Brain mechanisms underlying the tracking and localization of dynamic cues

Diego López Pigozzi

Universitat de Barcelona
Institut d'Investigacions Biomèdiques August Pi i Sunyer
Programa de Doctorado en Biomedicina

Brain mechanisms underlying the tracking and localization of dynamic cues

Doctoral dissertation by
Diego López Pigozzi

PhD. Supervisor: Dra. María Victoria Sánchez-Vives

Área: Neurociencia
Grupo: Neurociencia de sistemas
Línea de investigación: Neurofisiología y computación en sistemas corticales

"Sólo el que sabe es libre, y más libre el que más sabe.

Sólo la cultura da libertad.

No proclaméis la libertad de volar, sino dad alas;

No la de pensar, sino dad pensamiento.

La libertad que hay que dar al pueblo es la cultura."

-Miguel de Unamuno-

ACKNOWLEDGEMENTS

First of all, I would like to say how grateful I am to Dr María Victoria Sanchez-Vives for giving me the chance to do this project. Without her confidence, support and understanding this work would have not been possible. I have really appreciated sharing with her the last four years, which has given me the possibility of learning from her the importance of a constant dedication, a lucid mind and, in general, a positive mood and character in science. Thanks, I really appreciate it.

Next, I would like to thank a very important group of beings who have been involved in this work. I know this is not a common acknowledgement but, as I learnt from another person to whom I will refer soon, without them the present study would have never reached the objectives posed and therefore, out of respect, we always name them. They are Aurora, Borrasca, Ciclón, Destello, Eclipse, Fuego, Huracán, Incendio, Jungla, Krater, Lluvia, Mar, Nieve and Ocaso. Thank you!

It is also important for me to quote the institutions that have funded this research project and made it possible. In my case I would like to mention the EU Commission, in particular the funding of the Synthetic Forager project, the MICINN (Spanish Ministry of Science and Innovation) and the Generalitat de Catalunya (Catalan government). My gratitude for their support to this and many other projects.

There are two persons that I would like to especially thank and who were actively involved in the work developed while, at the same time, became close and loved friends for me: Lorena Pérez Méndez and Thomas Gener. Without your sympathy, support, patience and motivation, the road to achieve my goals not only would have been harder but also very less pleasant. Therefore, a special thanks to you!

One usual acknowledgement goes to the people one has met along the years of study in a particular professional environment. In this sense, I need to say that, every time I think about it, I am impressed about the people who have shared the lab with me. It really moves me. From the first time that I arrived to the lab of Dr. Sanchez-Vives, and I spent

maybe just one or two hours visiting it, I left the place with a deep feeling of friendship; everybody welcomed me warmly and I was, and I am still today, very grateful for that. Therefore, these lines are dedicated to Ramón Reig, Dani Jercog, Dani Pérez, María P. Zabalza, Vanessa Fernández, Thomas Gener and Juan Abolafia. Thank you very much for your nice welcome and for your continuous help.

During the development of the thesis new people arrived to the lab and each new incorporation was a chance to enrich the, already, fantastic group. I would like to say that each of them has been important for me, that they have shared many things with me and that I am very grateful for this. Starting from the moments in which we found together the way to overcome with humour all kind of issues that the research work gave us, and arriving to our never-ending scientific/philosophical talks during the meals, coffee breaks and time spent out of the lab. I am especially grateful for that, all of you are unique! Thanks to Thomas, María, Marcel, Lorena, Salva, Leandro, Laura, Julita, Lucila, Enrique, Núria, Bea, Julia and Patricia. You made everything happier.

Finally, I have to say that I would like to thank so many other people -I owe you a lot my friends!- that I would need pages and pages naming each of you and explaining the important things you have given me. Therefore, let me just say: “Friends, almost brothers in many cases, you are an important part of me and I hope that I am also a part of you. We have walked together through marvellous experiences and I will never forget you!!!”

And now, really ending these emotive lines, I would like to express to my family my infinite gratitude. You have taught me, you have shaped me, you have encouraged me and finally helped me in the road that I chose to follow, always believing in my skills and supporting me with your love. ¡Gracias! Grazie! ¡Ezkerrik azko!

TABLE OF CONTENTS

ACKNOWLEDGEMENTS 4
TABLE OF CONTENTS 6
ABBREVIATIONS 8
LIST OF FIGURES 10
SUMMARY 12
RESUMEN 14
1. INTRODUCTION 16
State of the art (Historical and Philosophical review) 16
1.1. Category of space in the brain 21
1.1.1. How space is encoded by the brain. 21
1.1.2. The hippocampus in rodents: a mammal model of spatial navigation. 24
1.1.3. Space representation across other species. 43
1.2. Dynamic cues 46
1.3 Single unit's recordings (Introduction to the method) 49
2. HYPOTHESIS & OBJECTIVES 52
2.1. Hypothesis 52
2.2. Objectives 53
3. MATERIAL & METHODS 56
3.1. Behavioural protocols 56
3.1.1. Subjects 56
3.1.2. Behavioural Setup 57
3.1.3. Protocols 63
3.1.4. Behavioural Data Analysis. 68
3.2. Electrophysiological recordings 70
3.2.1. Subjects 70
3.2.2. Tetrodes and microdrives 71
3.2.3. Surgery 72
3.2.4. Data acquisition 74
3.2.5. Data Analysis 75
3.2.6. Histology 90
4. RESULTS 92
4.1. Behavioural results 92
4.2. Hyppocampal rhythms 98
4.3. Single units 101
4.3.1. Spatial patterns of activity 102
4.3.2. Temporal patterns of activity 111
4.3.3. General examination of single cells 116
5. DISCUSSION 125
5.1. Behaviour 125
5.2. Oscillations 129
5.3. Single units 133
CONCLUSIONS 143
REFERENCES 145

ABBREVIATIONS

Ach: Acetyl choline.
AHP: After-hyperpolarization current.
A30: Area 30 is the surface covered by bins with a firing rate over 30% of maximum frequency.
A50: Area 50.
CA1: Cornus ammoni 1.
CA3: Cornus ammoni 3.
DDT: Direction discrimination task.
DG: Dentate gyrus.
EC: Entorhinal cortex.
EEG: Electroencephalography.
FF(s**):** Firing fields.
Fmax: Maximum frequency.
Fmean: Mean frequency.
fMRI: Functional magnetic resonance imaging
Fratio: Ratio between Fmax and Fmean.
FR: Firing rate.
HDC(s**):** Head direction cell(s).
I_m: Non-inactivating voltage-gated potassium current.
ITT: Inter trial time.
LIA: Large irregular amplitude.
LFP: Local field potencial.
LTD: Long term depression.
LTP: Long tern potentiation.
MI: Mutual information.
ODDT: Operant direction discrimination task.
OPDT: Operant position discrimination task.
PC(s**):** Place cell(s).
PDT: Position discrimination task.
PF(s**):** Place field(s).
PI: Positional information.
PtA: Parietal association cortex.
REM: Rapid eye movement.
RT: Response time.
Sb: Subiculum.
SEM: Standard error of the mean.
SI: Skaggs index.
SIA: Small irregular amplitude.
TTL: Transistor-transistor logic.

LIST OF FIGURES

Cover: Illustration of Fabricio Vanden Broeck © taken with permission from the …….0
book *El viaje*, México. Serpentina, 2007.
Figure 1: Example of a place cell recorded in the region CA1 of the Hippocampus. 23
Figure 2: Phase precession of place cells. 26
Figure 3: Border cells. 28
Figure 4: Grid cells. 29
Figure 5: The parahippocampal formation. 32
Figure 6: Ventral and dorsal pathways of visuo-spatial information. 34
Figure 7: Main hippocampal circuitry. 34
Figure 8: Layer organisation of the hippocampal *regio superior* (CA1 and CA3). 36
Figure 9: Hippocampal oscillations. 40
Figure 10: Tracking system. 57
Figure 11: The e-puck®, a commercial robot for educational purposes. 59
Figure 12: Behavioural scaffold. 60
Figure 13: Behavioural interface and algorithm. 61
Figure 14: Robot's interface and algorithm. 62
Figure 15: Discrimination tasks. 64
Figure 16: Operant Position Discrimination Task (OPDT). 66
Figure 17: Scheme of the different phases of the training. 67
Figure 18: Tetrode configuration. 72
Figure 19: Cluster isolation. 76
Figure 20: Time properties of an isolated cell. 77
Figure 21: Space and time relations of the spikes. 79
Figure 22: Assessment of the spatial activity. 80
Figure 23: Graphs for absolute positions and projections in one dimension. 81
Figure 24: Firing rate vs. relative positions. 82
Figure 25: Spikes, theta oscillation and speed correlations in relation to behavioural
phases. 84
Figure 26: Shuffled distributions. 88
Figure 27: Histology. 90
Figure 28: Learning curves for the discrimination tasks evaluated. 93
Figure 29: Pre-training phases in the OPDT. 95
Figure 30: Learning curve in the OPDT. 96
Figure 31: Distribution of response times. 97
Figure 32: EEG and velocity. 98
Figure 33: Natural correlation between theta's power and the speed. 99
Figure 34: Theta and velocity. 100
Figure 35: Firing fields 103
Figure 36: Skaggs Index quantifications. 105
Figure 37: Spatial parameters based on Skaggs Index separation. 106
Figure 38: Separation of the rat's position in behavioural stretches. 108
Figure 39: Firing rate vs. relative position. 109
Figure 40: Single unit activity across the trials. 111
Figure 41: Behavioural phase vs. Firing rate. 113
Figure 42: ON/OFF responses during the robot movement. 114
Figure 43: Cellular and spatiotemporal characteristics for a single neuron (I). 117
Figure 44: Left, center and right stretches. 119

Figure 45: Unidirectional FFs. 120
Figure 46: Cellular and spatiotemporal characteristics for a single neuron (II). 122
Figure 47: Cellular and spatiotemporal characteristics for a single neuron (III)..... 123

SUMMARY

Since the discovery of the place cells in 1971 by John O'Keefe and colleagues an extensive work over the hippocampus has been developed as the mammal model of spatial navigation. Place cells are rodents' hippocampal neurons whose firing is associated to certain locations of the environment. A majority of studies have focused on how the place fields (the area where the firing of a neuron is restricted) are generated in relation to the static cues of the environment (O'Keefe and Conway, 1978; Muller *et al.*, 1987; Gothard *et al.*, 1996). The present work assessed a similar question but regarding the dynamic cues surrounding the subject, and with the hypothesis that the hippocampus is also representing the position of other moving objects. In order to demonstrate if that was the case, we developed a behavioural protocol in which rats learnt to discriminate the movements of a robot in order to obtain reward, an Operant Position Discrimination Task (OPDT). Once the protocol was validated, the subjects were chronically implanted with tetrodes in the CA1 region of the hippocampus. In this way the activity of single hippocampal cells could be isolated off-line and the LFP of the area stored during the recordings. Using this method, the relationship between the firing of the cells and the field activity with the spatial parameters of the robot could be evaluated.

The results showed a modulation by the dynamic cue of the theta oscillation. While the locomotor activity of the subjects is directly related to the power of theta in natural conditions (Vanderwolf, 1969), during the movement of the robot such relationship was disrupted and the band power between 4-12 Hz showed a trough at this time.

The analysis of the single cells' activity showed neurons locked to several spatial features of the dynamic cue. First, the position of the rat and the robot where analysed by information content parameters. Skaggs Index and Positional information (Markus *et al.*, 1994; Olypher *et al.*, 2003) showed neurons locked to the position of the subject as expected in CA1 and also other neurons locked to the positions of the robot. Second, moving from the spatial analysis to the temporal one, we found responses to the movement of the robot like OFF/ON variations of the basal activity of the neurons.

Such changes in the firing patterns where quantified by the Mutual Information index (Nelken and Chechik, 2007) demonstrating that a large fraction of the neurons have a significant differential pattern of activity during the movement of the robot towards one side or the other. The use of the same index, MI, for the evaluation of the static or dynamic condition of the robot, also resulted in a set of neurons spiking with significant disparity during such epochs.

In conclusion, the present work has demonstrated the existence of neural correlates locked to a dynamic cue in the hippocampus. Both the field activity at the theta range, LFP between 4 and 12 Hz, and the activity of the hippocampal neurons were found to reflect and/or encode the spatial features of a dynamic cue. The present work has in this way enlarged the limited evidence present in the prior literature about the role of the hippocampus in the tracking and localization of dynamic cues with the use of a behavioural protocol where both the spatial and temporal dynamics could be assessed.

RESUMEN

La correcta localización y seguimiento de las pistas dinámicas que se encuentran en el ambiente es una tarea crucial para el individuo. Comportamientos fundamentales como la caza, el apareamiento o el escape necesitan una correcta identificación de la posición de presas, congéneres y depredadores para su correcta realización. El sistema cerebral encargado de localizar al propio sujeto en el ambiente se sabe que se encuentra en la formación hipocampal después de que diversos estudios hayan demostrado la necesidad del mismo para una correcta orientación (Morris *et al.*, 1982) y, aún más importante, tras el descubrimiento en roedores de neuronas que disparan únicamente en espacios restringidos del entorno, las células de lugar (O'Keefe and Dostrovsky, 1971). Si bien se conoce que estos procesos están fundados en una correcta representación de la posición de las pistas estáticas del ambiente (O'Keefe and Conway, 1978; Muller *et al.*, 1987; Gothard *et al.*, 1996), que sirven de referencia para la propia localización, poco se sabe acerca de cómo se integra la información relativa a los objetos y/o sujetos móviles que se encuentran en el mismo ambiente. Este trabajo tiene como objetivo principal intentar responder a esta pregunta, es decir, ¿en qué modo el hipocampo procesa la información relativa a las pistas dinámicas?

Para el desarrollo del estudio, primero, se diseñó una tarea comportamental que asegurara el hecho de que la pista dinámica resultase relevante para los sujetos de forma que los mismos prestaran atención a sus movimientos. Con este fin elegimos utilizar un robot cuyos desplazamientos pueden ser finamente controlados y asociar una recompensa a determinados patrones de navegación del robot. Después de probar con diferentes tareas de discriminación se llegó a una configuración (Operant Position Discrimination Task, OPDT) que permitía a los animales seguir los movimientos del robot desde un espacio separado en el cual recibían la recompensa en caso de discernir correctamente los desplazamientos de la pista. Una vez validada la tarea comportamental, a los sujetos que alcanzaron altas tasas de rendimiento se les implantaron tetrodos en la zona CA1 del hipocampo, lugar en el que se encuentran las células de lugar más estables. Una vez hecho el implante se procedió a registrar la actividad cerebral durante la ejecución de la tarea. Por una parte se aislaron los

potenciales de acción pertenecientes a neuronas únicas y el potencial de campo de la zona, LFP.

Respecto a la actividad de campo, LFP, se observó una disminución significativa de la potencia en la banda theta, 4-12 Hz, relacionada generalmente con la actividad locomotora del sujeto (Vanderwolf, 1969) durante el movimiento del robot. Durante el resto del registro la relación entre velocidad y potencia de theta se mantuvo y sólo en el periodo de discriminación del movimiento del robot esta relación se vio alterada con un mínimo de potencia observado en diferentes sujetos y registros.

La actividad de las neuronas se analizó en función de los parámetros espaciales y dinámicos de la rata y el robot. Mirando la especificidad espacial del disparo de las neuronas a través de los parámetros Skaggs Index y Positional information (Markus *et al.*, 1994; Olypher *et al.*, 2003) se encontraron células significativamente ligadas en su actividad a la posición del sujeto o del robot. La actividad de las neuronas también se analizó de forma temporal, tomando como referencia el inicio de los estímulos, es decir el movimiento del robot hacia un lado u otro. Utilizando como índice la Mutual Information (Nelken and Chechik, 2007) se encontró que una larga proporción de las neuronas tienen respuestas diferenciales durante el movimiento del robot hacia uno de los lados. A su vez, el mismo análisis, pero en esta ocasión comparando los periodos en los que la pista se encuentra inmóvil con los que está en movimiento, determinó que otra fracción de las neuronas tiene patrones de disparo diferenciales según sea la condición dinámica de la pista.

El conjunto de los resultados obtenidos indica claramente que el hipocampo se encuentra involucrado activamente en la localización y el seguimiento de las pistas dinámicas, siendo esto reflejado tanto en la actividad de sus neuronas como en la actividad de campo global. Los parámetros espaciales de la pista que resultaron modulados durante la tarea fueron su posición, la dirección de su movimiento y el hecho en sí de permanecer inmóvil o en desplazamiento.

1. INTRODUCTION

State of the art (Historical and Philosophical review)

This PhD thesis addresses how the representation of position and movement of moving objects is encoded in the brain, with the intention of revealing the underlying brain mechanisms that allow a correct localization of the surrounding moving objects. I will start by briefly explaining the history of the concept of space in order to give to the reader an introductory framework to the matter. The first theories came from philosophy, where the concept began to be analysed in the Greek classical philosophy times resulting in different theories and explanations about its ontology and development, and finally arriving at contemporary psychology and neurophysiology, where the issue became a matter of natural science thanks to the new knowledge acquired during the XX century regarding brain representation of space and new techniques that allow a deeper dissection of its function. This philosophical introduction tries to explain how the notion of space has evolved in history and how its interpretation has allowed the research of its basis in living beings (*).We can go back to Greek philosophy tracing the principles of space categorisation to show how, for instance, Socrates and Aristotle dealt with the concept of space. For Socrates, geometry, and therefore the notion of space, are inherent concepts to our immortals souls (Plato referred Socrate's thought of geometry in *Meno*, a manuscript dated between 386-382 b.C), while for Aristotle, space is an objective concept, it is continuous, filled with matter, and therefore any object is surrounded by matter of other kind, for example, void. Even only these two concepts of Socrates and Aristotle can give us an idea about the debate regarding the notion of space.

(*): This introduction uses as a predominant referent the illustrative first chapter of the book "*The Hippocampus as a cognitive map*" of John O`Keefe and Lynn Nadel, Oxford University Press 1978. Most of the quoted bibliography is therefore taken from this book without a deep knowledge of the previous literature. This decision was taken because an extensive documentation of the concept of space in philosophy and science is in itself a matter of another PhD dissertation. Overall, a brief review of it is important, in our opinion, to rapidly comprehend the state of the art in order to better understand the question posed by this work.

The first concept explains the innate *psychological space* and the second deals with the concept of *absolute space*. In spite of the importance of these two philosophers, who had a view of the space concept that influenced other authors in the subsequent centuries, we will not further explain their theories because the development of the concept of space only becomes really relevant for cognitive science in the XVIII century, even if, as we will see, the debate remains still unsolved and the positions taken by Socrates and Aristotle are a good synthesis of the differences found in contemporary thought, even if concepts and words have effectively evolved.

The debate about space in philosophy was basically reduced to these two different points of view: the point of view of the empiricists and the point of view defended by the rationalists or also called nativists. These two different theories deal, in a different way, with the relationship between the *physical space* and the *psychological space*. For the empiricists, the *psychological space* derives from the experience, that is, it is developed through our interaction with the *physical space*; for the rationalists, it is just a construction of the mind, an innate category that is present in the brain before its comprehension (a point of view closer to that of Socrates). The most eminent, or at least most famous, defender of the rationalist view was Immanuel Kant, who explained in the "Critique of pure reason" (Kant, 1781) that space is *a priori* intuition, a category *per se*, and therefore space is relative because, even if it independently exists, one cannot perceive it as it is. In the other front, Newton can be considered as the main exponent of empiricists; for him, space is absolute and continuous, it exists independently of the subject as an Euclidian space and only the experience can model its comprehension and therefore its mental representation. A relevant fact for our issue of how moving objects are encoded by the brain is the fact that Newton used the motion of other objects as one of the proofs of the existence of absolute space. He argued that this is so because the comprehension of external movements is only possible within an absolute reference frame and not in a relative one. As we can see, the debate between empiricists and rationalists was still unsolved because it was very difficult to assess the question about what is first: a scaffold brain structure underlying the notion of space or the experience that conforms from our senses and perceptions its notion.

After the XVIII century, new intermediate theories between empiricism and rationalism arose, until the advances in modern physics that changed drastically the knowledge of

the *physical space*. The *absolute space* was thought to be Euclidian before the discovery of the non-Euclidian geometries in the middle of XIX century by Riemann, Lobachevsky and others. The fact that the *physical space* should not be Euclidian posed a new challenge to the debate. Our intuition gives us the perception of a three-dimensional space and the experience further improves this notion while modern physics exposes a different reality. Our closer environment is well explained by Euclidian geometry, but if we consider larger distances, it becomes less precise than others geometries due to, for example, the curvature of the earth. The ideal *absolute space* was not so evident anymore and the theories of empiricists and rationalists needed a review.

Helmholtz, at the beginning of the XX century, was the first to take into account these new physical advances in his empiricist solution that explains how experience enhances the particular metric that we apply to our concept of space. In short, the Euclidian metric evolves intuitively because the subject has chosen it and the experience only reinforces the concept in a gradual way. Even if he was a declared empiricist, some nativist (or rationalist) ideas can be found in his theories; overall, Helmholtz declared the sense of touch as an innate intuition of space. For him, there is no need to separate the innate ability to understand space from the experience required to perform it more correctly. Actually, he argued that this issue can be pointed directly to the living being itself, to see which part is determined by its initial state and which one by its development through experience (Warren and Warren, 1968).

Poincaré's theory needs as well a review due to the fact that the subsequent empiricist theories used his framework as a starting point to develop their arguments. He agreed with Kant in the fact that the notion of geometrical space is not inherent to objects or senses but, moving away from Kant, he argued too that space is a notion restricted and egocentric, which means that it depends on our own movement. In his words: *'absolute space is nonsense, and it is necessary for us to begin by referring space to a system of axes invariably bound to our body' (Poincaré, 1913).* Poincaré also introduced the mathematical theory of groups in his model. For him, the visual field could change in limited manners and these comprise a part of the *representative space* or the whole. When the subject moves, the visual perception informs of a change of the point of view and the whole is affected by the egocentric changes, but when there is only a group

moving in the visual field, the mind differentiates between the invariant part and the moving group. This perceptive difference allows the mind to categorise the moving group as an independent object. In relation to the aim of the present work, such theory becomes highly relevant because it links directly the perception of our own movement, now called propioception, to the movement of external objects as an important factor for the right development of space representation in the brain. Summarising, Poincaré's theory assumes that the spatial concept arises from the properties of the senses by themselves while guided by a mental structure prepared to appropriately encode these modifications of the perceptions.

