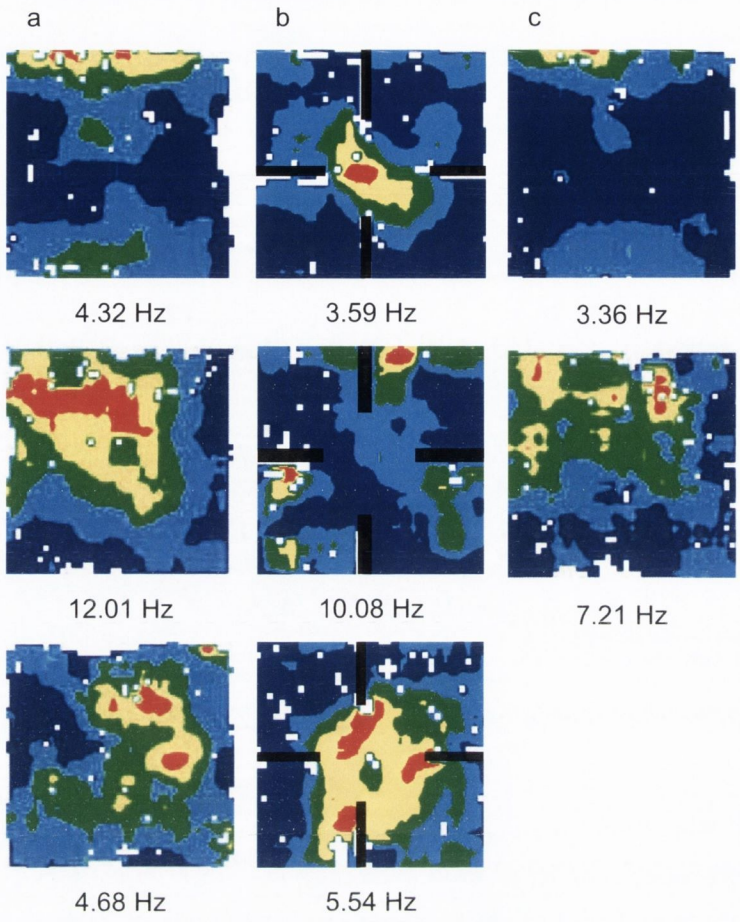
Unit A presented a firing field close to the south-west corner of the arena. In the four rooms condition firing happened in the south-west corner of the north-west quadrant, and it seems to be the same in the rest of the rooms except for the northeast chamber were the firing field drifted to the southeast corner producing symmetric representation in both arenas. Again firing field location returned to the same south-west corner in the last open field condition. Unit B presented peak of activity in the west barrier and in the east barrier. Moreover firing fields were quite sharp and well differentiated from the background firing. Response to barrier was complex. Firing field in the west barrier was reduced to a spot of activity in the south-west corner of the north-west chamber while firing in the south-east was increased. A new area of activity appeared in the north-east corner in the left barrier. Firing only returned partially to the original.

Figure 13: Firing Field Replication



Units above had equivalent representation in the four chambers. Unit **A** tended to fire in the north barrier. In the four rooms condition it can be seen how firing is replicated in the four arenas, in despite of lower in-field frequency similarity was present in all four sub-quadrants. Unit **B** displayed dominant firing in the south barrier with some dispersed firing in the northwest corner. It can be seen how firing happened in the south barriers of all 4 chambers in the four room's condition. Unit **C** tended to fire strongly in the whole arena with dominant activity closer to the barriers. Similarly firing happened close to arena boundaries in the four room's condition. It can be seen how the central are presented lower frequency of firing. In field maximum firing frequency is expressed for each map. In field maximum firing frequency is expressed for each map

Figure 14: Unit With No Firing Field Replication



Unit A, B and C were the only units which did not meet stability criteria for any of the comparisons. It cab be seen how unit A firing field drifted from the north and south barrier to the centre area between all chambers and then it drifted back to the original position firing in the north and south barriers. Unit B had a clear firing field in the north corner of the open field. Firing became more unspecific in the four rooms condition and then it drifted back to its original position in the last open field condition. Unit C was only recorded in the first initial baseline and four-room condition. Initial location of firing in the centre and north-west quadrant of the open field drifted to the centre area out of the four rooms. Unit A and C firing were attracted to the centre of the arena being exceptional response to those predicted.