After Poincaré, we must mention the theory of Piaget, who constructed a similar approach to Poincaré's theory using the group theory as well. Both works were very relevant and influenced the contemporary and subsequent experimental psychologists. Differing from Poincaré, who assumed that objects are identified *a priori*, Piaget thought that objects' identification and spatial principles arise together during development (Piaget, 1955). For instance, the concept of extension is useful for both categorisations, and without the comprehension of the extent of an object we cannot understand the distance separating different objects. The only innate knowledge that Piaget assumed in the neonates was the action systems that guide behaviour, which are practical and not in thoughts, as, for example, grasping, sucking, etc. Those behavioural patterns are only the seeds that guide the subject to a right comprehension of objects and space.

The stance of Gibson also needs a brief review. He was a declared nativist, but as we will see, his ideas differ completely from the older nativists, in fact being even closer to the last empiricists as Poincaré and Piaget. His theory starts dividing the *visual field* from the *visual world* as it was suggested previously by Mach. To do this, he included in the *visual field* the sensations and perceptions and said that this field consists in the direct experience of the world and therefore being unstable, bounded and composed by adjacent areas or figures. Oppositely, the *visual world* is a representation and therefore it is stable, unbounded and composed by edges, surfaces, solid objects and interspaces. Using one of his metaphors, even if we see the objects in the *visual field* with different sizes depending on the distance at which they are, we consider them by their real dimension and our mental representation transforms and interprets the perceived

stimulus. The basis of Gibson's theory is also supported by the concept of the *topological space,* where the mental representation shapes the *visual world*, and no metric is needed to establish the relations between objects, space and the subject. It is interesting to see that Gibson rejected the empiricist distinction between *exteroceptive* and *proprioceptive* sources of information. He argued that this distinction is not needed because the invariant framework of the *visual field* is enough for the subject to recognise when an object is moving. In the opposite case, the notion of own movement does not need further processing in order to explain the variant framework appearing in the *visual field*.

As we can see, the concept of space in the 20th century took a new direction, the classical debate between nativists and empiricists began to be moderated and the new theories of the scientists and philosophers accepted the fact that both the neural structure and its development during the experience are fundamental for a correct notion of space. Until now, this summary has focused on the most contemporary philosophical and scientific ideas and, as we can see, several authors have considered the detection of moving objects as one of the promoters of the notion of space and agreed that it is important for a correct development of this ability. These authors (Newton, Poincaré, Piaget, Gibson, etc…) have mentioned, for example, the need of object identification to understand the space separating objects, or the importance of a good discrimination between the movements of an object separately from the movement of the visual framework when it is produced by its own movement. Our aim with the present work is to try to shed light on the question of how dynamic objects in the mammalian brain are localized and represented. The next sections of the introduction summarise the most important experimental results and techniques found in the literature that were essential to the actual *state of the art* relative to the field concerned about the representation of space in the brain and the neural processing of environmental dynamic cues.

1.1. Category of space in the brain

1.1.1. How space is encoded by the brain.

The first studies assessing the question of how animals process the sensory information worked with the hypothesis that subjects learn to move in a maze by a correct learning of movement patterns, which are stimulus-response associations that will lead to a correct chain of movements (Watson, 1907). Subsequent studies demonstrated that complex mazes can not be solved with a simple rule of propioceptive guided rules (Dashiell and Helms, 1925) and thus suggesting that there is a more global space orientation system in the brain. In an article of Dashiell published in 1930 we can read: "…learning consists in the establishing not of a definite pattern of specific turns, but of some more *general orientation* function. This general function enables it to pursue new pathways from time to time, while remaining successfully oriented in the direction of the objective". After these first attempts to understand the question of how animals orient themselves in the environment, other authors began to share the view of Dashiell. One of the more formal models in this sense is Tolman's theory of the *cognitive map,* first mentioned in 1932 and more widely developed in 1948 (Tolman, 1948). It claimed for a view of the animal's notion of space based in internal and flexible maps rather than in a sequence of movements leading the subject to particular locations. This was the state of the art in psychology during the first half of the XX century; some authors held that animals learn to move and orient in a certain environment by chains of stimulus-response associations while others thought that animals use a more general brain representation of the environment leading them to correlate correctly the different areas and objects that compose the environment.

Spatial navigation was, in subsequent decades, studied mostly in birds' migration and human navigation. These studies were done with the aim of elucidating how animals orient themselves in the environment. Because birds' migration and human navigation are tasks in which the strategy used to reach the target position is crucial, several scientific groups assessed the question of how orientation works analysing them. Thus, the conclusions of these works shed light on the question of which are the strategies used during the navigation and not to the other question related to the brain areas or

mechanisms involved in the navigation. For example, Keeton found that birds use the stars, the sun and the magnetic fields in order to maintain a correct compass direction during navigation (Keeton, 1974), while Gladwin studied the habits of the Puluwatans, a human group of the South Sea islands, who use a map of stars and islands to locate the position of the ship during travels (Gladwin, 1970). These studies were important in order to know more about which are the possible behavioural rules that guide the exploration or navigation behaviours and that result in the identification of three main components: guidance, orientation and map following. This classification provided the needed framework to better design experiments that could answer which strategy is used by the subjects. Olton and colleagues made an important set of experiments during the 70's, in which rats were placed in radial mazes and trained to visit the different arms to get a reward without repeating the previous visited ones. The subjects did not use egocentric strategies while visiting the different arms without returning to any of them. Instead, the results showed the use of external clues to identify appropriately the arms visited (Olton and Samuelson, 1976).

An important feature of the behavioural studies in general is the fact that the subjects are usually trained to perform tasks in which the natural behaviour can be suppressed. In the study of spatial learning, a big step was achieved by a series of important studies which were performed by Morris and colleagues in the 70's and 80's with the introduction of the water maze. On one hand, the design of the water maze allowed the study of spatial memory in a new way thanks to the use of a hidden platform where the subjects go naturally to stop swimming without the use of a reward (Morris, 1984). On the other hand, these studies identified the hippocampus as one of the implicated regions in spatial memory, through experiments that produced lesions of the hippocampal complex (Morris *et al.*, 1982).

Almost at the same time, in 1971 the state of the art in the study of spatial navigation and its brain representation was totally altered by the presentation of a short communication in the *Brain Research* journal by J. O'Keefe and J. Dostrovsky explaining the discovery of individual neurons in the hippocampus firing in particular places of the environment (Figure 1) (O'Keefe and Dostrovsky, 1971). A posterior article in 1976 by O'Keefe confirmed again the presented evidence and called these neurons "place cells" (PCs) (O'Keefe, 1976). Two years later, in 1978, they published a

referent book with the title “The Hippocampus as a cognitive map” (O'Keefe and Nadel, 1978). These news changed drastically the contemporary debate of how space representation in the brain works, thus inclining the balance towards the theory based in cognitive maps as it was suggested by Tolman several decades before (Tolman, 1948).

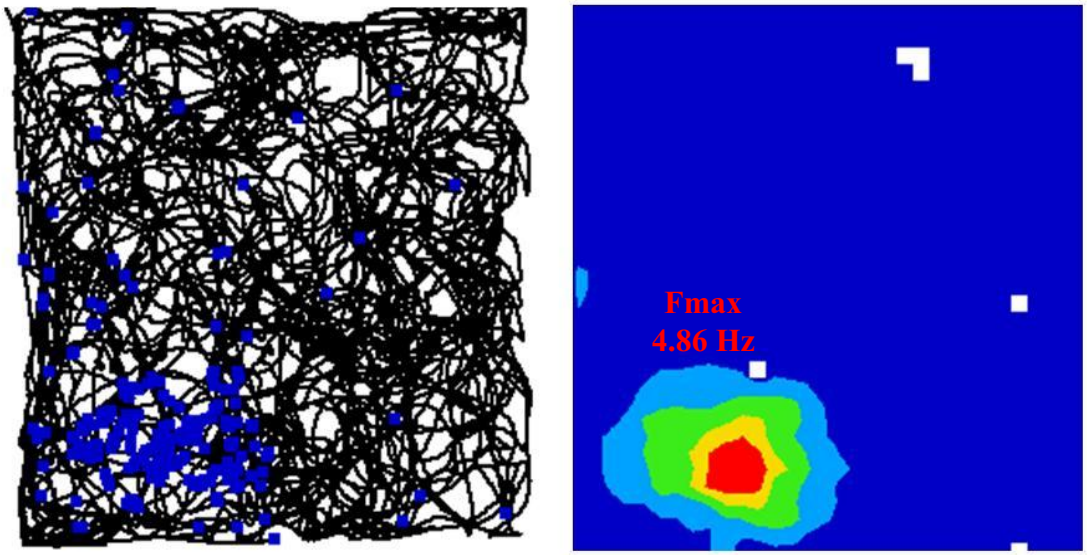

Figure 1: Example of a place cell recorded in the region CA1 of the Hippocampus in our laboratory. On the left (A) the position of the subject tracked during 15 min of recording in a square arena is shown with a black line while the spikes of a single neuron are represented by the blue dots. On the right (B) the firing rate of the neuron is obtained by normalizing the number of spikes for each considered pixel normalized by the time spent in it and applying a Gaussian smoothing filter. The colour code varies from 0 in dark blue to the maximum frequency in red (Fmax = 4.86 Hz). This neuron was recorded in our set up.

The next section focuses on the hippocampus as the reference brain structure for spatial navigation studies and more precisely in the rodents’ hippocampus because, after PCs discovery by O’Keefe and the identification of the hippocampus as the main brain area supporting spatial memory by Morris, the wider work done later was using rodents’ hippocampus as the main model of spatial navigation in neuroscience.

1.1.2. The hippocampus in rodents: a mammal model of spatial navigation.

In this section we will describe the neuronal and electrophysiological correlates of spatial information processing found in scientific literature. Thus, a summary of the knowledge that leads to the hippocampus as the main model of spatial navigation is exposed, from the neurons found in the hippocampus that carry spatial information, like the PCs for example and others that we will see soon, until the anatomy and general functions of the hippocampus which will explain how this processing is thought to be possible.

1.1.2.1. Hippocampal formation and the spatial information.

In 1971 the discovery of the PCs by O'Keefe and Dostrovsky altered the theoretical basis of spatial navigation studies, where the debate about the guiding rules of orientation was still open, pointing to hippocampal single neurons capable to encode the position of the subject in the environment (O'Keefe and Dostrovsky, 1971). This finding suggested that all the information needed for the creation of cognitive maps was available in this brain region and therefore this leaded neuroscientists to unravel the mechanisms supporting this function in the hippocampus. The subsequent experiments focused mostly in two questions about the activity of the PCs: How is the sensory information recruited and encoded in the brain in order to have neurons firing just in certain locations? And, how the own movement information perceived by the subject is incorporated in parallel in order to support the cognitive maps? In fact, these questions show how the issue of animals' navigation was assessed. The two main described strategies in animals' navigation are: the allocentric method, based in the identification of the current position supported by the relationship of the subject with environmental cues within an absolute reference frame, and the second is the egocentric strategy that uses propioception as the basis to know and update the current location.

The most of the experiments done identified the allocentric component of the PCs activity by the alteration of the environmental cues. They found that when the geometry of the environment was modified the place fields (PFs), the spatial receptive fields of

the neuron, were also altered in the same way (O'Keefe and Burgess, 1996). The principal method used to modify the environment in these experiments was the rotation protocol; it consists in the rotation of the maze in relation to the room where it is placed. If the firing fields of the neurons show a rotation according to the manipulation it can be concluded that they follow the environmental cues. Other experiments posed the question of how the local and distal visual cues are differentially used. The results showed that there were PCs locked to the local cues while others to the distal ones, being the second case the more common (O'Keefe and Conway, 1978). Therefore, the predominant idea at this point was that the major sensory input maintaining the PCs' activity is visual and the preferred landmarks are the peripheral ones. One important feature in relation to the activity of the PCs was also found during this time: the recording of the hippocampal EEG found the *theta* pattern of rhythmic waves, the predominant oscillation in the whole hippocampus and varying from 4 to 12 Hz, was found to be temporally related to the activity of the PCs (O'Keefe and Recce, 1993). In fact, the coupling has been later shown to be directly related to the phase of the *theta* cycle for almost all of the recorded hippocampal interneurons (Geisler *et al.*, 2010), spiking them in the same period of the cycle, while the active PCs in the environment showed a "phase precession" during the cycle (O'Keefe and Recce, 1993). The spikes of the PCs were precessing across the cycle and it was related with the location of the animal inside the PF (Figure 2). Because the *theta* oscillation in the hippocampus was previously seen directly related to the speed of the subject (Vanderwolf, 1969), the phase precession of PCs was soon interpreted as an evidence of the propioceptive input received by the hippocampus. In this way, the spiking of the PCs was related with a brain activity simultaneously related to the speed and therefore giving a framework for theories explaining how this integration of the information regarding internal and external cues can occur (Burgess and O'Keefe, 2011).

Another evidence of the important egocentric input received by the PCs was the experiment carried on by Save and colleagues, who found PCs in blind rats (Save *et al.*, 1998), demonstrating on one side, that the visual input is not the only one important input for their spatial stability and, on the other side, also reinforcing the theory of the cognitive map as a stable representation of the space developed by the exploration and the integration of multimodal sensory inputs. Another interesting topic in the debate about the egocentric input of the cognitive map is the proposed concept of path

integration (McNaughton *et al.*, 2006). In the absence of external cues that guide the subject in the space the idiothetic information coming form the vestibular system and the propioception is enough for the animals to orient in a certain environment.

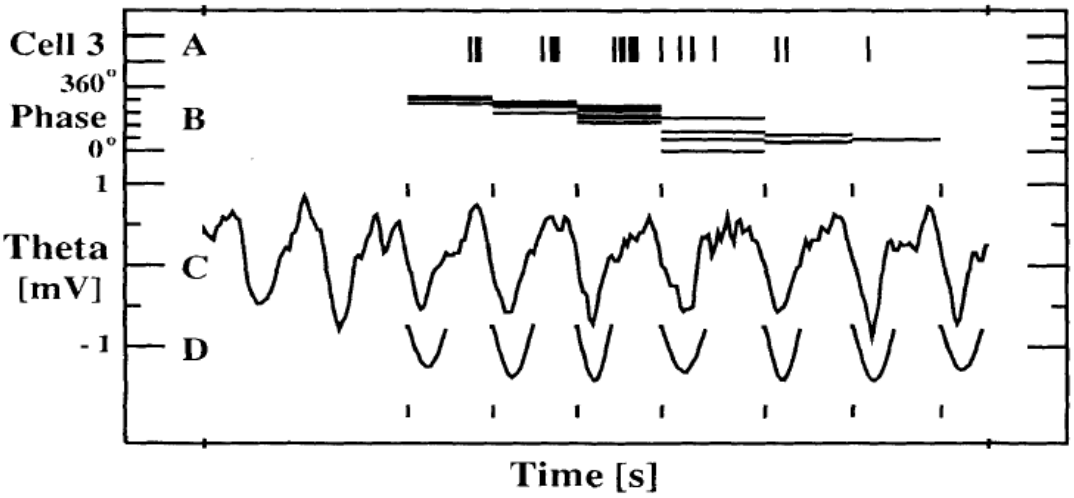

Figure 2: Phase precession of place cells.
The picture is taken from the original paper of O'Keefe and colleagues of 1993. It shows how the spikes of a PC precess in each subsequent cycle of theta oscillation. From the top: spikes of a PC (A), phase in which they occur (B), LFP oscillating in the theta range (C) and finally a fit of the oscillation (D). The figure shows how the phase in which the spike occur advances gradually during the crossing of a place field (in this case it is shown across time and not space).

As we have seen so far, the hippocampal neurons involved in the putative cognitive map are the PCs, but it is important to say that during the last decades new evidence have emerged pointing to a broader area implicated in the processing of spatial information. First of all, the activity of CA1 principal cells was not the only one found with properties as PC-like. There were found cells with similar spatial activity in other adjacent areas of the hippocampus, as CA3 and the dentate gyrus (DG)(McNaughton *et al.*, 1983). The activity of these cells is slightly different from the CA1 in the sense that the size of the PF was found to be different, being CA1 the region with sharpest firing fields, and also in these other areas the cells could have more than one receptive field (Leutgeb *et al.*, 2007). Because of the close location of these brain areas, the first interpretation of this data was to see the PC-similar activity as an evidence of the direct input between these areas, being them in different stages of the information processing flow. As we will see in the following anatomy section, that is the case and dentate gyrus and CA3 neurons are intermediate steps of the information flow arriving to CA1 from the entorhinal cortex (EC) via the perforant pathway to dentate gyrus first and via the Schaffer collaterals fibers to CA3 after. These interpretations were confirmed as well by

studies that presented functional differences between these cells. The PFs of CA3 neurons are, for instance, more sensitive to the remapping when the environment is altered, it means that the PFs change their location or were abolished in this environment more often (Muller and Kubie, 1987). Later on, the spatial processing research in the hippocampal formation advanced and more regions with spatial processing evidence were found. In the subiculum (Sb) and the entorhinal cortex, for example, not only neurons with similar PC activity were found but also head direction cells (HDCs) (Taube *et al.*, 1990) and border cells (Figure 3) (Solstad *et al.*, 2008). The presence of other spatial neurons across the hippocampal formation enlarged the putative area related to spatial information processing. The gap between the sensory information coding and PCs activity was then started to be filled with neurons providing important medium steps in the processing of the information, the HDCs in the form of an internal steering compass giving the correct angular input and the border cells as anchors of the PCs to objects and obstacles of the environment. The hippocampus and its surroundings of the hippocampal formation continued to give evidence of specific spatial computations making the theory of the cognitive map each time stronger.

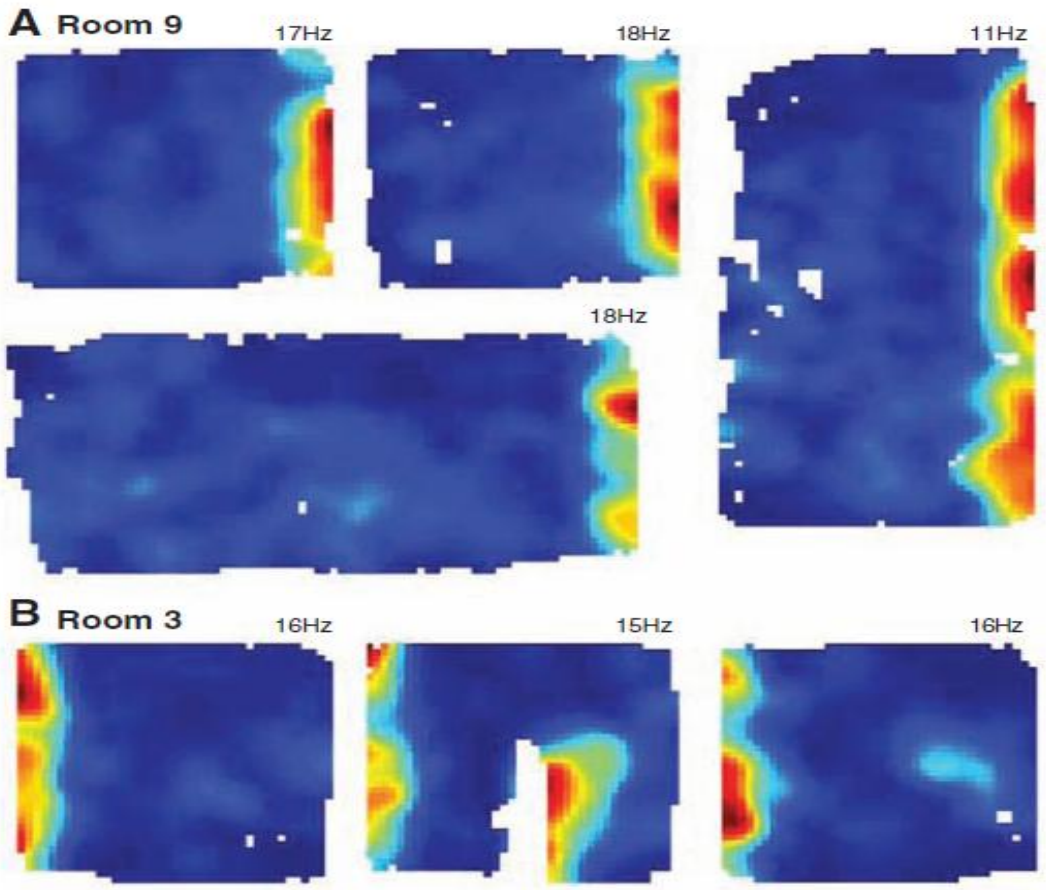

Figure 3: Border cells.
The illustrated firing fields are all relative to the same identified neuron. (A) The environment, a square, was enlarged in x or y coordinates and the cell maintained its firing in the same wall relative to visual cues. (B) The second test consisted in the introduction of a wall in the middle of the maze and the cell fired in relation to this new wall as a cathegory of "east limit". Adapted from the original work of Solstad et al. 2008.

However, other kind of very relevant spatial neurons were missed. Presumably, the most important finding regarding PCs and spatial navigation in the last decade was the discovery of the grid cells in the medial entorhinal cortex (Fyhn *et al.*, 2004) (Hafting *et al.*, 2005). The activity of these neurons is highly tuned to the space because they fire in a grid manner, creating equilateral triangles that tessellate the whole space covered by rodents (Figure 4). Theoretically, in physics and mathematics, this is considered the optimal way to cover a flat space with the minimum number of organised elements. When moving from the dorsocaudal to the ventral area of the medial entorhinal cortex, the grids become larger while the angle's orientation does not follow any specific brain topography. Importantly, the increase in the grid pattern resembles the gradient's size of the firing fields in the dorso-ventral axis of the hippocampus, suggesting that the known

projections that send the information from the medial entorhinal cortex to the hippocampus are involved in the building of the PFs that are integrating in some way the grid cells' activity.

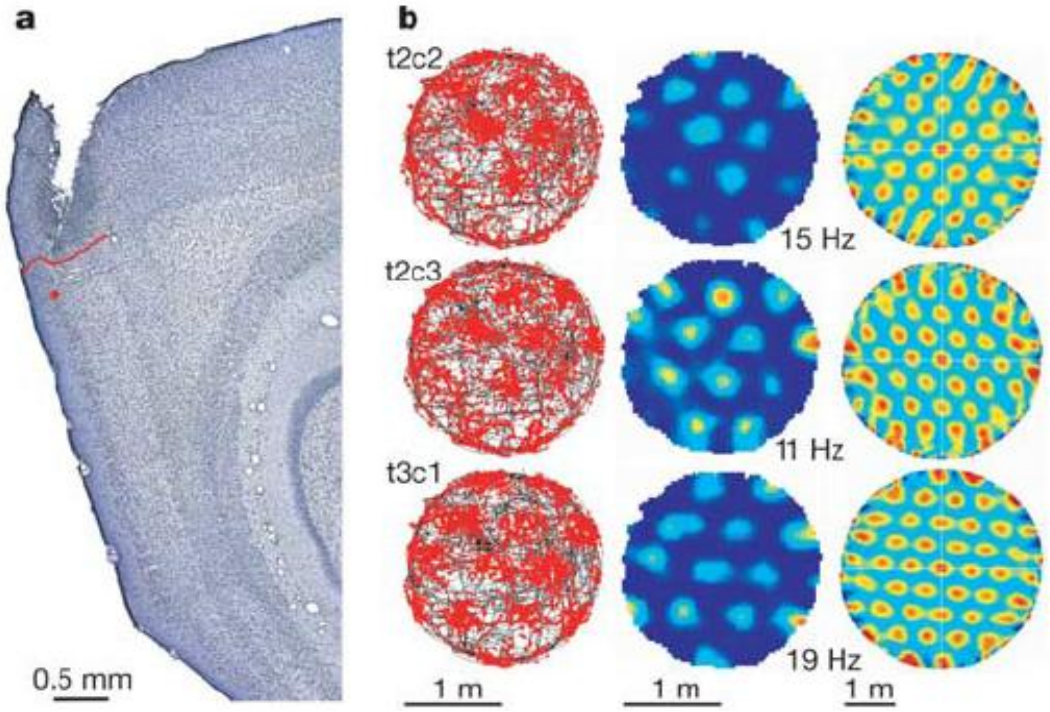

Figure 4: Grid cells.
Recordings from the medial entorhinal cortex done by Hafting and colleagues, published in 2005, found a kind of spatial processing neurons that tessellate the whole space covered by the animal with equilateral triangles forming a grid. (A) A slice stained shows the recording site in medial entorhinal cortex and in the right part of the panel an example of three grid cells isolated can be seen (B) with their raw activity showing the path of the subject during the recording in black and the spikes in red, then the overall activity is shown in a colour code plot (maximum frequency indicated) after applying a smooth filter and finally the spatial autocorrelation of each of the cells.

The grid cells discovery largely excited the PC's community. Not only because it attracted system's neuroscientists to the field, but also because several theories of how the brain integrates the information coming from the HDCs, border cells and grid cells result in the location-specific activity of PCs emerged in the subsequent years (Burgess and O'Keefe, 2011). These theories mostly argue that the parahippocampal neurons serve as the anchors of the PCs activity in conjunction with the theta oscillation as a pacemaker of the animal's translations in space. The grid cells do not depend of the environmental cues because it is not possible to establish the geometric firing pattern just by landmarks. Thus, they imply an internal computation of the animal's location by the brain in an egocentric way, a brain mechanism devoted to the quantification of

movements and angular positions, looking the grid cells as a kind of odometric control similar to those used for navigation in robots.

Another important debate about PCs is concerned with the fact that their spatial specific firing could be just an effect of memory process occurring in certain places of the environment. The hippocampus is an associative cortex where memory processes, as consolidation and retrieval, have been found to occur (Squire, 1987) and the PCs could be neurons participating in episodic memory just associated to certain locations. There is evidence supporting the cognitive map theory and the episodic memory as well. For example, PCs associated to reward, odours and in general to behaviourally relevant events have been found (Moita *et al.*, 2004; Leutgeb *et al.*, 2005), needing a context in order to fire in a particular location with not increased activity in their absence. Studies in recent years indicate that a solution to this debate may be emerging, because concepts such as episodic memory, context-dependent learning or learning of spatiotemporal sequences can be viewed as requiring an integration of explicitly spatial and non-spatial representations (Jensen and Lisman, 2000; Eichenbaum and Fortin, 2005). Thus, it seems that there is not a winning theory but rather a conjunction of them showing how the memory and the spatial learning are strongly interrelated processes.

Until now the proofs of the function and the characteristics of the neurons involved in the processing of spatial information have been reviewed in order to rapidly give to the reader a framework to understand the contemporary knowledge and debates concerning the representation of the space in the brain. However this aim needs a broader view of the brain area involved in those functions with special care about its inputs and cellular components.

1.1.2.2. Hippocampal anatomy.

The hippocampus is a bilateral symmetrical structure situated below the posterior and temporal lobes. Phylogenetically, it is older than the neocortex and its layer structure differs from the six layers found across the cortical areas because the hippocampal principal cells are packed densely in only one layer with their associated interneurons surrounding them. During its development, the hippocampus is formed from the

telencephalon and it is considered a part of the limbic system together with the cingulated cortex, the olfactory cortex and the amygdala. The limbic system is characterised by a structure in fewer layers than the classical 6-layers of the neocortex and is usually referred as allocortex or archicortex. The function of the limbic system is generally related to emotions, odours processing and memory associations (MacLean, 1952).

Anatomically, the hippocampus, is a particular area because while the neocortex has reciprocal connections between their adjacent regions, the hippocampus does not; Ramón y Cajal was the first to describe this characteristic in 1893 (Ramón y Cajal, 1893). Authors usually refer to this organisation as the hippocampal loop and the first step in this circuit are layers 2/3 of the entorhinal cortex (EC) as the main input to the hippocampus via the perforant pathway (Figure 5). The fibres from layer 2 of entorhinal cortex are unidirectional and project to the dentate gyrus (DG); the other fibres from the layer 3 of the entorhinal cortex project directly to CA1. Then the information flow that has arrived to the granule cells of the dentate gyrus from entorhinal cortex's layer 2 continues to the CA3's pyramidal neurons via the mossy fibres and once again the projections are not reciprocal. CA3 has a more complicated circuitry because, first of all, there are collateralized axons that terminate within CA3 forming a feedforward loop while the other axons of the CA3's pyramidal cells (fibres also known as schaffer collaterals) are directed to the pyramidal cells of CA1. Furthermore only the step between CA1 and CA3 has some reciprocal connections, prevailing the CA3 to CA1 direction. CA1 in turn project to the subiculum (Sb) and to the deeper part of entorhinal cortex; these are its layers 4 and 5. The Subiculum received from CA1 their inputs and it sends its axons mainly to the entorhinal cortex closing the loop on one hand and on the other hand some other projections are sent to the paraSb and the preSb. In short, the main circuitry of the hippocampal formation begins in the entorhinal cortex input passing trough DG-CA3-CA1-Sb as stages of information processing to finally return again to the entorhinal cortex (Figure 5B). This circuitry is highly conserved across animal species and therefore suggesting an important central role or function in the nervous system.

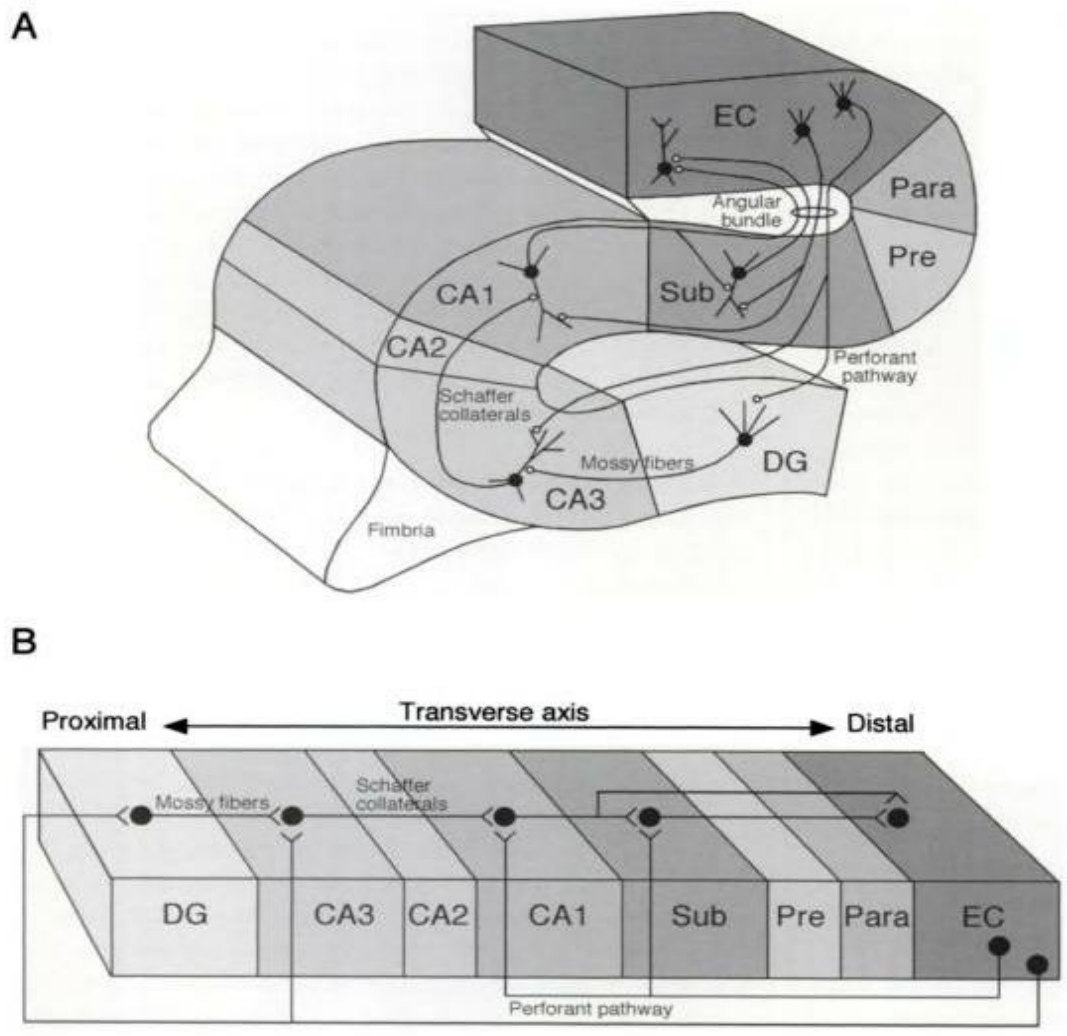

Figure 5: The parahippocampal formation.
(A) The figure shows the anatomical structure and the different regions in which the parahippocampal formation is subdivided. As we can see the principal input to the hippocampus is the entorhinal cortex that sends their axons mainly to the dentate gyrus. From there the information flow follows a step processing pathway, linearized in the lower plot (B), passing through CA3-CA2-CA1-Sb to finally return to the entorhinal cortex. Mostly, exception for CA1and CA3, there are not reciprocal connections as it occurs in neocortical areas being a unidirectional flow. Adapted from "The Hippocampus Book", Andersen et al. 2007.

The connectivity just described refers to the hippocampal main organization in relation to the surrounding regions which have direct connections to it. As we have seen the principal input is the entorhinal cortex and after crossing the hippocampal subregions the loop is close by the return to the entorhinal cortex. Some authors have proposed that this loop, comprising the perforant path fibres coming from the entorhinal cortex to the dentate gyrus and then passing through CA3 and CA1 before coming back to entorhinal cortex, is a functional unity of information processing (Andersen *et al.*, 1969; Andersen

et al., 2007) and the hippocampus is the core region organising it. The loop is repeated across the medio-lateral axis and the hypothesis of these authors see the hippocampus as a series of parallel units working independently. In the next section *1.1.2.3. Citoarquitectonic organisation* the few perpendicular fibres, known as commissural fibres, attaching these modules will be described.

For the effects of the present work, it is important to extend the brain circuitry a step beyond the hippocampal formation. This is important because the entorhinal cortex, the main hippocampal input, is divided in two sub-regions, the medial and the lateral sections (MEC and LEC), whose functions differ substantially. As referred above, the medial entorhinal cortex has spatial functions represented by the head direction cells (HDCs) and the grid cells, while the lateral entorhinal cortex has neurons with no stable and spatial specific PFs (Hargreaves *et al.*, 2005). Medial and lateral entorhinal cortices receive the information from different brain areas and they are the final output of the usually called dorsal and ventral pathways (Burwell, 2000; Witter *et al.*, 2000). The dorsal pathway conveys context-dependent information in primates, known as the "where" route, coming from the retrosplenial and parietal cortices, while the ventral pathway comes from the perirhinal cortex, which is connected to other visual cortical areas considered object-related ("what" route) (Figure 6). Thus, the information needed to process the object-in-place question converges in the hippocampus from the entorhinal cortex (Figure 7). For this reason, the tracking and localisation of dynamic cues, a brain mechanism essential to survive, seems to be integrated at this point of the spatial information flow: the hippocampus. The nature of the object and its position are fundamental features for its correct localisation; other visual mechanisms could help in the eye-tracking of the object, but the spatial representation of it needs an associative cortex that maintains and updates continuously its position without losing its identification.

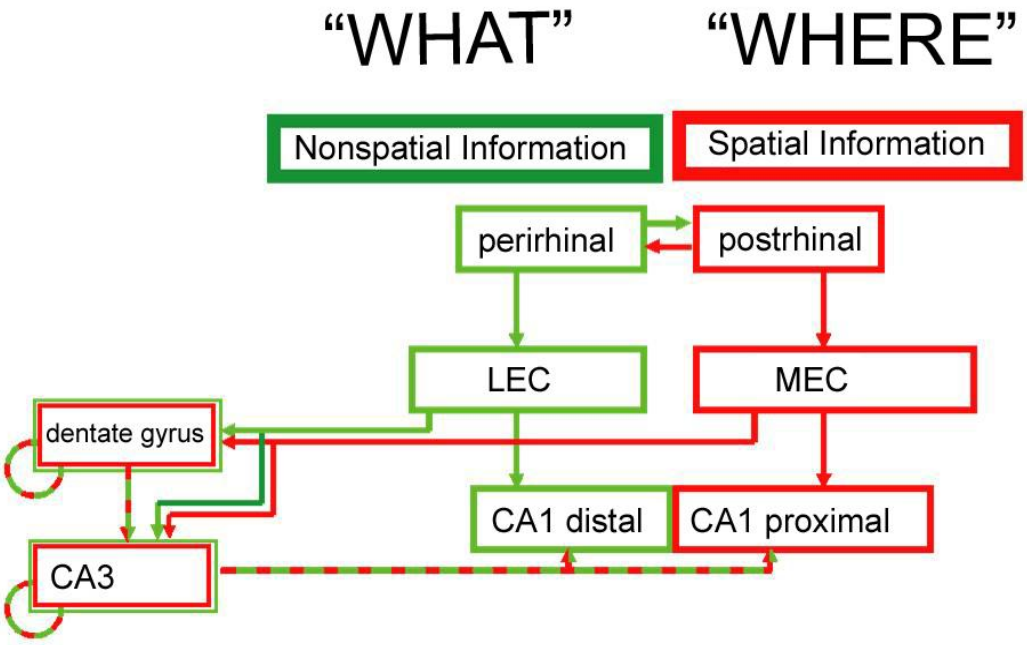

Figure 6: Ventral and dorsal pathways of visuo-spatial information.
The visual areas of the cortex send their information to the hippocampus via the entorhinal cortex but this flow is conveyed in two different pathways: the dorsal "where" stream to the medial part of the entorhinal cortex and the ventral "what" stream to the lateral part. Therefore these flows converge in the hippocampal formation where they can be integrated. Adapted from Knierim 2012.

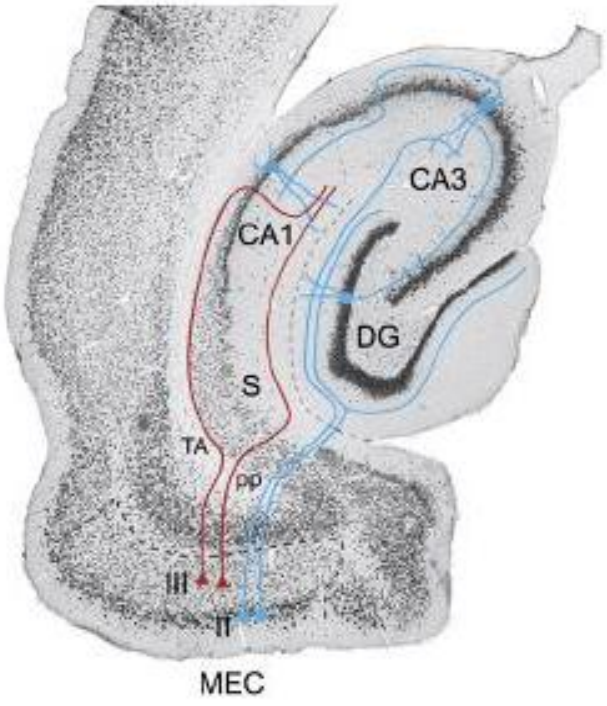

Figure 7: In this brain slice the hippocampus proper (CA1, CA3 and DG) and the hippocampal formation (MEC and S, subiculum) can be seen. The CA1 and CA3 zones have a continuous pyramidal layer U-shaped clearly revealed by the staining, so-called *regio superior*, and the dentate gyrus conforms another U-shape by the stained granular layer, *regio inferior*. In blue the inputs to dentate gyrus coming form layer 2 of MEC are shown and in red the ones coming form layer 3 to CA1.

1.1.2.3. Citoarchitectonic organisation.

As pointed before, the layer structure of the hippocampus differs substantially from the neocortical 6-layered organisation. Across the whole hippocampus there is a highly packed region of pyramidal cells, which composes a dense layer that is surrounded by different classes of interneurons, and thus the hippocampus is considered a brain region with a different citoarchitecture than the cortex. The hippocampus owns his name to the shape identified by the first anatomists, which reminded them of a seahorse, whose name in latin is "hippocampus". Posterior staining revealed that it is separated in two different U-shaped regions intersecting each other (Figure 7): the *fascia dentata* or dentate gyrus (DG) and the *cornus ammoni,* Ammon's horn (including CA1 and CA3). The dentate gyrus is a three-layered structure composed by: the *molecular layer,* the *granular layer* and the *polymorphic layer.* We will focus here in the layer and in the cellular description of the *regio superior* or "cornu ammonis" (CA), which is the region of interest in this work. There have been different attempts of a further layer classification of the CA and one of the more accepted is the next 7 layers classification (Lopes da Silva *et al.*, 1990):

- *Stratum moleculare (s.m.)*: this layer contains mostly fibres and dendritic terminals and it is adjacent to the hippocampal fissure. The perforant path fibres form synapses with the distal and apical dendrites of the pyramidal cells.
- *Stratum lacunosum (s.l.)*: it is a thin layer commonly referred together with *s.m.* as *stratum lacunosum moleculare (s.l.-m.)*. The main difference of the *s.l.* is the fact that there are not only fibres of the perforant pathway but also extrinsic ones, such as the fibres coming from the superficial layers of the entorhinal cortex.
- *Stratum radiatum (s.r.)*: it is characterised by a sparse number of interneuron's cell bodies (basket cells, bistratified cells and radial trilaminar cells). Different fibres are also found in this region, including schaffer collaterals (connecting CA3 to CA1, also known as associational fibres), septal and commissural fibres.
- *Stratum pyramidale (s.p.)*: it is the layer that contains the cell bodies of the pyramidal cells. When stained, this is the layer more dense-packed with neurons because not only the pyramidal excitatory cells are found, but also some

inhibitory interneurons as the axo-axonic cells, bistratified cells and radial trilaminar cells.

- *Stratum oriens (s.o.)*: it contains the tufts of the basal dendritic organisation of pyramidal cells and the cells bodies of basket cells and horizontal trilaminar cells. The basal dendrites of pyramidal cells receive input from other pyramidal neurons (recurrent connections are found especially in CA3), septal fibres and commissural fibres from the contralateral hippocampus (in rodents the two hippocampi are highly connected, while in monkeys these connections are weaker).
- *Alveus*: it is formed by the axons of the pyramidal neurons and incoming fibres from other areas. Some cell bodies can be found in it but they are equal to the ones of the *s.o.* and therefore considered part of it.
- *Epithelial zone*: it is the limit between the hippocampus and the ventricular surface of the hippocampus.

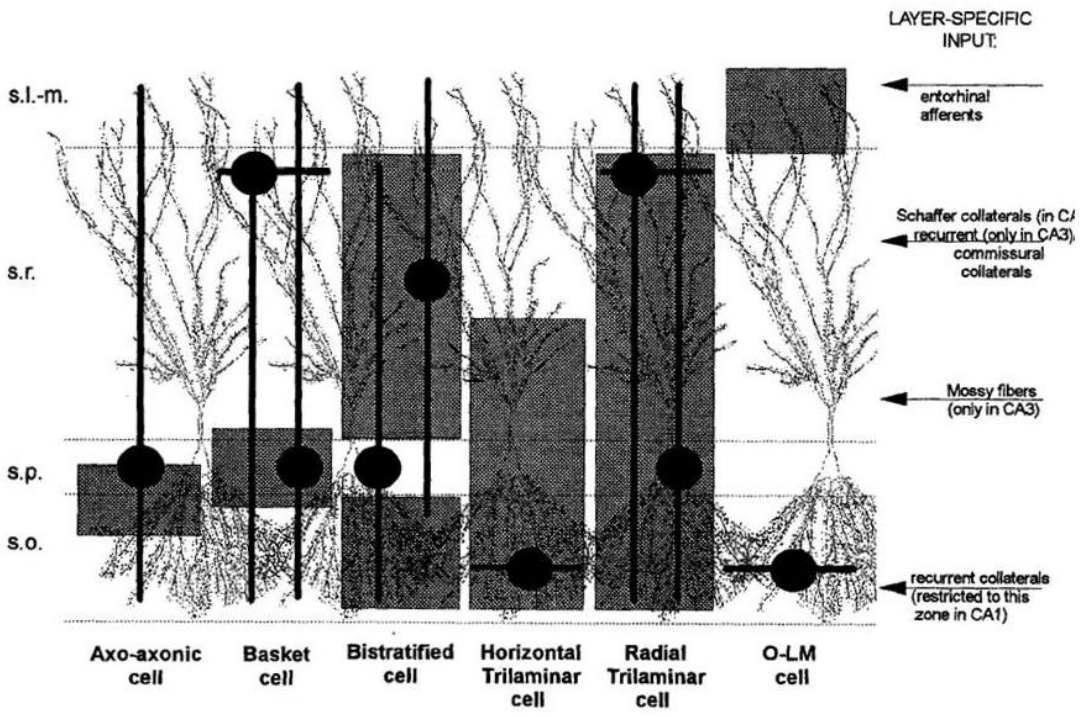

Figure 8: Layer organisation of the hippocampal *regio superior* (CA1 and CA3).
The nature of the interneurons found in this region and its localisation across the layers is depicted. In the right part of the drawing the different inputs receveid by each layer are indicated. Pyramidal cells are not shown in this figure but they have the cell bodies in the *stratum pyramidale (s.p.). stratum lacunosum moleculare (s.l.-m.); stratum radiatum (s.r.); stratum oriens (s.o.)*. Adapted from (Freund and Buzsáki, 1996).