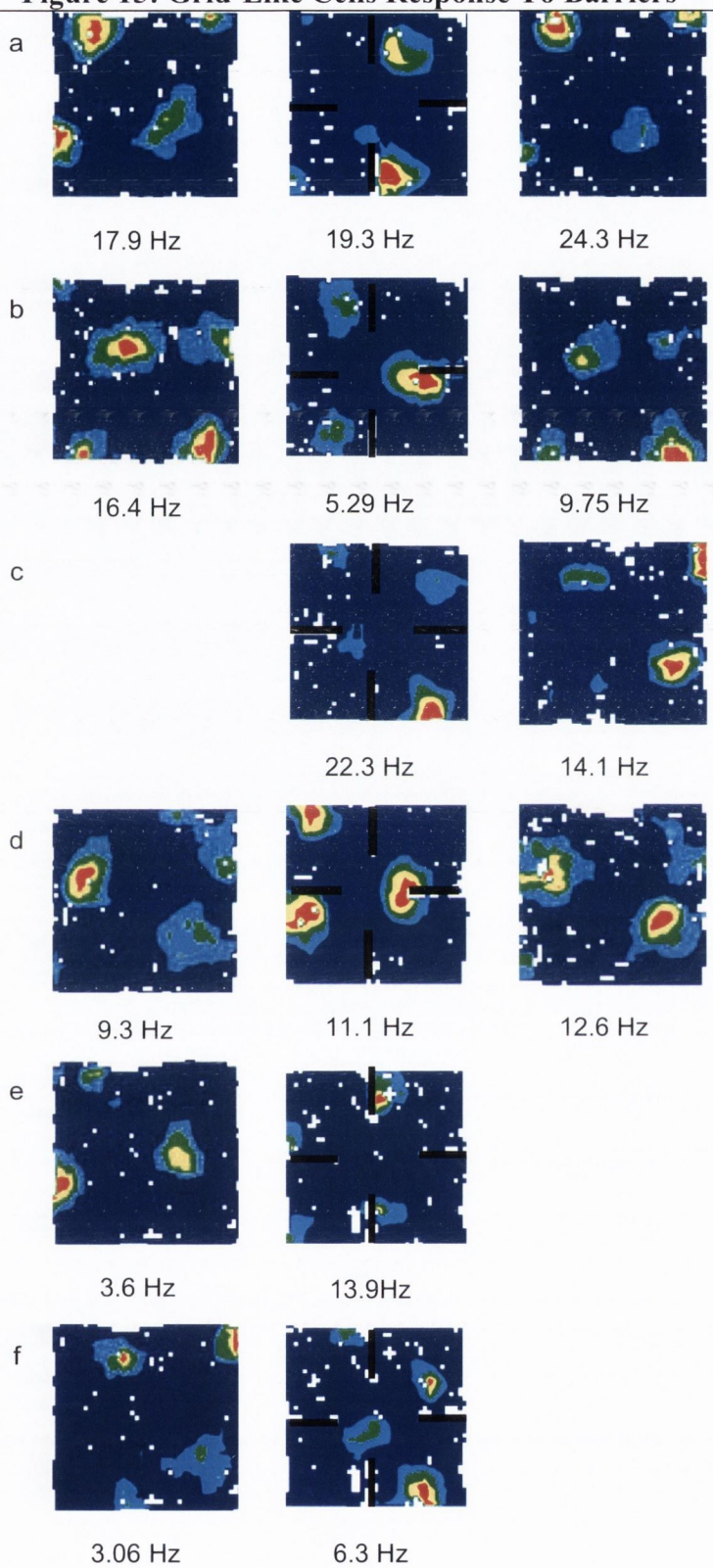
Grid-Like Cells Response To The Four Rooms Condition: Qualitative Description.