A scheme reviewing the described layers, the interneurons found in the *regio superior* and the inputs received by each layer is presented in Figure 8. The pyramidal cells are not shown in the figure but, as described above, they have the cell bodies in the *s.p.* layer. As we can see, the *regio superior* is an area rich in the kind of interneurons present, some works describe twenty one different types (Klausberger and Somogyi, 2008). Not only the layer distribution differs from the cortex, but the packing of the cells in the hippocampus seems also to be denser. Summarising, the hippocampus is an associative cortex with a special high number of neuron types and with a highly packed circuitry that differs substantially from the neocortex as in the layer distribution as in the lateral connections. Overall, the circuitry found suggests a unidirectional information flow which is conformed by the principal cells in the subsequent steps and simultaneously modulated by a high number of interneurons. The kind of excitation and inhibition processes occurring in the hippocampal synapses is varied, a large series of neuromodulators are involved in the circuitry. Next section will review the principal hippocampal synapses and their nature.

1.1.2.4. Neurochemistry.

The hippocampus variety of circuits and their neuromodulators is extensive and thus we will focus only on the transmitters involved in the main circuitry. Following, there is a description of the five main families of neurotransmitters or neuromodulators that act in the synapses of the hippocampus, mentioning the pathways that they mediate and the neuronal types involved (Lopes da Silva *et al.*, 1990).

1) *Glutamatergic pathways:* A big part of the synapses found in the hippocampus have the glutamate as the neurotransmitter. It produces an excitatory effect by depolarizing the postsynaptic cells. The intrinsic pathways of the hippocampus (mossy fibres and schaffer collaterals) are one of the examples in which glutamate mediates the synapses. Also, the major input coming from the entorhinal cortex, the perforant path, is glutamatergic. The NMDA are the main receptors for the glutamate; their distribution across the hippocampal cells is regionally specific and their activation depends critically

on the state of the membrane potential. Plasticity and memory processes are thought to be supported by their modulation via the control of the calcium influxes and the LTP.

2) *Gabaergic circuitry:* As a counterpart to these excitatory pathways, GABA is the main transmitter of inhibitory connections, arising not only from local interneurons but also from cells of the contralateral dentate gyrus and the medial septal nucleus. The physiological effect of GABA is essentially a hyperpolarization, either because of an increase of a Cl^- conductance (GABA-A type receptor) or of a K^+ conductance (GABA-B type receptor). However, some depolarizing effects, via the increase of Cl^- conductance, have mainly been found on the granule cells of dentate gyrus and on dendrites of CA pyramidal cells.

3) *Cholinergic inputs:* The third type of predominant neurotransmitter is acetylcholine (Ach), which is mainly found in fibre terminals coming from outside the hippocampus and this is why it is considered as a neurotransmitter mediating part of the hippocampal inputs. This transmitter modulates K+ conductances, causing a decrease of I_M (the non-inactivating voltage-gated potassium current) and a K+ outward current. The latter current underlies the after-hyperpolarization (AHP) that follows action potentials. In this way, ACh prolongs action potential discharges modulating the hippocampal activity.

4) *Neuromodulators (Dopaminergic, Serotoninergic, etc…):* The other substances, such as norepinephrine, dopamine, 5-HT, and histamine, act essentially as modulators of neuronal excitability of the hippocampal synapses, mainly through changes in K+ outward currents (AHP). They may have other specific effects, depending on the type of cell and on whether the site of action is mainly post- or presynaptic.

5) *Neuropeptides:* Finally, the physiological effects of the neuropeptides that are abundant in afferent fibres and/or in local neurons are a matter of intensive investigation. In relation to two of these, interesting findings have already been obtained. *Enkephalin* causes mainly membrane hyperpolarization and depression of granule cells but disinhibition of CA1 pyramidal cells mediated through a depression of local inhibitory interneurons. *Somatostatin* has hyperpolarizing effects on CA1 pyramidal cells through an increase in I_M, an effect that is opposite to that of ACh.

1.2.2.5. Hippocampal oscillations.

A special mention of the hippocampus activity refers to its oscillations. When the LFP of the hippocampus is recorded, four rhythmic activities are found: *theta* 6-12 Hz, *beta* 12-30 Hz, *gamma* 30-100 Hz and *ripple waves* 100-200 Hz. Another two non rhythmical activities are also present: the large irregular amplitude (*LIA*) and the small irregular amplitude (*SIA*). In Figure 9 an example of the different activities can be observed in real recordings; the first three rows show different cases of the *theta* rhythm (during REM sleep (1), jumping (2,3) or swimming(4)) and then other three examples of LIA activity are presented: animal sat (5) and during slow wave sleep (6, 7). In the last signal exposed of the figure (7) the ripples that can accompany the *LIA* are highlighted. Therefore, some of these activities can co-occur (*LIA* with *ripples*, *theta* with *gamma*) while others are mutually exclusive (*theta*, LIA and SIA). *Theta* is the most common of these activities, it varies in relation to the locomotor activity of the subject and also attentional states can modulate it (Vanderwolf, 1969). The rhythmic of *theta* is smooth and often sinusoidal, the sharper peak in the power spectrum is around 7-10 Hz and correlates with walking activity, while its harmonic usually appears at 16 Hz. The *LIA* pattern is much more random: its power spectrum is flatter and non relevant peaks in low frequencies are found even if the power across 1-5 Hz is higher than *theta*. During *LIA* the *sharp wave ripples* can occur: they are a high frequency activity that oscillates at 100/200 Hz. *SIA* activity is rare and characterised by low amplitude with a broad spectrum of high frequencies. *Beta* and *gamma* are the other classical oscillations found in the hippocampus and they can occur alone or simultaneously to *theta*, *LIA* or *SIA* (Andersen *et al.*, 2007).

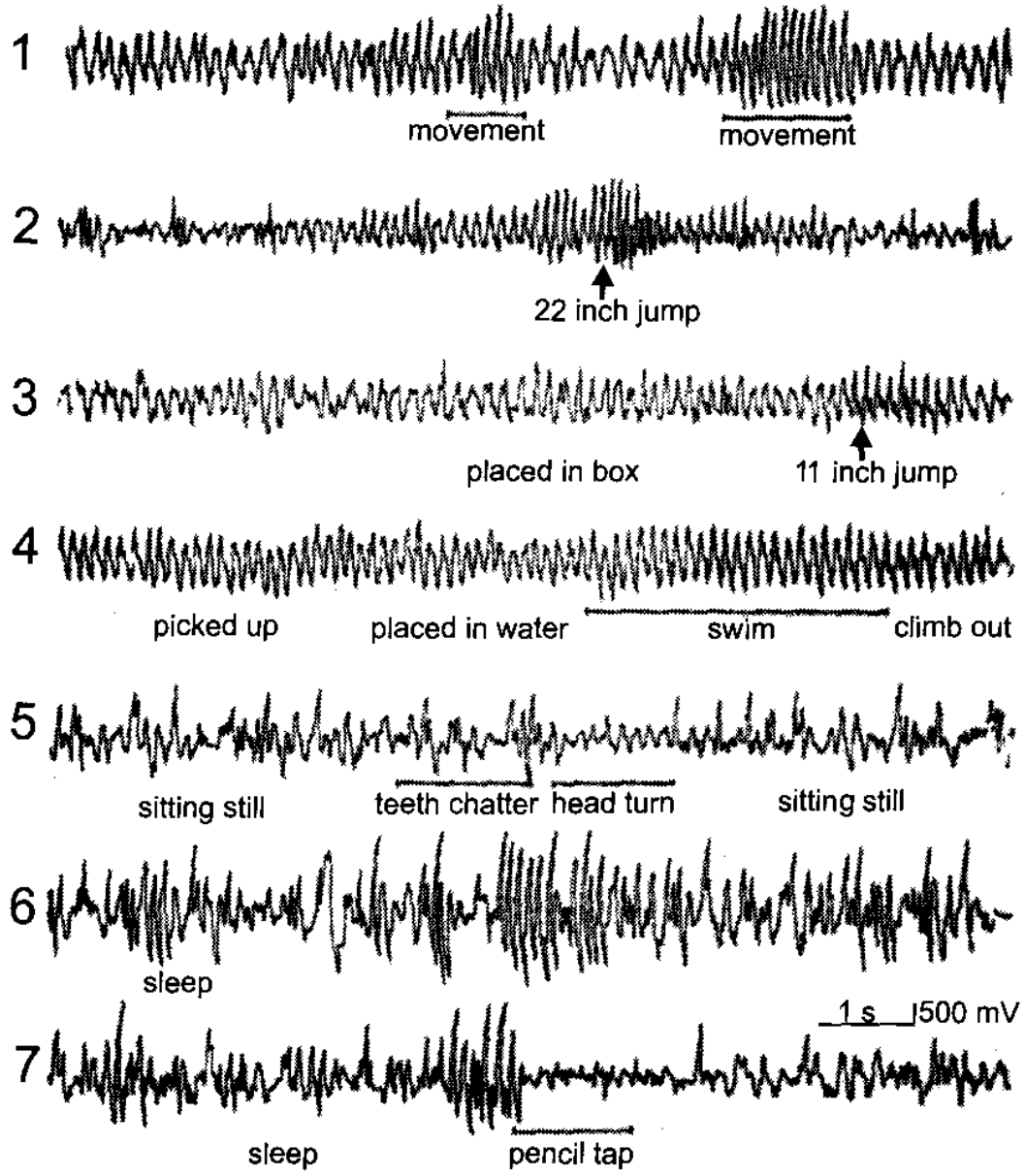

Figure 9: Hippocampal oscillations.
Theta during REM sleep (1), when the animal is jumping (2, 3) and swimming (4). The LIA activity is also showed in different situations: animal sitting (5) and durind slow wave sleep (6, 7). The ripple wave can be observed as the periods inside LIA with high amplitude and frequency. Adapted form "The Hippocampus Book", Andersen et al. 2007.

For the purposes of this work, only *theta* has been deeply analysed and therefore this section will review mostly the literature regarding *theta*'s characteristics and function. The other activity patterns aforementioned did not show important relationships with our focus of study. However a short mention of *ripple waves* and *gamma* concerning the processing of spatial information is needed. One of the features of the activity of the

PCs is their bursty behaviour during the entrance to the PFs (O'Neill *et al.*, 2006); this special pattern occurs as a *ripple wave* and its function is not clearly known. Some theories argue that they play a role in the consolidation and retrieval of memory. This idea is supported by different studies where the impairment of learning processes by abolishment of *ripple waves* was demonstrated (Jadhav *et al.*, 2012) or by other interesting works where the discovery of replay and preplay discharges of pyramidal cells have been shown to correlate respectively to the previous observed firing patterns of the cells or to the future ones (Foster and Wilson, 2006; Dragoi and Tonegawa, 2010). Concerning *gamma,* a similar function is hypothesised because its activity occurs in combination with the synchronisation of the hippocampus and other brain areas, entorhinal and perirhinal cortices are examples (Buzsáki, 2002). Thus, the function of *gamma* is thought to be a support of memory processes by the improvement of neuronal synchronicity across areas, and therefore as a rhythm underlying the information transfer and being cross-related to *theta* in neuronal computations (Lisman, 2005).

Regarding *theta,* a large amount of work has been developed because not only it dominates the hippocampal power spectrum but also correlates with a broad range of behaviours, varying from ongoing activity, arousal and attention (Vanderwolf, 1969; Buzsáki, 2005). The distribution of the oscillation varies across the hippocampal formation and it can not be considered uniform (Lubenov and Siapas, 2009). *Theta* activity can be separated again in two components (Kramis *et al.*, 1975), one of them related to arousal or attention (6-7 Hz), and the second one with voluntary movements or ongoing activity (7-10 Hz). Pharmacologically, these two ranges of frequencies can be separated as well; the atropine-sensitive *theta* is the one related to arousal or attention, *a-theta*, while the translational-movement *theta* is unaffected by cholinergic drugs, but the transmitters serotonin and glutamate mediating it, *t-theta*. Lesions of the medial septum and the associated diagonal band (medial septum diagonal band of Broca, MSDBB) eliminate both types of *theta* activity, while intraseptal injections of cholinergic antagonists disrupt both *theta* when the animal is stopped but not the *t-theta* associated to the movements (Kramis *et al.*, 1975). If the injections use cholinergic agonists instead of antagonists, the result is a continuous state with *theta* irrespective of the subject's behaviour. Oppositely, the lesions produced in the entorhinal cortex, the major input to the hippocampus, eliminate *t-theta* while *a-theta* is unaffected (Buzsáki, 2002).

A large debate still exists trying to unravel the real function of theta but three main functions are largely accepted. The first refers to the so-called "global synchronising mechanism", which means that the oscillation serves as a coupling between large areas of the hippocampus and also with other sensory and motivational areas locked too (Mitchell and Ranck Jr, 1980). The second one is the interpretation of theta as a "periodic clocking system" for the precise timing of spikes, organising them in phases of its oscillation and therefore improving the computations. In the case of PC activity, this timing effect has been calculated and results in a theoretical improvement of the spatial localisation inside the PF of a 40% (Jensen and Lisman, 2000). Finally, the third theory is the hypothesis that theta serves to provide temporal control over long-term potentiation (LTP) induction. Depending on the phase of CA1's *theta* cycle, when the inputs arrive they can produce synaptic potentiation (LTP) if it is in the positive phase or depression (LTD) when it is in the negative phase (Pavlides and Winson, 1989). One recent work supporting the last function argues that the two different phases, positive and negative, separate the information flow relative to the encoding or the retrieval periods between hippocampus and entorhinal cortex (Hasselmo, 2005). Experiments performed in subjects that were exposed for the first time to a new environment showed a change in the global activity of the *theta* rhythm that was independent of the locomotor activity (Jeewajee *et al.*, 2008; Lever *et al.*, 2010). These results support the interpretation of *theta* as an oscillation mediating either memory processes involved in the encoding of new information and as an integrator of sensory information from different brain areas.

Briefly, it is also important to say that the observed mechanisms of *theta* and its putative functions have analogies in other studied mammals and humans. Surface electrodes placed on the scalp's skin showed theta correlation with maze difficulty prominently in the temporal lobe of subjects performing navigation in a virtual reality maze, more specifically in the hippocampal formation (Kahana *et al.*, 1999). Using depth and subdural electrodes in a virtual taxi driver task, *theta* activity was found increased during virtual movement, exploratory search and goal-seeking (Caplan *et al.*, 2003). As exposed until now the *theta* activity seems to be an oscillation with functional characteristics preserved across animal species, highlighting its importance in the hippocampal coding of memory and its transfer to other areas.

1.1.3. Space representation across other species.

In spite of the fact that the rodents became the main model of spatial navigation after the discovery of PCs, a wide amount of work has been done in other species. The other main focuses of these studies have been birds, monkeys and humans. For birds, an important set of work was done before the establishment of the rodents' hippocampus as the main model of spatial navigation because they are species with perhaps the more talented navigational system known. The first studies were merely behavioural (Keeton, 1974) and only in the last decades electrophysiologists started to unravel the neural mechanisms underlying the birds' navigational skills, first by disrupting the local homing behaviour producing lesions in the parahippocampal region (Bingman and Yates, 1992) and more recently by the use of telemetry and electrophysiology in pigeons flying large distances (Vyssotski *et al.*, 2006). In the case of monkeys the approach has been totally different. Primates have been not a special focus in the spatial navigation field, however several studies assessing superior cognitive functions can not go to the neural level in humans and therefore there were used as models of these brain functions. As we can see, this was the case because even if in both, humans and monkeys, the hippocampus has been discovered as the core of spatial navigation as in rodents, the way in which it works seems to have slightly diverged from the inferior mammals to the primates and humans. This section will focus on the human brain and then it will discuss the other data obtained from monkeys that further support the interpretation of the human brain data.

As the major part of the neuroscientific advances in the discovery of the function and mechanisms underlying the human brain, the first studies that led to a deeper research of specific functions or areas were works derived from cases of patients with brain damages or injuries produced by strokes, illnesses or, in some cases, surgeries effectuated after the localisation of the brain's breakdowns produced by them. In the case of spatial navigation, the first works that found patients with impairments regarding spatial abilities were studies of amnesic people. One of the first descriptions explained the cases of people who have memory deficits associated not only to objects that have been seen recently, but also to where the objects have been seen (Smith and Milner, 1981). Almost in parallel the same conclusions were found for primates' studies; for instance, there were experimental studies in monkeys trying to define the

crucial structures to which damage produces memory impairments. Several studies showed that hippocampal damage produces deficits in learning about the place in which objects have been seen (Parkinson *et al.*, 1988; Gaffan, 1994). Therefore, the central role of the hippocampus in spatial memory and navigation was already described in these first studies. As we can see, the combination of human clinical cases and the experiments performed in monkeys started to reveal the brain regions involved in the processing of spatial information. Nevertheless, until the introduction of new technical approaches, the brain's spatial function in healthy humans was not assessed. A big step in this sense was the application of the fMRI to further reveal which are the regions used to solve spatial tasks in humans. An important work combined the fMRI results during navigation of humans in a virtual maze (Aguirre *et al.*, 1996) showing that the hippocampus and the parahippocampal region, principally entorhinal and perirhinal cortices, were involved in place learning. Thus, the evidence confirmed once again that the same brain regions dedicated to spatial information processing in rodents were conserved in primates and humans.

During the 80's there were a series of experiments performed by Edmund Rolls and collaborators that represented a step beyond in the study of primates' brains than the previous works, where only the spatial processing areas of the monkey were identified. Several publications of the group appeared during this decade (Foster *et al.*, 1987; Foster *et al.*, 1988; Foster *et al.*, 2000) and perhaps the most importants of them were the ones declaring the finding of spatial view cells in the monkeys' brain (Rolls and O'Mara, 1995; Rolls, 1999). These cells were found in the primate's hippocampal formation and, instead of firing when the monkeys were in a particular location of the environment, they fired whenever the sight of the subject was directed into a certain location of the environment. At first glance, the results could be interpreted as head direction cells, but the monkeys were actively moving across the environment and therefore the activity of the neurons was not related only to the head's direction but to the part of the environment that was at this moment observed. Interestingly, the neurons and the regions where they have been found correspond tightly to the system of rodents; the major part of the cells was in CA1 and CA3 with some cells in the parahippocampal gyrus and presubiculum. Furthermore, their spiking properties correlate with pyramidal cells as in rodents. These results point to a maintained spatial processing system for the hippocampal formation across mammals but with some little divergences in their

function for primates and humans. In rodents, the cells found are location-specific while in superior monkeys they are scene-related. Thus, the system seems to be conserved but the way in which it works has changed, probably due to other divergences in the previous processing of visual information. Recently, the same case was found for humans in a new set of experiments performed using environments of virtual reality (Ekstrom *et al.*, 2003). The fact that humans can immerse themselves in these artificial environments allowed the experimenters to have a corporal stability of the subject that assures a good recording of the neural activity during the tasks. In this way, Ekstrom and collaborators found analogies of the spatial view cells of primates in humans. The results pointed out that in the hippocampus and in the adjacent areas there were cells encoding information about as to the place virtually occupied by the subjects as to the visual direction of the subjects.

In the subsequent years several other articles were published confirming the results obtained in rodents and the combination of spatial tasks in virtual environments with fMRI studies extended the cognitive map theory to humans. The parallelisms began with the hippocampus identified in humans as the core of spatial navigation, but subsequently also a kind of head direction cells were found in the human entorhinal cortex (Jacobs *et al.*, 2010), the role of perirhinal cortex in object-context associations was discovered (Watson *et al.*, 2012) and the use of allocentric strategies in navigation showed to be supported by the parahippocampal region (Weniger *et al.*, 2011). Thus, the large amount of work done for the rodents' hippocampus showed to be a very powerful model of spatial navigation inspiring the work in humans once the techniques allow assessing the same questions with non-invasive methods in healthy humans. However, there are substantial differences between the rodents and the superior mammals. While the PCs seem to indicate precisely the position of the rat or the mouse in the environment, they do not mediate in rodents one of the functions in which primate's (including human) hippocampus is implicated, that is the role of the memory conveying the information about where in space an object has been seen, which can be remembered perfectly even when the human or monkey has never been to that particular position in space. Therefore, even if the similarities continue to appear when the work done in rodents is extended to humans, several questions will remain exclusive for our specie. Anyway, the model of spatial navigation in rodents has shown to be an important scaffold where important questions about the neural mechanisms underlying

the computation of spatial skills have been advanced in order to better point the focus when the same human function started to be investigated.

1.2. Dynamic cues

Until now, the introduction has focused on the previous literature related to the spatial processing and on how brain cognitive maps are created. Most of the work done in the past in relation to this issue has mainly evaluated the role played by the environmental static cues that lead the subject to orientate itself in space. The purpose of this section is to review the work done in relation to the dynamic cues across the neuroscience and, as we will see, the biggest efforts done in this sense were studies coming from the large field working in the visual system function.

The detection of movement in animals has been widely studied across different species revealing neural and computational mechanisms that work even at the level of the eye in the case of flies (Kimmerle and Egelhaaf, 2000). However these mechanisms explain just the clockworks underlying the function but not the behavioural outcome of them. For a predator it is very important to detect continuously the slight changes of the others in the space as it is for the prey in order to avoid the predator. Specific brain mechanisms should underlie these abilities. In the case of rats, the existence of neurons calculating the distance to a certain frontier or object has been found (Lever *et al.*, 2009) and it could sustain the correct localisation of an object in an egocentric framework. Nevertheless, for an allocentric system, map-guided, it is necessary the existence of neural mechanisms that will encode the representation of the location not referenced to the animal current location as it has been demonstrated for static elements. If we think about the importance of an allocentric system guiding the representation of dynamic cues there are several reasons supporting its putative existence. First of all, while an object is moving the brain can anticipate its trajectory and therefore infer its subsequent locations across time, so that an egocentric system should be updated continuously regarding the changes of the own subject's position, while for an allocentric it will be

easier to maintain the representation guiding the encoding with the other static characteristics of the environment already processed in an allocentric way. Thus, the objective of our work is precisely to find these interactions between the allocentric representation of the own subject position and the relevant dynamic cues encountered in its environment.

Some works have already advanced this idea and subjects were trained to discriminate the relative position of a dynamic cue respect to others that are static in a screen (Nekovarova and Klement, 2005). The results show the capability of rats for discriminating the relative position of a dynamic object respect to static ones, but even if the subjects learned easily the task the effect of their own position and movement in the discrimination was not assessed. In a different approach, this effect was better clarified, when the rats were placed in a special environment in which they should differentiate between static cues and global dynamic cues in order to avoid punishment (Cimadevilla *et al.*, 2001). The set-up used a stationary arena surrounded by a set of cues rotating during the task. Animals learned to avoid specific regions of the stationary part where there were placed in relation to the rotating cues. This work demonstrated the fact that, on one hand, rats can use different reference frames simultaneously and, on the other hand, that these frames can be dynamic. The aforementioned works opened a new branch by showing how rats are capable to discriminate dynamic cues by their relation with other statics; nevertheless, a more natural paradigm where the subjects should discriminate the dynamic cue and perform a natural behaviour was missed at this point. Subsequently, new paradigms filled this lack. The capability of the rats in attaining success correctly the identification of another moving object or a robot in an open arena has been demonstrated (Pastalkova and Bures, 2001; Telensky *et al.*, 2009) and the results revealed that the task was easier for the subjects when it was done with a robot, which was possibly perhaps because it avoided the social interactions derived of the presence of a conspecific. The inactivation of the hippocampus during these protocols severely impaired the ability of rats to perform correctly the task (Telensky *et al.*, 2011).

As we can see, the issue of spatial localisation of other moving objects has been researched in the last decade and until now the presented previous literature shows behavioural results and inactivation paradigms where the involvement of the

hippocampus is once again demonstrated. In the last years, coinciding with the development of this PhD thesis, the first electrophysiological studies assessing the question of other moving objects' localisation have been shown. Few but relevant works have been done in this sense. The first of it used as approach a circular arena where a rat chronically implanted in CA1 with tetrodes should avoid a toy car placed within the same arena (Ho *et al.*, 2008). Subjects learned to keep a safe distance from the car in order to avoid a mild electrical foot shock. In a parallel task without the car, this punishment was delivered every 150 cm travelled to control the effects it could produce. The results show a different pattern of activity for the PCs in the car dependent task: the firing fields of the neurons tend to remap more frequently in this case. Furthermore, analysing the effect of the moving parameters of the car in the cells' activity, the authors found a significant modulation of their spatial firing due to the toy car's movement. Finally, the information content of the spikes was calculated in both protocols regarding the movement parameters of the car and there were significantly different. A similar approach was used in another study where the implanted subject was accompanied by a second rat trained as well in the foraging of randomly scattered food pellets (Zynyuk *et al.*, 2012). The results of this work confirmed the subtle effects in the PCs' activity, more precisely they found a degradation of the firing fields, but oppositely to Ho and colleagues, they did not find increased remapping due to the introduction of the second rat. An important remark of this publication concerns the fact that they found that degradation of the location-specific firing is distance dependent, which means that the closer the second rat is, the higher is the effect produced in the cells' activity. Finally, there is another relevant publication recently appeared where the authors tested if the hippocampus is encoding information relative to the recognition of conspecifics (von Heimendahl *et al.*, 2012). The aim of this work was slightly different in the onset, but both the results and the analysis that they performed can shed light to our own focus of study. The set-up consisted in a rat placed in a platform from which two other elevated spaces are available but not reachable, which means that the subject could make contact with the vibrissae but was not allowed to jump into the other platform. The aim of this set-up is to provide two different places where other rats can be presented to the subject and thus to determine if the hippocampus is involved in the recognition of individuals. The results showed surprising results: the hippocampal cells did not show specific firing to certain individuals while the neurons fired differentially for rats or inanimate objects and the neuronal responses were more strongly modulated in general by objects.

As we can see, several works published in the few last years add support to the hypothesis that the spatial characteristics of own-self moving objects are being processed in the hippocampus. Moreover, these works point to a relevant role of the hippocampus in the recognition of the object's nature and its position or other spatial parameters while not for a social processing where other individuals can be identified and localised. Therefore, our particular set-up in which the use of a robot instead of a second subject was selected is a good choice according to the results of von Heimendhal. Another important question supporting our experimental design, which will be further commented in the discussion section, is the fact that the previous works do not have a temporal restriction in the discrimination of the dynamic cue's movements, while ours does, as in classical behavioural tasks where the presentation of the stimuli is finely controlled.

1.3 Single unit's recordings (Introduction to the method)

The acquisition of electrophysiological data comprises a wide field of research. A large amount of techniques have been developed since the first experiments of Galvani passing electrical currents trough biological tissues in the final part of the XVIII century. The variety of those is enormous but a first classification of them could be based in the distinction between invasive a non-invasive approaches. Even if the invasive techniques give better results because the signal to noise ratio is higher and the activity of tighter regions is recorded the non-invasive methods have developed as well because they are largely preferred for humans' brain research. Inside the invasive techniques we can separate again the techniques in intracellular and extracellular ones. The intracellular methods need a very stable set-up that allows recording continuously the same cell, avoiding the vibrations produced by other devices and having as supports fine manipulators. Instead of that, the extracellular recordings are easier maintained mostly because the source of the signal is wider and the emplacement of the electrodes should not be so precise. Thus, depending on the purpose of the recording the intra or extracellular method is chosen. A different extracellular method was designed during the end of the last century: the stereotrode technique (McNaughton *et al.*, 1983) by the

simultaneous use of several electrodes showed to be useful to infer which the source of the spikes that are detected is. Each of the electrodes receives the changes of the potential that gradually decreases depending on the distance to the source; thus, having more than two electrodes it is possible to isolate in clusters the activity of single cells by selecting different parameters of the recorded waveforms (peak, trough, amplitude, etc…; further details in Material and methods). The fact that these electrodes are extracellular assures at the same time the stability of the recording during days or weeks. The use of the stereotrode technique rapidly encouraged many scientists to implement it in different studies. Today, thanks to the scaffolds designed for this purpose, it is possible to move independently each electrode or even select distant brain areas to record. In general, the stereotrodes are the most used technique to record during behavioural protocols in which the level of single cell activity wants to be reached. Only in the last years, intracellular techniques that are stable enough to be used in awake animals have been developed trough patch clamp in awake animals (Harvey *et al.*, 2009) and they require very sophisticated equipments with a stability of the recordings that is still far from the stereotrodes.

Thanks to the stereotrodes, important questions regarding circuits can also be assessed. The isolation of different neurons during the same recording in close or distant areas is a potential tool to analyse the relations between these neurons. The analysis of the data recorded has largely advanced from the pioneer work of McNaughton and colleagues (McNaughton *et al.*, 1983) and today the signal acquired could be used either to identify neurons (Csicsvari *et al.*, 1999) or to correlate the spiking patterns between them. A debate was opened in relation to the real possibilities that the tetrodes could offer in the study of circuits, because even if the neuron classification is precise, the pretension of a disentanglement of the circuit is far beyond the reach of the technique. That could be the case when the firing activity of different neurons is very close and, even more important, when these neurons have been not classified before with intracellular techniques. In this sense an important contribution to the debate are the studies where both techniques were combined: intracellular and tetrodes. In this way, the extrapolations derived from the stereotrodes can be confirmed or not by the intracellular recording. An example of this work is the article of Henze and colleagues published in 2000 where they performed both techniques simultaneously in CA1 (Henze *et al.*, 2000).The results obtained were conclusive for several aspects as, for example, the fact

that some features of the action potential can be deduced from the extracellular correlates (width and amplitude). Another important question concerned about the number of neurons active was in part solved by this work because one of the absences of the tetrode's method is the missed information of non active cells. When one makes intracellular recordings, usually the emplacement of the electrode is done with a microscope that allows seeing in parallel the neurons population. In case there is no such microscope, the simple ratio of neurons patched and non active while recorded gives an approximate idea of how many neurons are active or silent. For tetrodes the technique is blind in this sense, no conclusions can be reached of the silent population, and therefore a previous knowledge of the brain area is required in order to maximise the use of the data recorded. Regarding CA1, the cell population is well known and it is probably the most characterised area of the hippocampus. The proportion of active PCs, neurons effectively firing in a specific location for such environment is around one third of the entire pyramidal cells population (Thompson and Best, 1989).

Another important technical aspect of the stereotrode's technique is the range of the area recorded. Before the implantation of the microdrive the impedance of the electrodes is adjusted. The impedance determines at least two characteristics of the obtained signal. On one hand, the range of the electrical changes detected in the adjacent volume is due to the resistance of the electrode and therefore a lower impedance result in the detection of the activity in a broader area. On the other hand, the impedance simultaneously affects the signal to noise ratio because the fact that a broader area is recorded also produce a higher level of variability in the signal which can mask relative close events. Because of that the impedance of the electrodes should be adjusted before the surgery and previous studies have determined as a correct range a value between 200 and 300 KΩ (McNaughton *et al.*, 1983). Thus, the signal stored with this impedance assures a good number of neurons in the recorded area, estimated in 50 microns of diameter, while the signal to noise ratio is high enough to well recognise action potentials of putative neurons.

2. HYPOTHESIS & OBJECTIVES

2.1. Hypothesis

The main hypothesis of the present work was that *the hippocampus is actively involved in the tracking and localisation of dynamic cues by the processing of information relative to their movement parameters.*

Behavioural experiments where the subjects were asked to track moving objects require the hippocampus because its inactivation produced a severe impairment in the execution of the task (Levcik *et al.*, 2012). The aim of the present work was to find the electrophysiological correlates of this function. Prior studies have shown that the information needed to process the object-in-space question converge in the hippocampus (Knierim, 2006). The major input to the hippocampal structure comes from the entorhinal cortex, which is subdivided in two functional sections, the medial and lateral regions. These areas have been characterised by their distinct role, the medial entorhinal cortex contains neurons with spatial processing characteristics as the grid and border cells (Hafting *et al.*, 2005; Solstad *et al.*, 2008) while the lateral entorhinal cortex has been related to information more relative to the nature of objects (Knierim, 2006). The medial and lateral entorhinal cortices are the final output of the dorsal and ventral pathways which have been respectively related to the processing of visual information about spatial characteristics, also called as the "where" content, or identity characteristics, the "what" content. Thus, only in the hippocampus such information is properly integrated, a fact that suggests the hippocampus as the candidate area to study the brain representation of movement parameters of dynamic cues.

2.2. Objectives

Main ➔

Our aim is to determine which are the movement parameters of the dynamic cue modulating the hippocampal activity and how such modulation is reflected either in the neural firing patterns or in the global activity of the network reflected by the local field potential. This will be achieved by means of the exploration of single neurons activity and the local field potential during a task where subjects pay attention to a dynamic cue associated to reward,

Scientific goals ➔

1) To determine a behavioural paradigm where subjects pay attention to a dynamic cue. In order to compare to the known spatial processing of the subject's own position such task should allow a certain level of spatial exploration by the subject.

2) To determine if the activity of the hippocampal cells is modulated by the spatial parameters of the dynamic cue. How is the proper activity of cells already coding the subject's position affected by the dynamic cue?

3) By the examination of the different cells isolated in CA1, to identify cells with activity related to the spatial parameters of the dynamic cue.

4) Extracting the predominant band of the LFP in CA1, theta oscillation around 4-12 Hz, see if there are relationships with the movements of the dynamic cue.

Technical goals ➔

1) The design and development of a behavioural task in which rats can learn to track the movements of a dynamic cue.

2) The control of the behaviour of the dynamic cue in concordance with the subject's behaviour. This way the shaping and conditioning of the subjects could be reinforced by the robot's behaviour.

3) The use of a tracking system allows on one hand to record the position of the rat and the robot during the task and, on the other hand, to use this information on-line in order to control the behaviour of the robot.

4) Finally, during the execution of the task is important to stably record the activity of single neurons and the local field potential in the region CA1 of the hippocampus. We selected the use of microdrives carrying tetrodes that allow a continuous recording of single unit activity.

5) The last step is the correct synchronisation of all the systems used during the task: behavioural apparatus, tracking system, robot's control and electrophysiological recordings.

3. MATERIAL & METHODS

3.1. Behavioural protocols

3.1.1. Subjects

Lister-Hooded male rats (N=5) with a body weight varying from 220 to 360 grams were tested. Rats were housed on a reversed 12/12 hrs light/dark cycle with a stable temperature of 22 +- 1 ºC. Animals were housed in clear Plexiglas cages measuring 60x40x30 cm (Rody Cavia, SAVIC) with Plexiglas tubes in order to enrich minimally the environment. All experimental procedures were attempted during the animal's active period. When the animals were not in experimentation phases they were housed in groups of 2 or 3 subjects, then just previously to the experiments they were separated individually and handled for a week in order to prevent stress originated by their manipulation.

During the behavioural protocols animals were water deprived. Body weight was daily supervised and to prevent an excessive body weight loss, superior to a 15 % of the initial weight, at the end of the journey subjects were allowed to drink ad libitum during 10 min.

All these measures were supervised and approved annually by the University committee (CEEA, Universitat de Barcelona) and were in accordance with the present law of animal care. These are the EU guidelines on protection of vertebrates used for experimentation (Strasbourg 3/18/1986) and the local law of animal care established by the Generalitat de Catalunya (decreto 214/97, 20 de Julio).

3.1.2. Behavioural Setup

The design of the behavioural protocol where assessed how the tracking and localisation of moving objects in the brain works was focused on the generation of salience of these objects to guarantee their tracking by the trained animals. To assure a clear view on the moving object a white cylinder was constructed on top of the robot that increases its size (the height of the robot changes from 7 to 35 cm), which creates higher visual contrast against the black walls of the maze and is seen equally from every point of view. Next, for the animal to follow the robot, it has to be behaviourally relevant. For that to occur the rats were water deprived and the correct determination of the position/direction of the robot will result in water delivery. In that way, paying attention to the robot is relevant for the animal. We developed several protocols in which the subjects need to track the direction of movement or the position of the robot later explained (see section *3.1.3. Behavioural protocols*). However all the protocols share in common the fact that the robot's behaviour determines when the subject can receive reward or punishment.

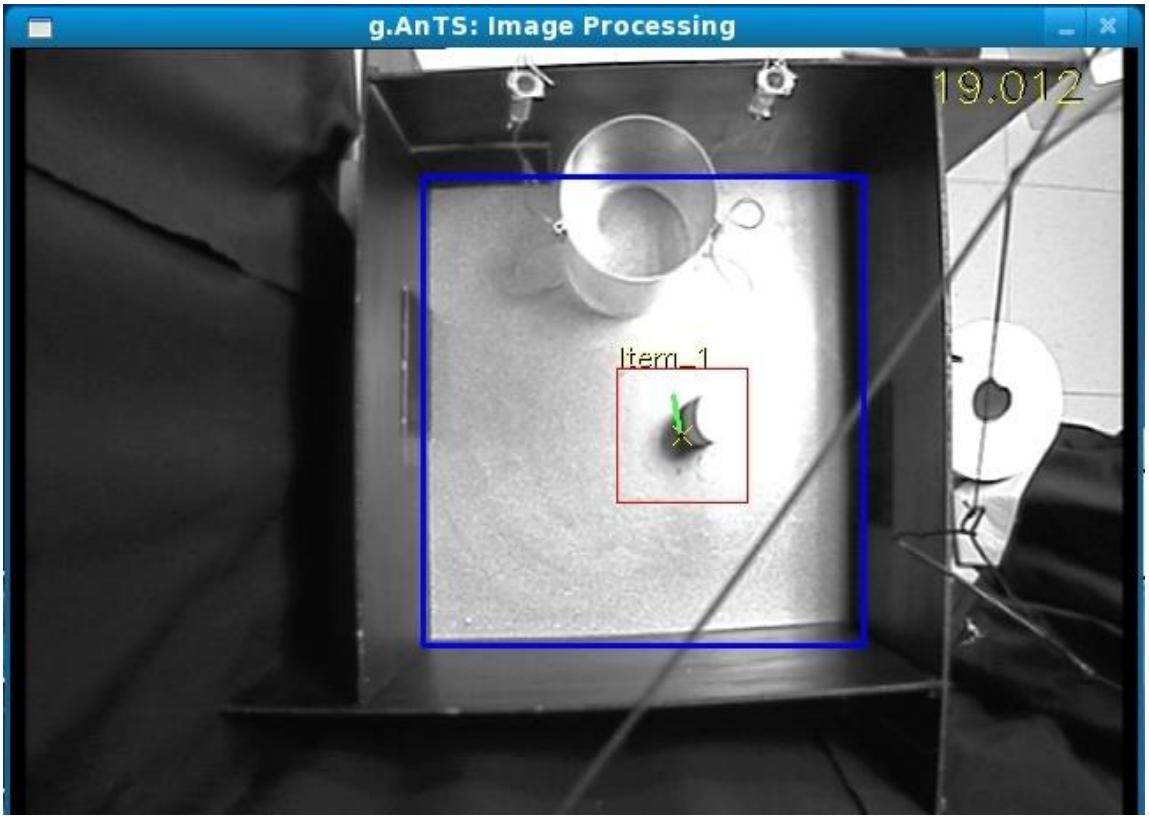

Figure 10: Tracking system.
View of the setup from the top view camera used to track the items. The blue square represents the region considered by the tracking system while the red square is detecting the robot covered with a white cylinder. The subject, not present in this photo, is placed inside the transparent cylinder where two water deliverers control its behaviour.

The tasks cannot be described as a simple visual discrimination paradigm. Even when we have chosen a rat strain with good visual accuracy the subjects should detect the behaviour of the robot also by other cues, such are auditory ones or even the floor's vibrations generated by the robot's movement. Our study is not aimed to focus only on the role of the visual system but on a multi-sensorial task that better resembles the natural conditions. In the tracking of moving objects not only visual information should be involved, especially in rodents, animals that do not have a visual predominance as humans do.

The behavioural setup (zenithal photo in Figure 10) is conformed by the next elements:

- Robot (e-puck® of the EPFL).
- Open field (80x80 cm^2).
- Behavioural scaffold (see-trough cylinder where the subject performs the task).
- Behavioural hardware: 2x infrared sensors, 2x water deliverers and the acquisition card NI® USB-6009.
- Behavioural software: Labview® custom made algorithms.
- Tracking system: g.AnTs® of g.tec® company.
- Robot's control: g.smartcontrol® of g.tec® company and MatLab® scripts.

A more exhaustive description of each of these components separately follows this line.

Robot

The robot used in our experiments is the commercial e-puck® developed by the Ecole Politechnique Federale de Laussane (EPFL, Switzerland, Figure 11). This robot is designed as an educational tool with several open code programs that allows an easy control of it as well the development of new software. The mechanics are very simple and the different parts can be changed easily with standard pieces. The software that we are using in the current experiments to control it is the g.SmartControl® developed by g.tec®, it consists of a logical base for the Bluetooth command constructed in the Simulink® graphical interface of MatLab®. Other software (e.g., ePic freely downloadable from the EPFL webpage) has been used to test the different sensors and devices carried by the robot but not used in the experiments.

Figure 11: The e-puck®, a commercial robot for educational purposes developed by the EPFL. Several sensors, speakers, leds and other hardware are constructed in the robot. The software used in our case was a modified version by g.tec® of the open code ePic. The final approach used in our protocol covered the robot forming a white cylinder in order to increase the size and the contrast of the stimulus.

Open field

The arena consists of an open square field of 80x80 cm surrounded by black walls with a height of 60 cm. In order to control the external references the maze was isolated by a surrounding black curtain and speakers also covered the area with white noise when needed. The room containing the maze is separated and the experimenter should be outside during the protocols in order to prevent possible disturbing noises. A video signal is transferred into a screen where the experimenter can see the subject from the adjacent room in order to control the protocol.

Behavioural scaffold

The space where the subject performs the behavioural protocols is separated from the open field by a plastic transparent cylinder with a diameter of 24 cm and a height of 39 cm. On one hand the cylinder allows the subject to see through the exterior where the robot performs the movements and on the other hand it serves as a scaffold where the nose-pokes and their water deliverers were constructed. A photo of the cylinder and their components can be seen in the Figure 12 during the execution of a protocol.

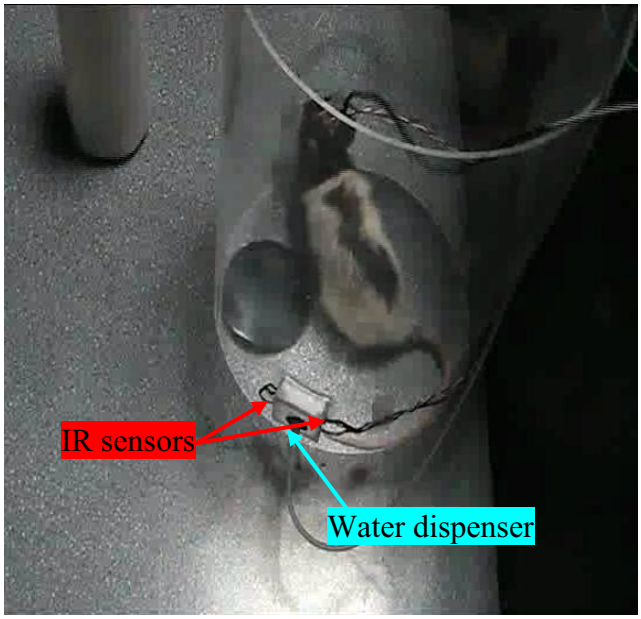

Figure 12: Transparent cylinder where the subject performs the task. In the photo the rat is drinking in one of the nose-pokes according to the robot position. The other nose-poke can be better observed with the water tube attached frontally and the infrared sensors, emitter and receptor, placed laterally. The operant platform which serves to the subject to mark the onset of a trail can be observed as well in black. The subject carries the headstage and the cable need to record the electrophysiological signal.

Behavioural hardware

The hardware used to control the rewards delivery is controlled with a NI® USB-6009 acquisition card. There are two different devices that we are controlling with the card, first the motors that allow the water to be delivered to the reward wells and second the infrared sensors that monitor if the rat tries to drink. The motors are simply switched on or off by a TTL signal sent from the acquisition card (square pulse of 5mV and 100 ms). The infrared sensors were mounted manually into the scaffold and they work with energy supply of 12 V. When the subject crosses the space between the emitter and the receptor change in voltage is detected by the acquisition card and the entrance to the nose-poke recorded. With this information and the triggers coming from MatLab® the control of the protocols is done by a Labview® program described below.

Behavioural software

The behaviour of the animals is monitored with infrared sensors. Two nose-poke receptacles are inside the transparent cylinder where the rat is placed in and the infrared

sensors can detect the rat's approaches to the nose poke sites. These sensors are connected to the acquisition board that also controls the water delivery. With a Labview® program we can design a logical interface that manages these devices (Figure 13).