Grid-like cells tended to modify their firing structure in the experimental condition in a different way as those classified as classic subicular place cells. It can be seen in figure 12 how unit A had 4 well defined firing fields in the two open field conditions being stable in both recording sessions. Two firing fields were located close to the north barrier while the other two were situated in the south half of the arena. Firing drifted positions in the four rooms. Two were located in the south-west corner of the two south quadrants while a third firing field was found in the north-east chamber. A small peak of activity was found in a similar position to the original close to the north-west corner but in this case firing was lower. Distance between firing fields seemed to be constant in the open field while is not as clear in the four rooms. Unit B presented 5 firing fields. Two close to the south corners and two in the half north of the open field. A fifth firing area could be found in the north-west corner, although firing was less strong. In the four rooms firing field not only drifted but also the numbers of firing fields were reduced to 3. One FF was located in the north-west quadrant, another in the shout-west quadrant and a third in the north barrier of the south-east corner. In this case distance between firing fields seemed to be more stable between firing nodes. Unit C was not detected in the initial recording session but it was stable across the two last conditions. In the open field three clear peak areas could be observed. One was located in the north-east corner, a second was located in the north meddle area of the arena and the last one in the south-east quadrant. A forth spot of activity was also found in the south-centre area. In the four rooms arena firing fields also changed position. Four clear firing areas could be seen although stronger firing was found in one of the FF. One in the north-east corner, a second one was found in the north-east quadrant although sifted south in respect to the other north located firing field. Another firing field was located in the north limits of the south-west barrier while a forth firing field was observed close the south barrier of the

south-east chamber. The forth firing field was located in the south-east chamber. Unit D had 4 firing fields structured in a similar way as the rest of units, four firing fields, two closer to the north and two to the south. Again barriers produced firing field drifting leaving only 3 firing fields. Units E and F presented similar firing structure as the other units. Unit E produced an extra firing field in the four rooms condition and like unit F drifted position. In all cases units drifted their firing pattern in the four rooms but they also showed strong stability in the two open field conditions, except for units which could not be recorded in both. In one case unit was undetected during the initial recording in those cases in which units could not be recorded in the last condition was due to electrode movement after small hit of the headstage against one of the wall. Activity was not recovered after a few days.

General firing pattern of grid-like cell seemed to followed similar structure to those of EC, (Hafting et al., 2005) although it was observed that distance between firing fields was not always equal. Also firing was clearly affected by barriers. Therefore it could be suggest that firing of grid-like cells is affected by modification of the arena structure. They could be using information from the proposed BVCs to adapt their firing to arena size.

Figure 15: Grid-Like Cells Response To Barriers



6.5 Discussion

Response To Arena Manipulation

As mentioned in the result section, direct comparisons between open field firing fields and four rooms conditions allowed understanding firing field change from a general point of view. Plastic Vs stable units presented significant difference for centre of mass and correlation values between group of cells. Interestingly significant differences were found for correlation values between cell groups and conditions except when the two open firing fields were compared. This clearly suggested that units responded to manipulation of the environment, being stable in open fields recordings. Units which underwent further trials in the experimental conditions proved strong stability, but more refined firing fields were observed in the last conditions. This result is similar to that described by Sharp, (1999a), who observed a tendency for units to vary their firing fields in the latest recording sessions in a new environment.