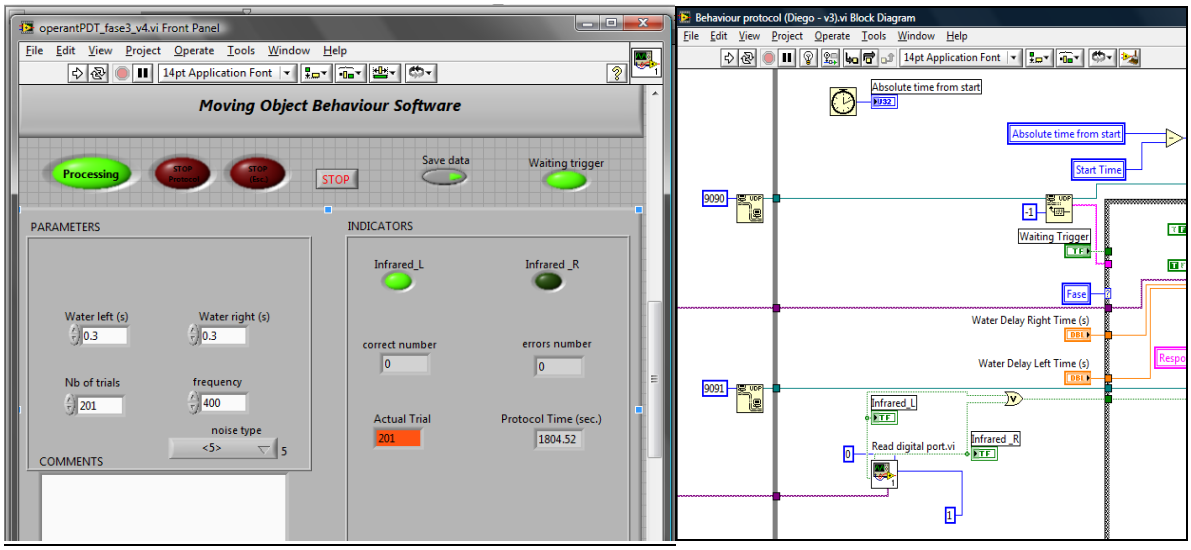

Figure 13: Behavioural interface and algorithm.
Two print screens of the Labview® interface are shown; on the left is the flow diagram where the acquisition and hardware control are developed. On the right is shown the user interface derived from the diagram. The initial variables (water deliverers' time, number of trials, kind of sound used, etc …) can be manipulate by the experimenter before the beginning of the protocol.

The program receives information from MatLab® via the UDP protocol to respond correctly to the movement of the robot with the appropriate response (for example water delivery at the nose pokes). The different time stamps are collected in recording files in order to know the precise time of the behaviourally relevant events. We recorded the start point of the trials, the moment at which the rats enter into the nose poke receptacles and the exit. These events in relation to the robot's behaviour determine the different kinds of response of the animal: correct, incorrect, premature or missing responses. The analysis is done offline and basically consists on the calculation of the performance, response time, etc… (percentages of right and wrong responses, time outs per session, further description in the next section 3.1.4. Behavioural Data Analysis).

Tracking system

The software used to track the animals' behaviour is the g.AnTs® program developed by g.tec® in collaboration with UPF. The time resolution of the system is 30 frames/sec. The system permits the user to follow two or more objects. Filters are applied to the video input recorded from a top-view camera (SONY®) to improve the tracking of the objects; first a background substraction and next a threshold filter adjusted manually to the light and contrast conditions.

Robot's control

The position of the robot is registered via the tracking system g.AnTs® described just above. The movement is tracked with a camera above the maze and the position matrix is sent to another computer with the UDP protocol. We can track more than two objects and then create different interactions between them, following or avoiding behaviours, etc.

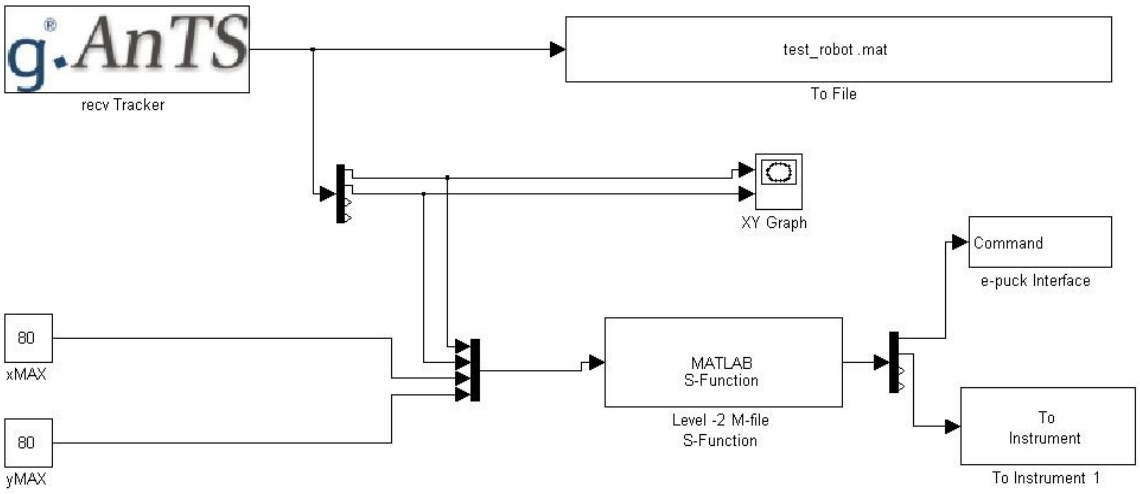

Figure 14: Robot's interface and algorithm.
Simulink® model used to control the robot depending of the items' position. The tracking signal is recorded via one route ("recv Tracker") and then sent to a function that processes it into commands for the e-puck and finally triggers for the hardware control ("To instrument" block).

The MatLab® software receives the data of time and position of the tracked objects. In Figure 14 we can see the Simulink® model that controls this part of the protocol. The input

is the received tracking signal that is recorded in a file with the correct format to be read and processed by Matlab®. Following the arrows the information flow arrives from the g.AnTS® in the upper left corner and is splitted in the file recording the positions and sent to an algorithm that converts the received data into commands for the robot. The communication with the e-puck® is done via Bluetooth, without processing inside the robot. Hence the computer does all the processing of the data in the block represented by the MATLAB S-Function of the diagram; in this case the inputs are only the x/y location of both items, rat and robot. The outputs are, on one side, the instructions of movement sent to the "command block", especially adapted by the g.tec® partners, and, on the other side, the standard block used to sent data to external devices (in this case are the triggers for the Labview® program). An important part of the protocols is the response of the rat to the robot's behaviour. For example when there are incorrect trials the robot is demand to stop for a time-out period until it can start a new trial. For this reason we also send a control signal from the hardware program to the computer carrying MatLab®, which permit the robot to change its behaviour according to the rat one.

3.1.3. Protocols

The design of a behavioural task where the subjects pay attention to the dynamic cue was the first effort to be accomplished in this work. Without a correct experimental design in which the subjects are tracking and localising the movement of the robot it is not possible to examine later the neural correlates that could be related to the movement parameters of the dynamic cue. We have proved different protocols in order to choose one that the animals could learn well enough in a short period. Some of the protocols uses the direction of the robot's movement as the parameter that the animal must to discriminate (direction discrimination task, DDT) and others the position (position discrimination task, PDT). In order to increase the percentage of the correct responses we introduced an operant onset of the trial (operant position discrimination task, OPDT). In this way the animal must place itself into a platform during half second and then the robot moves randomly in one of the two possible target positions. These protocols were called operant discrimination tasks. A description of all the tasks tested is written below.

Position Discrimination Task (PDT)

In this task the animal learns to associate the position of the robot with the reward. The rat is separated from the space in which the robot moves by a transparent cylinder in order to constrain its movements. The robot moves along an imaginary line and there are only two stop positions at the borders of the segment. Each stopping point corresponds to the water dispenser at the same side as illustrated in the Figure 15A. In the task, the subject should attend the movement of the robot and only when it stops the rat can drink water if he chooses the correct side. The time of the stops lasts 4 sec and the interval between stops changes randomly. The sequence of stops is random too and is not an alternation protocol, in this way we can assure that the subject does not learn to drink alternately on each side.

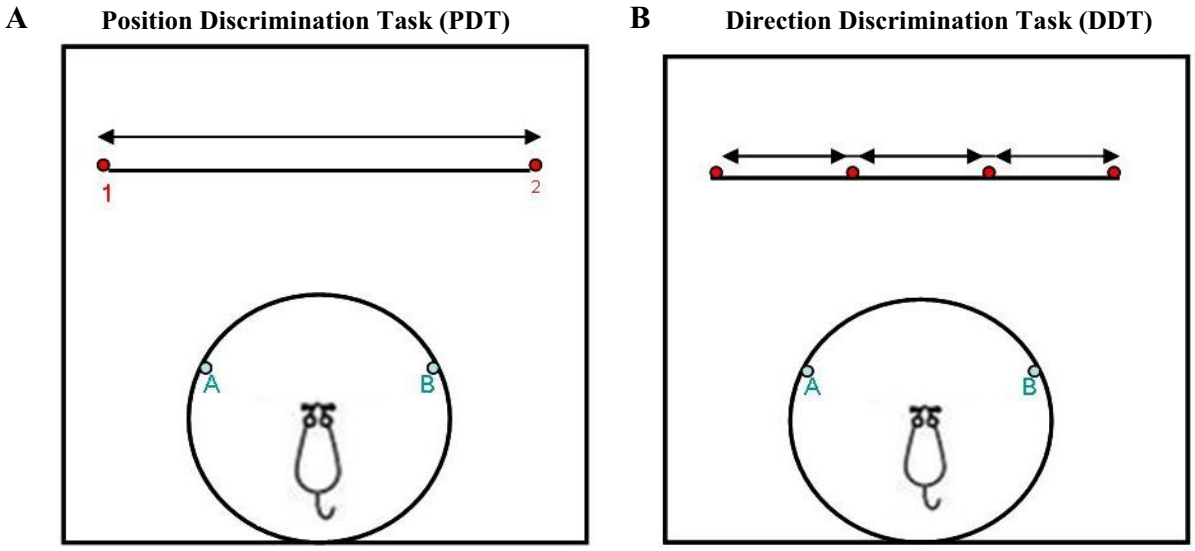

Figure 15: Discrimination tasks.
(A) Position Discrimination Task (PDT), the red dots correspond to the stop positions of the robot. The stops at 1 are associated with water delivery at A and with stops at 2 the rat can receive water at B. (B) Direction Discrimination Task (DDT), the movements from left to right are coupled to delivery of water at point B and the leftwards' movements are coupled to delivery of water at A.

Direction Discrimination Task (DDT)

The subjects trained on this protocol learn to associate reward with the direction of movement. Once again the robot moves along an imaginary line and each water dispenser is activated according to the direction of its motion. In this case we cannot create a random sequence of directions with only two stops because this is spatially impossible. To solve this problem we subdivided the line into different segments with a little stop between them (Figure 15B). Except for the farthest points the robot can go either to left or right randomly. Each trial consists of a movement from one point to another and not necessarily to an adjacent point. The stop lasts 0.5 sec and the trial duration depends on the segment crossed because the speed is constant but never being less than 4 sec.

Operant Position Discrimination Task (OPDT)

In this task the animals associate the obtaining of the reward with the position, but how I will explain other movement parameters could help the animal for the correct choice. A see-through cylinder separates the animal from the space where the robot is placed, in this way the movements of the animal are constrained and the learning of the task is easier.

In the Figure 16 the configuration of the task's phases is represented by a scheme. The movement of the robot is depicted like an imaginary line where the red dots are the stop positions and the black arrows show its possible movements. The robot stays in the central position before the start of each trial. The subject can start the task going to the black platform placed in the front of the cylinder and remaining there for half second, then the tracking system sends the trigger to the integrator software and the robot starts a movement. The objective of this first stage of the trial is to assure the attention of the subject into the robot. The position of the platform inside the cylinder implies the fact that the animal need to be pointing the robot when its head is above it.

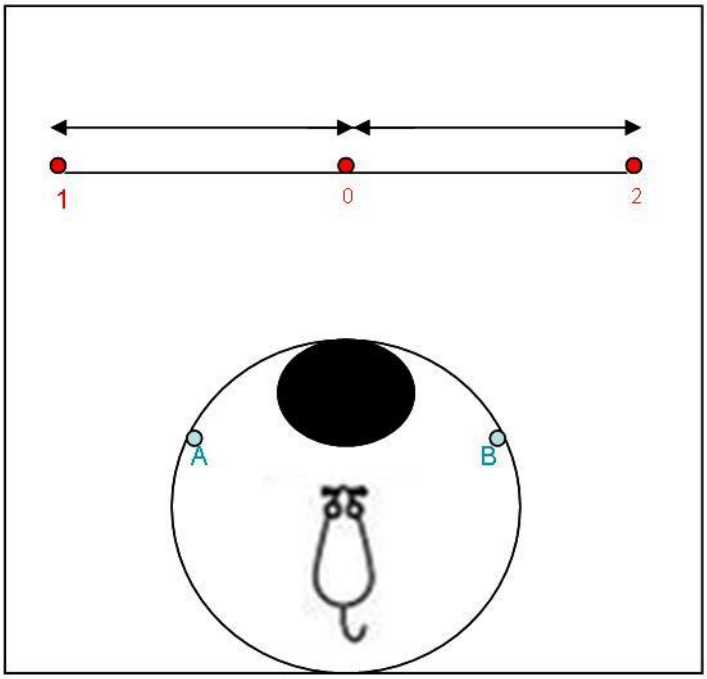

Figure 16: Operant Position Discrimination Task (OPDT). The picture shows the rat inside the cylinder where carries out the protocol. There are two water deliverers, A and B, the black platform for the operant onset and the animal. In the upper area three red dots are numbered, they correspond to the stop positions of the robot. A trial begins with the object in the dot 0 waiting that the animal goes to the platform, then the robot goes to the right or the left and only when arrives to the border dots the water is delivered if the correct one is chosen.

After the half second spent in the platform the robot begins it movement randomly to one of the sides. During the motion the animal can not drink and must wait the arrival of the object into the border dot. At this moment an infrared sensor detects if the rat is in one of the nose-pokes and if the chosen water dispenser corresponds well with the robot's movement. Therefore three different situations could be: correct response, incorrect response or missing response. The consequences of the kind of response are respectively water delivery (0.05 ml), aversive stimulus (white noise at 80 db) or just a longer period of robot's stop.

Previously to the random stage of the protocol the animals were trained in the *shaping* and *conditioning* phases of the protocol. Following there is a more in detail explanation of this *pre-training period* only explained for this protocol because as one can read in the results section (*4.1. Behavioural results*) this protocol was the finally selected one to do the electrophysiological recordings. A scheme of the different phases is shown in Figure 17.

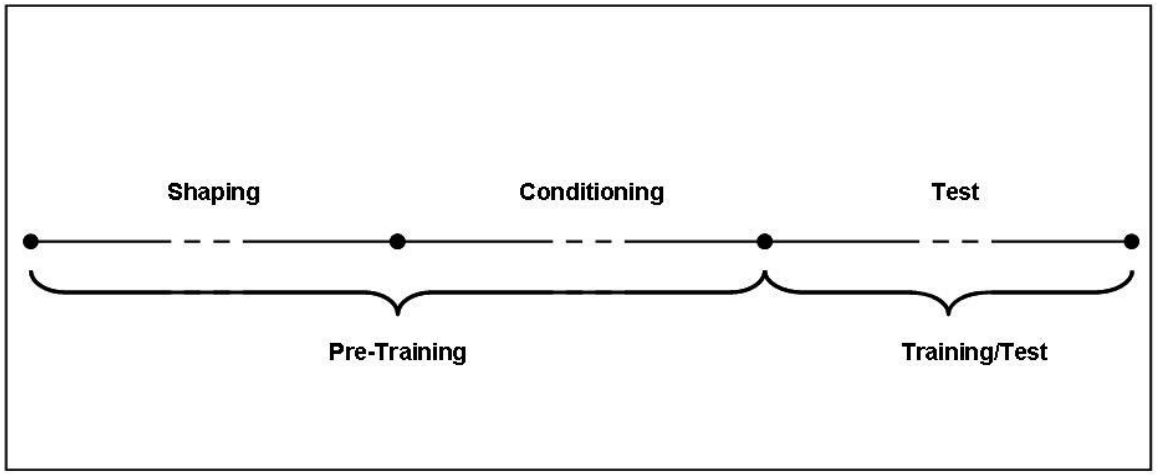

Figure 17: Scheme of the different phases of the training.
During the shaping the stimulus is presented always paired to the reward and therefore independently of the subject's behaviour. The conditioning phase consists in the introduction of the reward/punishment according to the behaviour and finally the training or test phase is composed by the random presentation of both stimuli.

The *shaping phase* comprises a set of sessions where the animal learns to associate its own positioning above the operant platform with the onset of a robot's movement and a water delivery in the correspondent nose poke. After some sessions the subject creates a behavioural pattern. The subject learns to go from the platform to a water deliverer while the robot is performing the stimulus. The sessions at the beginning have long trains of equal stimuli, left toward movement or right toward movement. The opposite dispenser, it means the one that is not used for this session, is blocked by a Plexiglas tap. When the subject easily changes from session to session its behaviour respective to one stimulus or the other the tap is removed and the trains of stimuli are changed during the same session (it varies between animals and it is subjectively decided according to the observed behaviour).

The *conditioning phase* demands to the subjects to well perform the task, it means that the reward is not delivered in case the subject performs wrongly the trial, by selecting the opposite nose poke, and an aversive stimulus is presented (80dB of white noise). During these sessions the animals begin to really associate the reward or the punishment to the robot's movements. Sessions need to be supervised continuously because the subjects can develop patterns of behaviour that prevent a correct learning of the task. An example, the most common observed, is the repetitive behaviour in one of the water deliverers that could be solved by giving trains of the opposite stimulus or just incrementing the water delivered in the opposite deliverer. At this point of the training is very important to well determine

when the trains of equal stimulus should be replaced by a random presentation of them. Some animals failed to reach the random phase because the repetitive behaviour was never abandoned and they performed just a motor task without paying attention to the stimulus.

The last of the entire pre-training period differs between animals because it depends on the performance of each animal. During the shaping the stimulus is always paired with the response independently of the subject's behaviour, trains of stimuli into the same side are alternated and once the rat understands the onset of the trial and goes to the correct nose-poke a series of random stimuli are presented (once again this moment is determined subjectively for each subject). The behavioural recordings of the protocol (time stamps collection, etc…) begin when the rat does random protocols with an average of correct trials over the 60%.

3.1.4. Behavioural Data Analysis.

The format of the files acquired by Labview® is a matrix containing the relevant time stamps of the behaviour. The different vectors acquired in the matrix contain the next behavioural events: the trial number, the onset of the robot's movement, a left trial rewarded, a right trial rewarded and an error response. Therefore there is an on-line processing of the data done by the Labview® algorithm that controls the behaviour set-up and it allows the assignment of the time stamps adequate to the animal's response. Each of the trials of the task is randomly selected in the central algorithm running in the Simulink® model but an output informs the kind of stimulus presented and thus the time stamp collected could be directly recorded as correct left, correct right or error response. If not time stamp is collected the trial is a time out. In a second step this information allows the processing of the data by custom made MatLab scripts. Once the trials are assigned the performance of the subject, as percentage of correct trials, and the response times can be calculated as the difference between the onset of the trial and the delivery of the water or the aversive sound.

Other behavioural parameters can be extracted from the recorded positions by the tracking system during the protocols. In this sense two important parameters were extracted from the behavioural protocols: the velocity and the relative position. Using the position matrix as starting point a derivative of the position in time is done resulting in a first vector containing the speeds of the items during the protocol. A smooth filter is the applied to eliminate artefacts and peaks of velocity due mainly to missed points of the tracking. Respect to the relative position it should be analysed for two main reasons. The first is the fact that the way in which the subject determines the position of the robot could be egocentric, in relation to its own position, instead of allocentric and therefore if just the absolute positions are analysed one of the possible strategies used by the subjects will be not examined. The second refers to the attentive periods where the rat directly pointed its attention to the robot. The only way to determine when these moments happened is to link them to the behaviour. The subject detects the position of the robot or the movements that it performs by directing its head towards the robot. Our tracking system infers the head direction by the movement of the subject's position and this is not an effective measure. A correct determination of the head direction requires the use of at least two different led lights aligned to the head or a tracking system capable to detect the shape of the head. Because of these reasons we will not refer to the head's direction but to relative positions of the items during the protocol whose calculation is explained in the 3.2.5.3. Data processing section.

3.2. Electrophysiological recordings

We consider that the animals performed the protocol in a stable way when they have over a 75% of correct responses in three consecutive sessions and then they were chronically implanted. The aim of the surgery is to record the electrophysiological signal of CA1, the dorsal area of the hippocampus. In this brain area we found the above described PCs, pyramidal neurons that fire when the animal is in a certain location. In order to well isolate the signal of these neurons the implant must be done with a multielectrode technique. We use 16 channels mounted in a microdrive, a scaffold that allow us to move down the electrodes and find the signal coming from specific neurons.

3.2.1. Subjects

Lister-Hooded male rats (N=5, all of them used in the behavioural protocols as well) with a body weight varying from 220 to 360 grams were tested. Rats were housed on a reversed 12/12 hrs light/dark cycle with a stable temperature of 22 +- 1 ºC. Animals were housed in clear Plexiglas cages measuring 60x40x30 cm (Rody Cavia, SAVIC) with plexiglass tubes in order to enrich the environment. All experimental procedures were attempted during the animal's active period. Before the animals carried on trainings they were housed in groups of 2 or 3 subjects. Previously to the experiments they were separated and handled individually for a week in order to prevent stress originated by their manipulation.

During the behavioural protocols animals were water-deprived. Body weight was daily supervised and to prevent an excessive body weight loss, superior to a 15 % of the initial weight, at the end of the journey subjects were allowed to drink ad libitum during 10 min.

All these measures were supervised and approved annually by the University committee (CEEA, Universitat de Barcelona) and were in accordance with the present laws of animal care. These are the EU guidelines on protection of vertebrates used for

experimentation (Strasbourg 3/18/1986) and the local law of animal care established by the Generalitat de Catalunya (Decreto 214/97, 20 de Julio).

3.2.2. Tetrodes and microdrives

The electrodes used in the experiments were HM-L-coated 90% platinum-10% iridium wire of 17 or 25 μm diameters (California Fine Wire, Grover Beach, CA). These wires were twisted in groups of four in order to obtain a tetrode, a strand that preserves their geometric configuration during their movement along the dorso-ventral axis. The microdrive is the scaffold structure that allows the movement of the tetrodes and is very light (1.5 grs) to do not bother the subjects during the recordings. Basically, the microdrive consists in a screw fix part and a moveable one that carries the electrodes, look at the Figure 18 to better understand the configuration. Each full turn of the screw moves the electrodes 200 μm and therefore allowing precise steps of 50 μm when only a quarter of a turn is done. Once the microdrive is ready to be implant, actually just before the surgery, it is immersed in a gold plating solution. The aim of this procedure is to adjust the impedance of the electrodes by passing an electric current varying from 1-100μA at 1KHz. Depending on the impedance the recorded signal comprises wider or thinner areas of neural activity. The initial impedance of the electrodes used can vary between 1-3 MΩ and they are adjusted to an impedance of 200-300 KΩ. These values are chosen because previous studies have shown that this range is the optimal to obtain a good signal to noise ratio and simultaneously having a good area of record comprising many neurons (McNaughton *et al.*, 1983).