Chamber-by-chamber comparison proved a strong elasticity of subicular units to transfer the same spatial representation in to the different rooms of the new divided arena. These results indicate that subicular PC are strongly modulated by environmental barriers being compatible with the Boundary Vector Cell model, which proposed that subicular units will be boundary vector cells modulating Ca1 place cells. It is still no clear the way in which way subicular units could drive firing of hippocampal PCs. Anatomical bases of the interaction between BVCs in the subiculum and CA1 need to be elucidated. One possible via in which subicular units could drive firing of CA1 units could be through the EC. It has been proved the existence of a projection from subiculum to deep layers and also superficial layers of the EC (Kloosterman et al., 2003). Also further interaction could be provided by connections between units in the deep layers which reach superficial layers of EC (van Haeften, Baks-te-Bulte, Goede, Wouterlood, & Witter, 2003). Although this hypothetical pathway still needs to be

elucidated, data support the independence of subicular PCs firing from CA1 input (Sharp, 1997; Sharp, 1999b).

Units not only duplicated but also generated 4 equivalent representations in the different chambers following in this way the proposed predictions. In certain cases it could be seen how more disperse firing in the large open field became better modulated in the smaller chambers of the four rooms arena As proposed in the initial BVC model Hartley et al. (2000).

Contrary to the models described above, unpredicted remapping was also detected. It could be suggested that subicular firing is boundary and directional dependant but their firing could be set to different preferred distance and angular direction whenever the new arena boundaries are modified.

Grid-Like Cell Response To Environmental Manipulation

Initial characterisation of grid cells in the EC suggests a robust and fixated representation of space which doesn't seem to be affected by size, shape or lack of external cues (Hafting et al., 2005). It is not clear if grid-like cells were also topographically organised, presenting small variation between units recorded in the same electrode, differing more clear when units were compare across different electrodes. Grid cells compose a complex structure in which firing fields were the vertex of equilateral triangles. Results above described suggest that barrier had an impact on grids. None of the recoded units were stable across conditions. Hafting et al., (2005) suggest that grid cells used external allocentric cues to set their firing. Rotation of visual cues generated rotation of the grids but also grids were stable across light to dark transitions. It is not clear if units recorded in this study share exact characteristics to those recorded in the EC. However, firing field peak distance seems not to be constant in all cases, as judge by eye. For example unit C and E in figure 12 presented 3 FF which clearly seem to shape as an equilateral triangle although a forth firing field

seems not to keep the same distance to the rest of the grid. The firing structure changed with barrier insertion modifying the number of firing fields and the distance between them. As mention by Hafting et al., (2005) grid cells use external cues to set their firing but no predictions in relation to barrier insertion is made. Barrier insertion could simple produce a change in the phase at which cell fire therefore displaying part of the grid which normally would be out of the limits of the arena, also distance between firing fields changed. This suggests that grid-like cells recorded in this work presented more plastic responses to arena modifications than those units recorded in the dMEC . The mechanism underlying this plasticity could be related to a more complex estimation of distance in a complex environment and possible due to an interaction with subicular PCs.

Chapter 7 : General Discussion

Chapter Contents

1. Summary
2. Experimental main results
3. Implication to theories
4. Future directions

7.1 Summary

As mentioned in the introduction section, the subiculum's role in spatial navigation and in other types of information processing is not yet understood. Different models and theories attributed to the subiculum different roles in spatial navigation and memory processing but also in the control of the hypothalamic-pituitary-adrenal axis (HPA), (Barry et al., 2006; McNaughton et al., 1996;. McNaughton et al., 2006; Sharp, 1999b; O'Mara, 2005 ; Redish & Touretzky, 1997; Eichenbaum et al., 1999; Deadwyler & Hampson, 2004; Hampson & Deadwyler, 2003; Hampson et al., 2000).

This thesis was aimed to understand better the electrophysiological characteristics of subicular units, but it was mainly an attempted to clarify better the spatial properties of dorsal subicular units. Properties of subicular units as described by models were experimentally tested in order to shed more light in the role of the subiculum in spatial navigation. Experimental protocols were designed to engage the animal in behavioural tasks manipulating environmental conditions which would allow us to evaluate SPCs properties.