During the task the subject will carry the microdrive attached to the recording cable. Even if the microdrive per se is light the weight of the recording cable and the possible tension produced by their turns could bother the subject during the stay in the arena. Several strategies were used to balance this effect, in our case the tension of the recording cable can be adjusted by the experimenter. At the beginning of the protocol, before the subject is connected, the cable is measured in relation to the floor. An optimal distance let the microdrive at the height of the animal's head while the cable is free. In order to adjust it a second elastic cable fits this distance attaching the recording cable to a fixed point in the middle of the arena. Then the tension of the cable at the

beginning of the session is almost zero and the movements of the animal produce a minimal change in it.

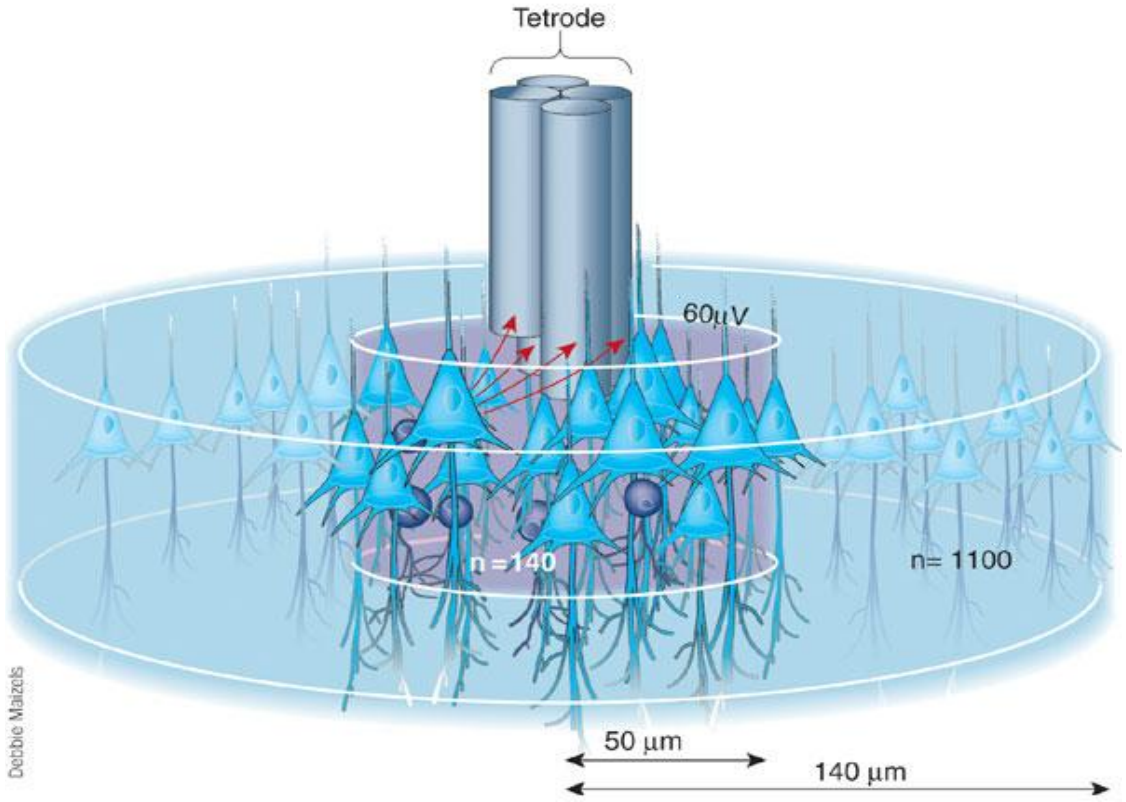

Figure 18: Tetrode configuration.
A schematic illustration shows the geometry of a tetrode with its four electrodes recording signals from the adjacent volume. There are two highlighted areas, one with a radius of 50 µm where the signal of one cell can be isolated and containing approximately 140 neurons in total and a second region enlarging to a radius of 140 µm with an estimation of 1100 neurons with their spikes affecting to the LFP recording on the electrodes. Adapted from McNaughton 1983.

3.2.3. Surgery

Animals were food deprived just the day before the surgery to better control their metabolic state prior to the anaesthesia induction. The drugs used to induce the anaesthesia were ketamine (60 mg/kg; Imalgene 1000, Merial Laboratorios SL) and medetomidine (0.5 mg/kg, Domtor, Pfizer SA) by an intraperitoneal injection. After 5 to 10 minutes the subject was placed in the stereotaxic and the surgery began exposing the skull and injecting a subcutaneous dose of atropine (0.05mg/kg; Atropina Braun 1mg, B. Braun Medical SA) to prevent excessive secretions during the surgery. The body temperature of the subject was continuously monitored by a rectal thermometer and

maintained constant by an electric blanket. The heart rate and the oxygenation level of the blood were also monitored with a capnograph (SurgiVet, Wisconsin, USA) giving an idea of the anaesthetic and healthy states.

To support the microdrive, six steel screws (Precision Technology Supplies LTD) were anchored to the skull and an additional screw was placed in the parietal bone as the ground for the electrophysiological recordings. The tetrodes enter by a hole done with a trephine of 3 mm diameter; this craniotomy was done in the stereotaxic coordinates that correspond to the parietal association cortex (PtA): at -4 mm anterior-posterior and 2.8 mm medium-lateral from bregma (Paxinos and Watson, 2005). The dura was then carefully removed, with special caution for the blood capillaries taken to prevent blooding during the manipulation. The electrodes were inserted at an intermediate depth of 1.5 or 1.8 mm and therefore not reaching yet the pyramidal layer of CA1. The whole configuration, it means the screw and the microdrive, was then cemented with dental cement (Simplex Rapid, kemdent®) to well stabilise it. The ground screw was connected to the 17^{th} channel of the microdrive and the subject was removed from the stereotaxic. After several hours of recovery, determined by the behaviour of the animal (normal locomotion, drinking, licking, etc…), subjects received some drugs in order to prevent infection, inflammation and pain: antibiotics (enrofloxacin; 10mg/kg; s.c.), topical application of neomycin and bacitracin in powder (Cicatrin®), analgesic (buprenorphine; 0.05mg/kg; s.c.) and finally an anti-inflammatory (methylprednisolone; 10mg/kg; i.p.). It is important to wait for a good recovery of the subjects before the administration of all these drugs because to avoid the interaction with the anaesthetics. During the next 5 days a special careful of the subjects was need to prevent infections or other complications derived from the surgery. The administration of antibiotics and anti-inflammatories continued during the recovery period while the electrodes were slowly advanced ventrally in order to avoid excessive cicatrisation in the tips which could affect the quality of the recordings.

3.2.4. Data acquisition

After the recovery from the surgery the recordings can start with animals well trained and correctly implanted. To start a recording the microdrive with the 16 channels relatives to the tetrodes and the ground channel is connected to a headstage. In order to stabilise the connection a metal peg is attached to the microdrive and an infrared led overhangs from it to allow a better detection of the animal's position by the tracking system. Light and flexible wires transmit the signal from the headstage to the preamplifier (Axona, St. Albans, UK) that increases the signal with a gain of 1000x. From the preamplifier to the recording system (also form Axona, St. Albans, UK) a tape containing the wires sends trough it the electrical signal. The recording system first further amplifies the signal (10.000-40.000x), then a high-pass filter is applied (above 360Hz frequencies) and finally the signal is acquired at a sample frequency (Fs) of 48 KHz. The recording method could be continuous but a big demand of memory disk is required if the Fs is 48 KHz, therefore the recording system gives the possibility of a single unit recording method. This technique stores the data only when the signal overpasses a threshold determined manually in each channel, the aim is to avoid the noise and store only neural activity reducing the amount of data acquired. Then, a window of 1millisecond is recorded for all the electrodes of the tetrode where neural activity was detected by the threshold (0.2ms before the threshold and 0.8ms after). In order to further diminish the noise to signal ratio, the signal of each electrode is subtracted to the signal of another one, this method is known as the recording differential mode. Even if there is a ground channel attached to the skull and touching the brain surface with the purpose of noise elimination the differential mode abolishes most of the local/intrinsic noise often due to the LFP activity which is not important for the single unit recording. By all these procedures what we obtain finally is the waveforms of the signals for each tetrode during a millisecond, a time window enough to see the whole action potential dynamics, and as it will be described in the next section (3.2.5.2. Cluster isolation) that allows the isolation of single neurons activity. One of the 16 channels is selected to store EEG signal, this channel has not filtering and is stored by default twice, one with a sample frequency of 250 Hz and other at 4.8 KHz. This is important to correlate the spikes of the neurons isolated to the local field potential (LFP).

3.2.5. Data Analysis

As described before we obtained different kind of data that must be prepared and integrated to analyse. To resume: first, the *behavioural data* consisting in the classification of the rat´s behaviour with the relevant time stamps collected; second, the *positional data* as a matrix containing the path of the items (rat and robot) during the whole recording and finally, third, the *electrophysiological data* just described above (15 channels acquired in single unit method plus the EEG signal). To do that we used some commercial software and other hand-made software designed in MatLab®. We can separate the whole analysis as follows:

- **Behavioural analysis** explained in previous section 3.1.4 Behavioural Data Analysis (simple MatLab® scripts calculate the percentage of success during the task, the response time, number of timeouts, etc…).

- **Cluster isolation** (the electrophysiological data obtained need to be clustered in the activity of single neurons with the commercial software TINT® (Axona, St. Albans, UK)).

- **Data processing** comprises the biggest analytical effort because all the collected data need to be well synchronised and integrated in order to elucidate if there are neural correlates of the tracking or localisation of dynamic cues (this part was done with custom-made MatLab® scripts).

3.2.5.1. Behavioural analysis

During the protocols the relevant behavioural time stamps were collected and, as explained in section 3.1.4 Behavioural Data Analysis, there were analysed with homemade MatLab® scripts. All this data need to be well synchronised with the electrophysiological recordings and the position data derived from the tracking system. In order to do not repeat the analysis description it is integrated in a following section (3.2.5.3. Data Processing) where a detailed explanation of the analysis steps required synchronising the different recordings is exposed.

3.2.5.2. Cluster isolation

The recorded data is analysed by the commercial software TINT® (Axona, St. Albans, UK), specifically designed for multi-unit recordings. A capture of the program running is shown in Figure 19. The interface allows the user to plot different parameters of the signal against others. It works tetrode by tetrode, therefore the user can choose between the following measures for the electrodes comprising this tetrode: amplitude, voltage at a selected time, value of the peak, value of the trough, time to peak and time to trough. As illustrated in the Figure 19 the resulting plots are clouds of spikes depicted according to the variables selected. The cluster is done manually selecting spikes with almost identical waveforms and adding them to it.

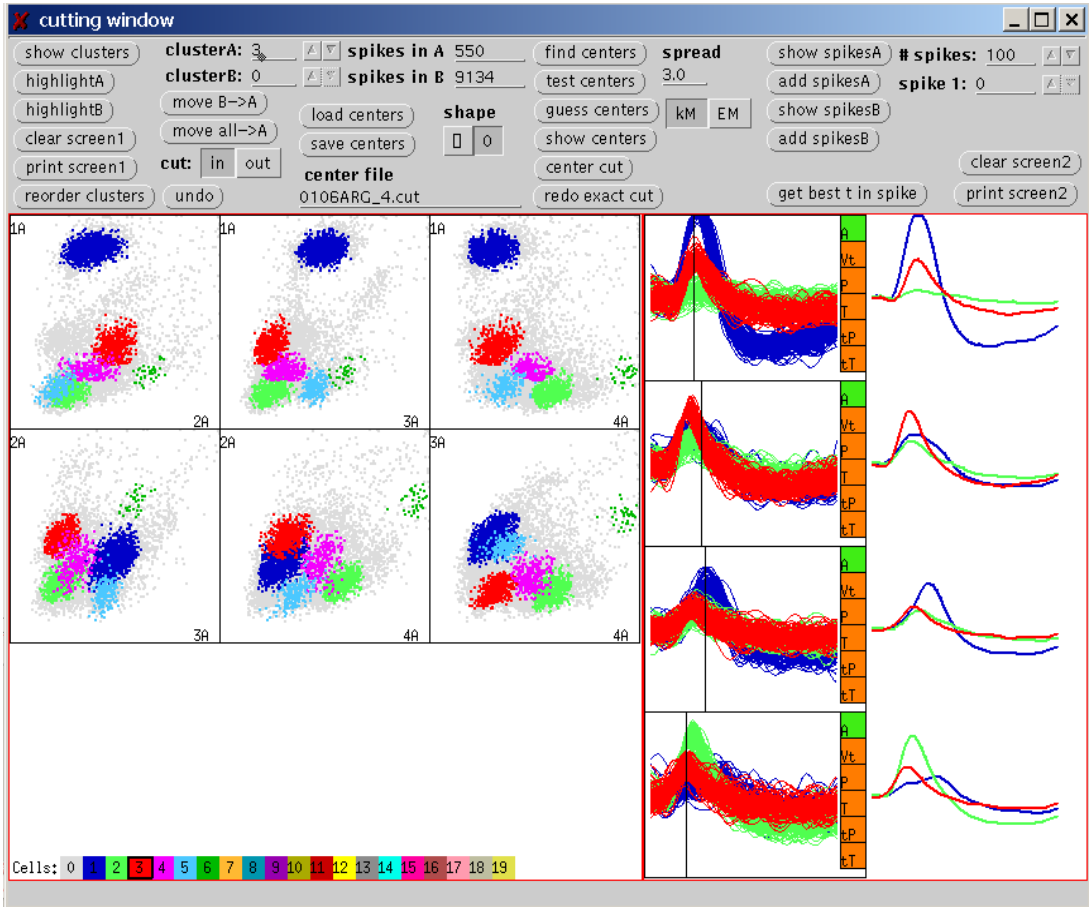

Figure 19: Cluster isolation.
Cutting window of the TINT® software (Axona, St. Albans, UK). The interface has two main graphics, on the left the spikes parameters allow the user to isolate the units by them (peak and through values or times, voltage at time t and finally amplitude) while on the right the selected spikes are first plotted and then averaged.

The software also has a time window (Figure 20) where the autocorrelation histogram can be observed in order to determine if the cell is bursty (a characteristic of pyramidal cells), if the refractory period is respected (minimal interspike interval assuring that the activity is just for one neuron) and if there is a phase-lock with a hippocampal oscillation (theta and gamma are the predominant ones). Finally there is the fields' window, a practical tool to see the spatial dispersion of the spikes in the maze. This allows a fast identification of the neurons with a firing restricted to a specific location of the environment (Figure 22, next section).

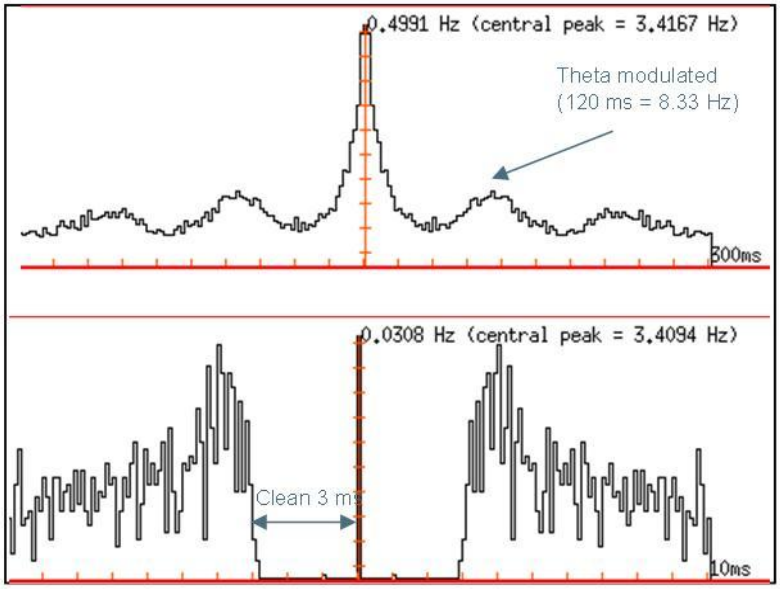

Figure 20: Time properties of an isolated cell. Above the autocorrelation histogram showing in this case a modulation in the theta range (4-12 Hz). Below the same autocorrelation plot is calculated but the temporal window comprises 10 ms; this way one can see if the neuron spiking respects the minimum refractory period (that is an indicator of the quality of the cluster because if other spikes are encountered durin the refractory period thay should be noise or the activity of a second cell).

3.2.5.3. Data processing

Once the units are well isolated by the identification of the time stamps for each of the spikes occurred during the recordings, the data needs to be integrated with the positional and behavioural data. With this purpose the different formats of the acquired and pre-processed files were transformed to data's structures in MatLab®. Resuming the files acquired contain the following information:

- *Behaviour*: the time stamps of the relevant events during the execution of the protocol (onset of the trigger, entrances to the different nose-pokes, phases of the task) and the result of the previous analysis which contains the behavioural output of each trial (details in section 3.1.4. Behavioural Data Analysis).

- *Position*: the coordinates occupied by the rat and the robot are continuously recorded at a sample frequency of 30 Hz. An on-line processing of this data is done during the protocol to adjust the behaviour of the robot to the rat's one.
- *Electrophysiology*: the results of the cluster isolation of single units using the TINT® software (Axona, St. Albans, UK) plus the recorded EEG signal during the recording are ready at this point. The activity of each of isolated neurons and the LFP fluctuations are then prepared to export them to MatLab®.

All this data is loaded by a handmade script in a new MatLab® structure. Then the data is available to access it from the same analytical programme. The assignment of the behavioural output is yet ready to analyse differentially the correct trials (left or right), the errors (once again they can be separated in left or right ones) and time outs. Once all the starting variables are assigned the analysis performed was separated in the spatial and temporal domains. In order to illustrate the analytical challenge of this separation the Figure 21 shows the spikes of a neuron in relation to one dimension of the space and time (respectively Figure 21A and Figure 21B illustrate it for the rat and the robot). The space coordinate, x, in the case of the rat represents its position across the axis separating the two water deliverers within the operant platform in the center, or, for the robot case (Figure 21B), the position of the robot across the axis connecting the two target positions. The time coordinate, y, is centred in the onset of the trials with time 0 equal to the onset of the robot's movement. The spikes are depicted in violet for correct trials and red for error ones while the trajectory of the items is shown in shaded grey behind the spikes. Histograms surrounding the central plot collect the percentage of spikes occurred in each spatial and temporal coordinate to see if there are significant accumulations of them. This plot allows a fast glance of the different variables that could affect the spiking of the neurons: time/phase related, spatial location specificity or behaviourally modulated.

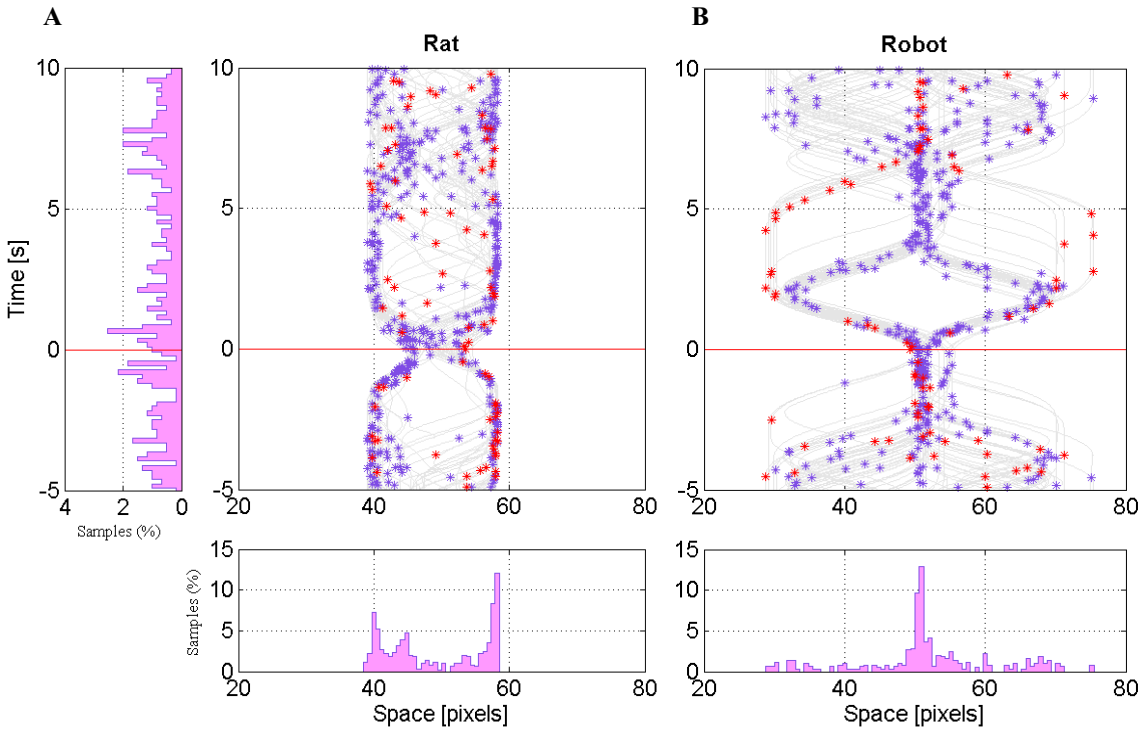

Figure 21: Space and time relations of the spikes.
The activity of a neuron is shown in relation to the spikes fired within a certain location (x axis, projection of the positions across the line between the water deliverers) and to the time of the task when they happen (y axis, trials centred in the onset of the robot's movement). The histograms bordering the plots represent all the spikes occurred for each sample while the plot itself contain the spikes separated for correct and incorrect trials (violet and red respectively).

Analysis in the Spatial Domain

In order to see how the activity of the isolated neurons is distributed in the space the first step was the generation of the classical firing fields taking into account where the spikes occur in relation to the rat and robot positions. According to the time stamps obtained by the cluster isolation each spike is assigned to a certain location of the items during the recording. The region of interest manually selected for the tracking system, with 80 cm each side of the square selected and coinciding with the open field configuration, is subdivided in 50 equal bins (bin area results in a square of 1.66x1.66 cm^2). Then, each of the spikes is assigned to the spatial bin occupied by the item at the moment in which the spike occurred and the obtained matrix is normalised by the total time spent in each bin. This way one can draw a map where is easy to judge the preference of a neuron to fire for a certain location. The Figure 22A represents in pink the spikes of an isolated neuron superposed to the path travelled by the rat in black on

the open field (80x80 cm^2). This information is combined as aforementioned; it means correcting by the time spent (dwell time map, Figure 22B) the number of spikes obtained in each bin (raw activity map, Figure 22C) and finally applying a Gaussian smooth filter of 5 bins to create the firing map of the cell (Figure 22D). The firing rate is colour coded with 0 in deep blue and red as the maximum frequency (Fmax), in this case the maximal activity found for one spatial bin was of 8.4 Hz (red central area of the firing field).