Path integration capabilities of subicular PCs were tested in the L-D-L experiment. Scaling properties of subicular units across different arena size and mapping of PCs after insertion of multiple barriers in an open field in the four rooms experiment. Tetrode recording techniques were applied to obtain better unit separation while the animal foraged for food in different arenas. Results produced were coherent at different levels to the predicted role proposed in different theories (Barry et al., 2006; Redish & Touretzky, 1997; Sharp, 1999b) but also emergent properties were detected such as grid-like units and head direction cells.

7.2 Main Experimental Results

Main results obtained across the experimental chapters are described below:

a. Electrophysiology

i. Electrophysiological tentative classification yielded four different groups further divided in 5. Bursting units, strong-theta-modulated bursting units, fast-spiking units, non-bursting theta-modulated units and regular spiking units.

ii. Although no correlation with EEG was performed, units seem to be clearly influenced by theta rhythm in their autocorrelograms.

iii. It is not clear if FS units correspond to previous units described as putative interneurons, since many units here described as FS had small firing fields, high spatial selectivity and high Skaggs values. As described by Gold, Henze, Koch, & Buzsaki, (2006), electrode proximity to the soma is directly related to shorter spike duration.

iv. Units proved to fall in a continuous to propensity-to-burst: bursting units, fast spiking, strong-theta-modulated bursting units, Strong-theta-modulated units and regular spiking units.

v. Theta frequency range was dominant in subicular EEG.

b. Firing patterns of SPCs recorded in this thesis were similar to those described by previous work (Barnes et al., 1990; Sharp, 1997; Sharp, 1999b; Sharp & Green, 1994).

c. Unit Response To Light-Dark Transition:

i. Place cells in the subiculum seem to be mainly stable across light-dark transitions.

ii. On the contrary to hippocampal PCs (Quirk et al., 1990) order of conditions did not seem to affect PCs firing stability.

iii. Spatial parameters such as firing field size, spatial coherence, mean frequency and spatial selectivity were not different between light and dark conditions or when protocols were compared. Only Skaggs values were different, being higher in the dark.

iv. None of the recorded cells switched off firing during recordings in the dark, probing more robust firing in the darkness that hippocampal PCs and therefore being independent, to a large extent, from allocentrical visual cues (Markus et al., 1994; Quirk et al., 1990; Save et al., 2000).

v. Place cells plasticity produced during light-dark transitions did not follow a light or dark pattern. Firing field drifted randomly across different transitions.

d. Subicular Response To Arena Size Manipulation:

i. In the same way as in the light-dark experiment, units showed similar firing fields properties to those described in the literature.

ii. About 50% of units were stable across different arena size while 50 % had specific firing fields for the large arena or small arena or remapped across conditions.

iii. Firing field shape and location followed description made by other authors (Sharp & Green, 1994)

iv. Four different cell types were determined after evaluating scaling of the firing field size and stability across conditions:

1. Good scaling units: units which were stable and which scale firing field size across conditions.

2. Imperfect scaling units: cells which although being stable across conditions did not scale across different arena size.

3. Scaling remapping units: neurons which were stable in the two first control conditions but which remapped in the different arena size keeping their firing field size constant.

4. Size-specific units: units which were stable or presented firing in the large and intermediate arena or in the small arena.

e. Subicular PCs Response To The Four Rooms Arena

i. Barrier insertion produced PCs remapping when many cells were compared between the open fields and the four rooms.

ii. Units which underwent further experimental recording sessions presented high correlation across repeated recordings although it was observed how in some units, firing fields became better defined across repeated experience in the environment. Similar results were also underlined by (Sharp, 1999b).

iii. Barrier insertion produced units to replicate firing in different degrees across the four different sub-quadrants. Only 3 units had no equivalent representation across the different sub-chambers while most of the units were similarly in more than two comparisons between chambers.

iv. In almost no case units did not stop firing after barrier insertion.

v. A relation between firing field location in the open field and the different chambers of the four rooms was found.

vi. In some cases more dispersed firing in the large firing field was better spatially tuned producing more specific locational firing.

vii. If units are set to fire in a determined distance to a barrier, this seem not to be constant. Similarly, directional firing modulation could be affected if boundaries are not present.

f. Emergent properties of units in the subiculum were encounter.

i. As described in chapter 5 and chapter 6, units with a grid-like firing pattern were detected. These units tended to multiply firing in larger arenas keeping the same general grid-like structure and were apparently equal to entorhinal grid cells (Hafting et al., 2005; Sargolini et al., 2006).

ii. Head direction cells were also found simultaneously with grid-like cells and classical subicular PCs. Head direction cells and grid cells have been also found in the entorhinal cortex (Sargolini et al., 2006).

iii. Interestingly, grid-like cells recorded in the subiculum did seem to modify their firing structure by the insertion of barriers in an open field, see chapter 5.

7.3 Implication For Theories

Results obtained in chapter 4 suggest that subicular units are capable of keeping a stable representation across light-dark transitions. In previous work, no such stability was ever found in hippocampal PCs (Quirk et al., 1990), although it was suggested that sensory cues generated by the animal while navigating could improve spatial stability (Save et al., 2000). It appears, unless uncontrolled cues were available, that firing field stability was the result of a path integration mechanism as proposed by Redish & Touretzky (1997), who suggested in their model, that all structures involved in path integration should be capable of keeping their firing during dark periods. Interestingly, the fact that trial order did not affect spatial stability suggests that sense of direction, and distance to the different barriers, possibly calculated by using idiothetic cues such as motor input or vestibular information would have helped the system to keep firing field stable. As reported by (Sharp et al., 1990) angular position in relationship to external cues predict rotation of firing field in relationship to cue rotation, further supporting these results, Knierim et al. (1995) demonstrated that repeated experience in an environment with constant orientation of the animal at its entrance to the arena would lead to stable, unique associations between visual stimuli and units representing space. This could explain how repeated exposures of the animal to the same environment produced stable representation in the dark. Since no disorientation or disruption of the vestibular input was produced, it seems that, movement by direction by position, was enough for the system to update spatial representation in subicular PCs (Sharp, 1999b, McNaughton et al., 1996). Sharp, (1999b) proposed the existence of a universal representation of space which would be held in the subiculum and the entorhinal cortex. It has been proved that entorhinal grid cells are capable of keeping their firing fields across light-dark transition in the total absence of external cues, similarly to subicular place cells here described. The fact that both unit types represent the environment in a very similar way suggests

that both structures, at least partially, represent space by processing spatial information in a similar manner. Further research should be carried out to understand both areas interactions.

McNaughton et al., (2006) in their update of the path integration model, suggested that firing field size and the internal metrics used by the brain are somehow controlled by the speed at which the activity moves in an attractor layer. In a movement x direction x location layer, strong input from the movement layer would generate a fast change in the location attractor layer producing a fast change in the activity and therefore a small firing field. Signal about speed of movement are assumed to be generated outside the hippocampus by a combination of visual input, vestibular input and motor input. Previous work suggests that firing field size changes across the dorso-ventral axis of the hippocampus Jung et al., (1994). This dorso-ventral segregation is explained as result of a variation in the movement gain factor throughout the dorso-ventral axis of the hippocampus. Although the model explains how the hippocampal scale changes, it is not clear why subicular units produce larger firing fields that CA1 units. The same gain-movement mechanism could explain different firing field size between the subiculum and CA1, although this doesn't explain why different structures have different resolutions.

Interpreting results obtained in the Light-Dark experiments under the light of the BVCs model, (Barry et al., 2006) generates different contradictions since this model doesn't include any sort of path integration mechanism making more complicated to explain firing field stability in the darkness. The model proposes that units are tuned to fire at certain allocentric directional angle and at fixated distance to the barrier (Barry et al., 2006; Hartley et al., 2000). The fact that firing is modulated by direction may indicate that in absence of visual information, idiothetic information may be enough to keep a continuous up date of the angular position of the animal in respect to external cues.

Indeed, calculation of distance movement in the darkness would require idiothetic information to be used continuously. Therefore, although barriers could set initial firing in the light, it seems that the two parameters determining firing of subicular PCs in the darkness had to be updated through some sort of path integration mechanism coordinated with the allocentric representation of the environment. In this way, subicular units seem to be integrating allocentrical and path integration process, therefore linking both necessary elements for an efficient spatial navigation.

Sharp´s (1999b) proposal, suggests that subicular and EC PCs would transfer a universal representation of the environment across different arenas. McNaughton et al., (2006) proposed that this universal representation is not only held by EC unit, but also by subicular units as described by Sharp (1999b). According to this model, it was predicted that spatial representation would be stable across different arena size and that firing fields would scale in proportion to arena size, something already suggested under certain conditions (Sharp, 1999b). The BVCs model would initially predict not perfect scaling across different arena size unless the distance to which cells are set to fire was not constant (Barry et al., 2006; Hartley et al., 2000). Results obtained in the arena size experiment suggest that cells are able to scale across different arena size, but in some cases, units were not stable across conditions: although 50% more or less of units basically scaled across arenas, another 50% had arena-specific firing or they produced a new more complex representation in the larger arenas. Sharp, (1999b) initially proposed that subicular firing could scale across conditions. A second explanation to firing field enlargement is based on the assumption that subicular units would represent space using one unique map and different parts of this complete map could be observed depending on the size of the arena. This later idea would possibly fit better with the description of entorhinal grid cells (Fyhn et al., 2004; Hafting et al., 2005; Sargolini et al., 2006), but also with grid-like units described in this. On the other hand, units remapping are not

explained by models here described. It could be suggested that alteration of the original arrangement of the arena would disturb allocentrical relationship between the barriers, the animal and initial setting of the system. New arrangement of distal cues produces the system to select a new set of allocentrical referents which allow units to reset their distance and preferred angle of firing. This would generate, in some cases, multiple firing fields not equivalent in size, location or directional preference, producing a differential representation by the same unit of different areas of the environment. In this way, in a similar manner to EC grid cells, (Hafting et al., 2005) the same unit would connect different points in the same arena facilitating spatial navigation and path integration. Under this view the BVCs model would need to consider that direction and distance to the barriers could be reset under some circumstances generating more complex response than it had predicted. The second hypothesis proposed by Sharp (1999b), by which, different parts of the firing field would be observed depending on the arena size could be also an alternative explanation for apparent remapping in the large arenas.

Data obtained here provide some limited support to the BVCs but also to Sharp, (1999b) model of the universal representation of space by the subiculum, although in both cases remapping observed is not easy to explain. In the more recent proposal of McNaughton et al. (2006) grid units would have periodic boundaries, meaning that units would replicate their firing fields without end, if the animal was navigating in an open field without boundaries. In this case, grid cells would self-replicate their firing continuously. On the contrary BVCs model suggests that the limits of an arena are essential to set PCs firing, this element seems not necessary in McNaughton et al. (2006) model but would be up to some point necessary in Sharp (1999b) model. Results obtained here show that in some cases more dispersed firing became more precise in smaller arenas than in larger arenas, but also showed that when recordings were

compared between the small and the large arenas, perfect scaling was observed. Therefore, different units seem to have different responses to arena manipulation. One hypothetical mechanism to explain subicular firing field expansion would assume input from entorhinal cortex units to dorsal subicular classic PCs would drive SPCs sustained firing and expansion. Basically, multiplication of firing fields in EC grid cells, and possible in subicular grid-like cells, would produce enlargement of subicular FF in the larger arena. In some cases, if input is not too selective, multiplication of EC firing fields, would produce subicular firing to be more dispersed. On the other hand, if input is specific and firing field replication is limited, sharper firing fields would be produced by SPCs. Other explanations of this phenomenon could be made. McNaughton et al. (2006) proposed that direction and movement input to the location layer defines firing field size. It was proposed that speed signal generated outside the hippocampus would modulate firing field size. Speed of movement signal would be configured by vestibular input, visual flow and motor input. If speed of movement up dates the metric of the brain, estimation of speed in the larger arena could be somehow less precise than in a smaller arena were translational movement is more limited producing in this way larger firing fields.

Results obtained in the four-room experiment support the BVCs model (Barry et al., 2006) and to proposed model (Sharp, 1999b). Units generally replicated their firing structure across the different sub-chambers. Assuming the BVCs model predictions: firing fields located in corners coinciding with no boundaries in some of the chambers would not display firing peaks in those locations, a prediction normally supported, but not totally. In some cases, firing fields appeared independence of physical boundaries being present in a specific sub-chamber. Assuming Sharp's (1999b) universal map theory, it was expected that firing fields would be replicated across the different chambers with more independence of the barrier being complete or open. This result

would be predicted on the basis that path integration system would set the firing at equivalent distance and direction across the different chambers with independence of a real barrier being present.

Emergent properties of the subiculum suggest that although widely studied, the hippocampal formation has still much to tell us about how spatial processing is performed at the different levels. It also confirms the general idea that the subiculum is still a quite unknown structure. Interestingly, grid-like cells recorded seem to have different properties than those described in the EC (Fyhn et al., 2004; Hafting et al., 2005; Sargolini et al., 2006). While EC grid cells seem to generate a quite perfect triangular grid structure, which seems not to be altered by external conditions, such as arena shape, size or darkness, units here described seemed to be affected by the presence of barriers. Firing fields tended to reorient themselves after barrier insertion. This is a phenomenon not yet described in EC grid cells (Hafting et al., 2005; Sargolini et al., 2006), and not predicted by McNaughton et al. (2006) models. Two different explanations of grid-like cells remapping could be proposed. The first one would include a possible interaction with classic SPCs which by means of barrier detection would induce correction in the distance between firing fields of grid-like cells. A second possibility is that barrier insertion would produce a change of phase of the grid rotating firing fields clockwise or anticlockwise. In this way, it is hypothesized that some firing fields displayed under the barrier conditions were previously out of the arena range. It is also possible to consider that both explanations could be interacting.

7.4 An Alternative View Of The Subiculum And The Hippocampal Formation

It is proposed similar to Sharp, (1999b) that EC and subiculum would be providing the basic representation trough which CA1 generates stable representation of space.

Subicular PCs would generate a coarser map, and therefore a general framework where distance, movement and direction would represent space. As suggested by McNaughton et al. (2006) a thresholding operation and the summation of multiple grid cells could produce unique firing fields as those observed in the hippocampus. This summation and association to external cues would be performed within the stable representation generated by the subiculum. Morris et al. (1990) found that animals with subicular lesions or EC lesions were able to finally learn to escape in the Morris watermaze while hippocampal lesions produced stronger spatial deficits. It was observed that animals with EC and subicular lesions had different deficits to those with hippocampal lesions. Not only could lesions in the EC have produced an inability to relate the internal representation with the external cues, but it could also have produced incapability for path integration. This would force the system to rely only on external sensory cues to generate a stable representation of space. Similarly, subiculum lesions could have disrupted the facilitation role of the general representation and would have also altered path integration capabilities. The fact that the subicular spatial representation seems to be stable during dark periods, indicates that this area is path of the path integration system, but being strongly modulated by external elements such as barriers, indicates that the subiculum is linking internal path integration systems and external allocentric cues.

7.5 Future Directions

a. Further research should be performed to fully characterise the spatial role of the subiculum in spatial navigation.

b. Future recordings should be specifically aimed to detect grid-like cells, establishing the overall proportion of the population they constitute and their general characteristics.

c. Dual recordings in CA1-subiculum and subiculum-EC combined with temporal inactivation or different disconnection techniques would shed light in the interdependency of these structures to generate different spatial representations.

d. Combined recordings under specific spatial task or spatial environmental conditions would allow a better understanding of the impact of different conditions on spatial representation, possible accounting for spatial events being coded by units.

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