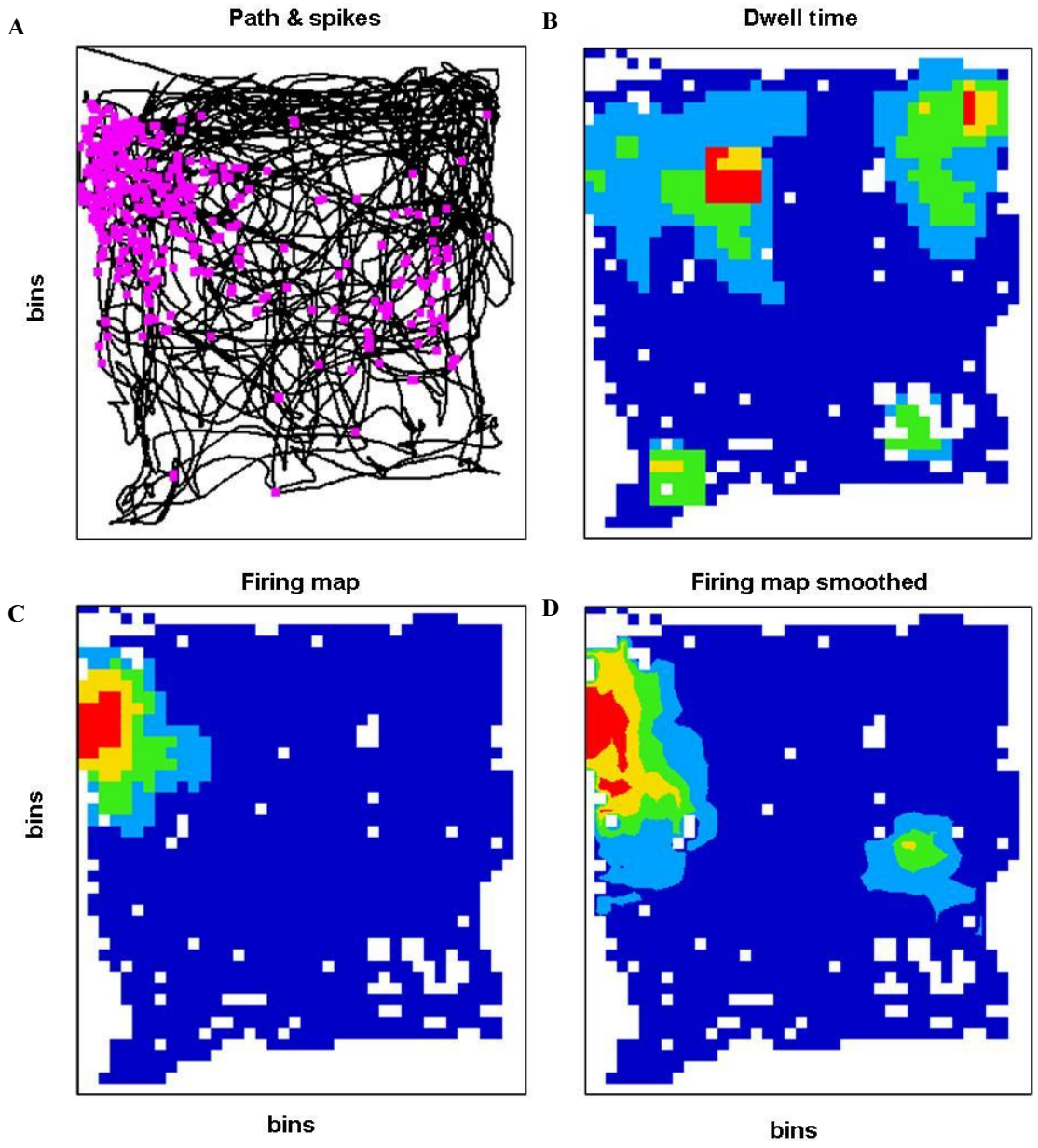

Figure 22: Assessment of the spatial activity.
A single neuron activity recorded in an open field of 80x80 cm². On the left the path of the subject during the recording is shown in black while the isolated spikes of a neuron are blue dots (A). On the right the derived firing field of the neuron after the use of Gaussian filters to smooth it (B). The colour code varies from 0 in dark blue to the Fmax in red, 8.4 Hz

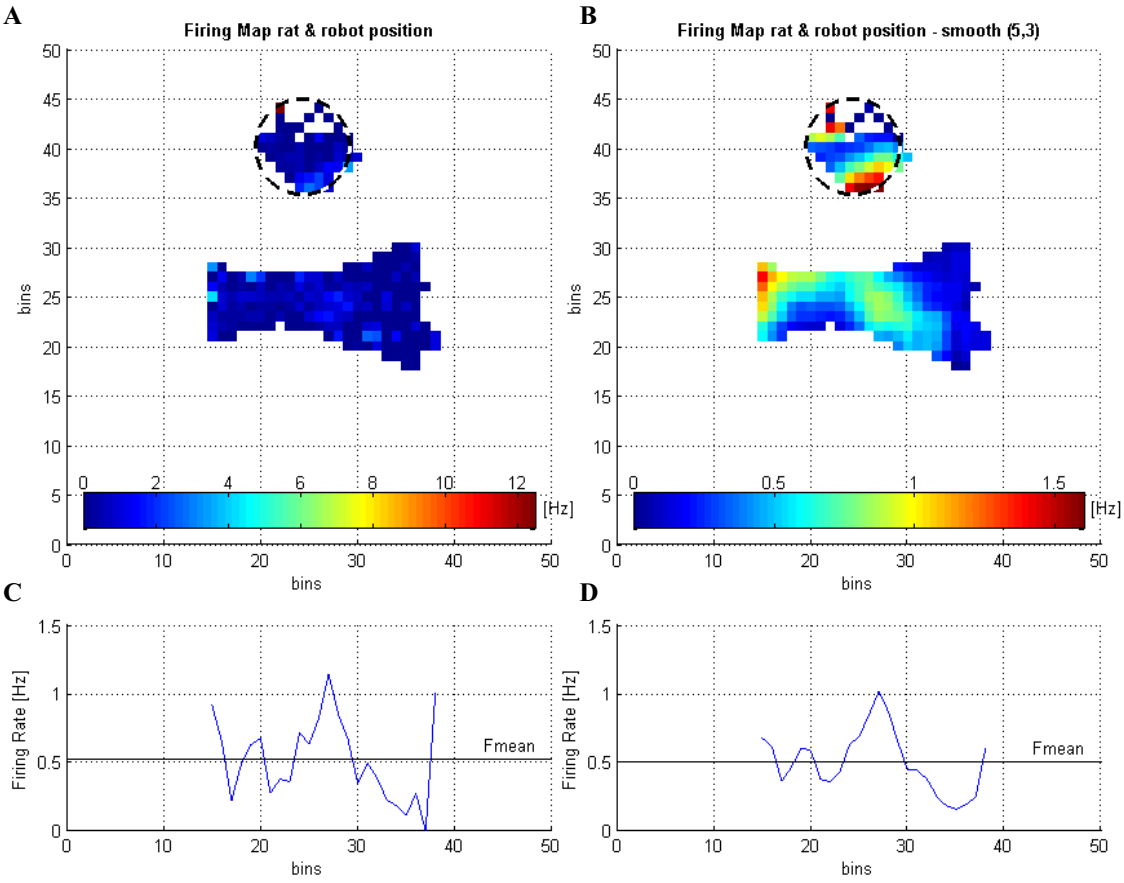

Figure 23: Graphs for absolute positions and projections in one dimension.
The activity of a cell is shown for different spatial frameworks. First is represented in relation to the positions of the items (A-B, 2 dimensions wit raw and smoothed maps respectively). Then the obtained map for the robot is projected obtaining a firing rate, in blue, for each point travelled in one dimension (C-D) and compared against the mean firing rate of the cell during the recording.

In our case the same procedure is applied to the robot path giving a plot where the spikes of a neuron can be observed not only in relation to the rat's position but also to the robot's one. In the Figure 23A is shown on the left the firing field without smooth filter applied and on the right yet smoothed (Figure 23B, the cylinder where the rat performs the behaviour is superposed to the firing map in dashed lines to better understand the protocol configuration). This way a first look to the spatial properties of the cell activity is appreciated for both items. Here we can find several cases varying from neurons spiking specifically for a rat location to other neurons with a first glance show a sharper response to the robot.

A simplification of the robot firing map was done because its track is almost linear during the recording and it can be projected in one dimension. Due to the fact that for the subject the position of the robot physically, and even more important behaviourally,

is the same in each of the coordinates for this dimension the bins occupied by the robot were projected into one line. Examples of the projected representations are depicted in Figure 23C and D. Once again showing respectively the unsmoothed result on the left and smoothed results on the right, the firing rate is the blue line with the mean frequency of the recording marked in black.

Finally the relative position of the subject in relation to the robot was analysed to see if there is a correlate between the spiking and the angle formed by the items instead of a correlate between their absolute positions and the activity. In Figure 24 a polar plot represents in blue the firing rate at each of the angles formed between the items and the black line marks the mean frequency over time for the analysed cell. The angles formed change between each of the sessions but because of the restriction imposed to the items during the protocol they vary approximately between 30º and 150º. The restrictions are due to the fact that, on one hand the subject is enclosed in the behavioural apparatus and, on the other hand, the robot is performing linear movements between to target positions.

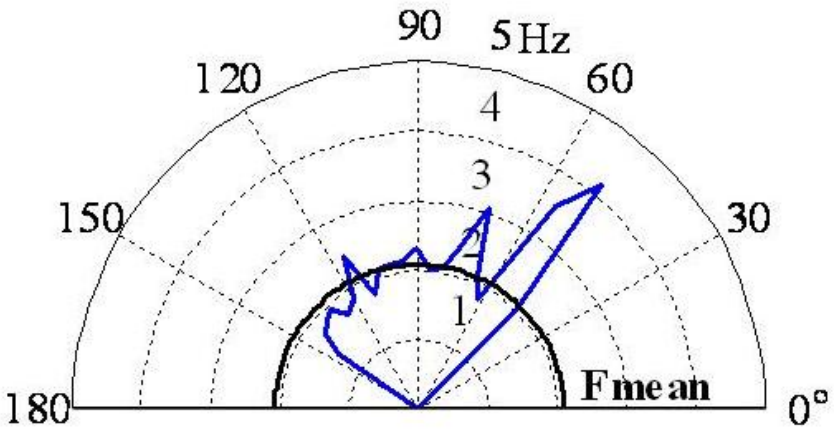

Figure 24: Firing rate vs. relative positions.
A polar plot shows the activity of a single cell in relation to the angles formed by the rat and the robot during the protocol. In blue the firing rate for each of the angles (scale indicated by the dashed circles; 1, 2, 3, 4 and 5 Hz respectively) and in black the inner solid circle marks the Fmean of the neuron.

The analytical variables examined in the spatial domain were with the aforementioned methods covered. Resuming, the firing patterns of the neurons were analysed in function of the position with three different methods: absolute positions of the items

during the recording (as in the classical PC's analysis), projections on linear tracks merged in one dimension (only for the dynamic cue's displacements) and finally the relative positions. With these techniques the spatial analysis was considered completed and gives way to the temporal examination of the firing patterns explained in the following section.

Analysis in the Temporal Domain

The data processing presented since this point worked mostly with the positional data obtained by the tracking system. The next steps here described focus on the temporal and behavioural features of the isolated cells. In order to do that the onset of the stimulus served as the reference temporal point in which align the trials to compare them. The onset of the stimulus corresponds to the beginning of the dynamic cue movement, detected by the trigger which was stored during the protocol and reviewed by the changes in velocity of the robot.

The Figure 25 is an example of the temporal analysis, several plots analysing the speed, the cell's activity and the LFP are shown. The 0 time of all the representations coincides with the onset of the trials and is marked with a red vertical line. The plot includes only correct trials performed to one side and therefore the behavioural information previously recorded and the assignment of response's type is needed to perform the analysis. The first plot (Figure 25A) shows the average velocity of the rat and the robot across the trial with their SEM errors shadowed in the same colour. As first appreciation one can notice that the SEM error is higher before the onset because the alignment of the locomotor activities of the rat and the robot are not as homogeneous as after the onset. The activity of a single cell is presented with a raster plot (Figure 25B), each dot represents the identification of a spike at this moment. In Figure 25C the percentage of spikes detected in each temporal bin (100 ms windows) for all the included trials is collected within a histogram. This way the possible accumulations of spikes in certain phases of the trial can be easily found. Not only the activity of the neurons was analysed in the temporal domain but also the theta oscillation was analysed in relation to the temporal phases of the protocol. A perispectrogram (Figure25C), a spectrogram centred

on the trial's onset) of the theta frequency shows the mean power of the recorded EEG signal in the selected trials (once again just the correct ones for one of the sides).

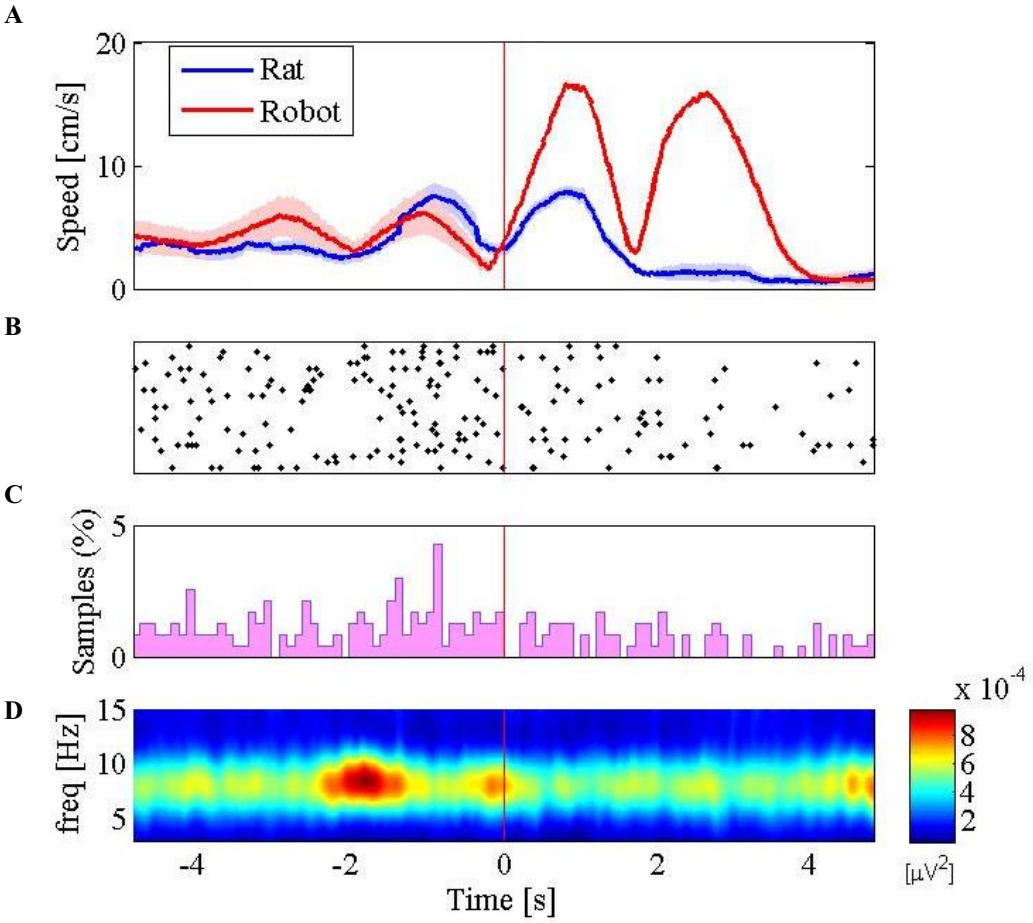

Figure 25: Spikes, theta oscillation and speed correlations in relation to behavioural phases. (A) The average velocity of the rat and the robot during the protocol with its SEM error were calculated around the onset of the trials. For this case the 0 time of the plots, marked with a red vertical line, refers to the onset of the stimuli and only left correct trials were included. (B) Raster plots of a single cell are shown during the left correct trials of one recording session. (C) Then are presented the histograms collecting the percentage of spikes per temporal bin. (D) A perispectogram also centred in the onset shows the averaged power of the theta oscillation across the selected trials.

Parameters

The analysis presented until now in the spatial and temporal domains can reveal the tendencies of the neurons, or of the brain's oscillation, to become more or less active in certain item's locations or task's periods during. Then, this analysis and the figures presented served us to better point our efforts after a qualitative inspection of the data

by the firing maps and the raster plots, however to conclude if the tendencies and the effects observed are significant several parameters of quantification have been used.

Respect to the spatial distribution of the spikes of a neuron in relation to the position of an object, this is a matter of study well known thanks to the extensive research and analysis developed since the discovery of PCs more than 40 years ago. Thus the parameters used are the ones more accepted in the previous literature that contemporary were useful for our work. One important consideration could be to remember the reader that all these parameters were usually applied just to the rat's position while in this study there were also taking into account for the robot's positions. A list with the different parameters and a brief description of their calculations is here presented:

- **Fmax:** Maximum frequency corresponds to the firing rate in Hz for the bin with the biggest firing rate without smooth applied.
- **Fmean:** Mean frequency is the average of the firing rate found in each bin and therefore it gives an idea of the basal level of neuron's activity.
- **Fratio:** Is the ratio between Fmean and Fmax, this parameter gives an idea of the sharpness of the putative firing field.
- **A50:** Area 50 is the percentage of the covered bins with a frequency superior to 50% of Fmax. While Fratio quantifies the sharpness of the firing field by the relation of its peak to the basal activity A50 quantifies the sharpness more in relation to the covered space because the value is more affected by the decay of the firing rate.
- **Skaggs Index (SI):** This is an index widely used in the literature because its capacity to reflect the amount of spatial information derived from the spiking of a neuron (Markus *et al.*, 1994). The equation presented below is a summation of the probabilities to find spikes in each bin of the maze. In a totally uniform matrix the Skaggs Index is equal to 0. For the opposite case, it means when only one bin contains spikes the Skaggs Index is the maximum one for this condition.

$$Skaggs = \sum_i P_i \frac{R_i}{R} log_2 \frac{R_i}{R}$$

- **Positional Information (PI):** While Skaggs Index calculates the probability to find in a certain bin spikes the Positional Information index calculates the probability of a spike to be in a certain bin. At the first glance Skaggs Index and Positional information are very similar but the second reflects the amount of spatial information in a certain bin and therefore "the extent by which uncertainty about the spike count is reduced if the rat is in a known (and interesting) position" (Olypher *et al.*, 2003).

$$I_{\text{pos}}(x_i) \equiv \sum_{K \geq 0} P_{k|x_i} \log\left(\frac{P_{k|x_i}}{P_k}\right)$$

Those were the parameters used to quantify the spatial specificity of the neurons activity. However, in the temporal domain we needed to quantify the modulation of the firing rates of the neurons during the trials. With this purpose different strategies were used. From the parameters calculated to compare the firing of the neurons in specific moments of the task one close to Skaggs Index and Positional information was used. This parameter is the **Mutual Information (MI)**, which reflects the variability of the neural response in the selected time window by its entropic component and not as a simple variance (Nelken and Chechik, 2007). In such way MI reflects the consistence of a signal in relation to different stimuli or conditions. Another way to interpret it is as a parameter measuring the strength of association between two random variables. The formula used to calculate is:

$$I(S;R) = \sum_{s \in S, r \in R} p(s,r) \log_2\left(\frac{p(s,r)}{p(s)p(r)}\right)$$

In the formula *S* are the stimuli and *R* the analysed response, which could be firing rate, latency, band power, etc… Only if the joint distribution of *P(S;R)* is known the exact value of MI can be calculated. If not the measure could compare the results derived by the calculation of the marginal distributions of *S* and *R*, p(s) and p(r) respectively. In our case the MI was used to compare the firing rates of the neurons during three different conditions relatives to the robot's dynamics: leftwards (L) or rightwards (R) displacements of the cue and static periods (S). This way the differential patterns of

activity during the movement of the robot can be assessed by the comparison of (L)/(R) periods. The merging of these last two conditions gives the dynamic state (D) and facing it vs. the (S) period the firing rates of the neuron during moving and resting states of the robot can be compared as well, (S)/(D) comparisons. In this case free software was used in order to the calculations of the MI in MatLab® instead of handmade scripts (Magri *et al.*, 2009).

Statistics

All the parameters presented until now try to quantify the spatial or temporal properties of the neuron's activity but the analysis needs to go one step beyond to conclude if their values are significant respect to a random spiking distribution in time or space. For the determination of the significance we have used in this work two main different approaches: the first method is a shuffling procedure and the second one is composed by classical statistical tests. These two approaches complement each other, because the statistical methods used do not imply the same quantifications performed by the shuffles, and therefore giving a framework from which have good controls of the statistic performed.

The idea to use shuffled distributions was motivated, first, by the complexity of the data itself and, second, by the even higher issue of the crossed analysis between the subject's and dynamic cue's positions in relation to the neural activity. A shuffled distribution consists in a hypothetical case where the obtained data is randomly shifted in time, that's why it is called shuffled, and that means in our case to desynchronise the positional and the electrophysiological data resulting in fake spatial distributions of the spikes. The process is repeated 1000 times and the shift is cyclic, meaning that the spikes move from the end of the recording to the beginning as if there is a continuous in time. This way the possible artefact effects due to the spikes' distributions in time are avoided because the obtained shuffles serve as control of the contributions due to particular spikes distributions or to the positions distributions obtained in each recording. The value of a parameter in the real case should be in the distribution over the 95 % of the values obtained by the shuffles and, thus, it can be considered significant ($p<0.05$). Some examples of results obtained by this method are shown in

Figure 26. The parameters calculated in the example refer to spatial properties of two neurons, one in the upper row and the second in the lower row. Each graph shows the real value of Fmax, Fmean, Fratio, Area50, Skaggs or PosInf with a vertical black line and all the values of the shuffles in a coloured distribution. Below the graphs the significancy level is indicated and highlighted in red for those that were over the 95% of the shuffled distribution.

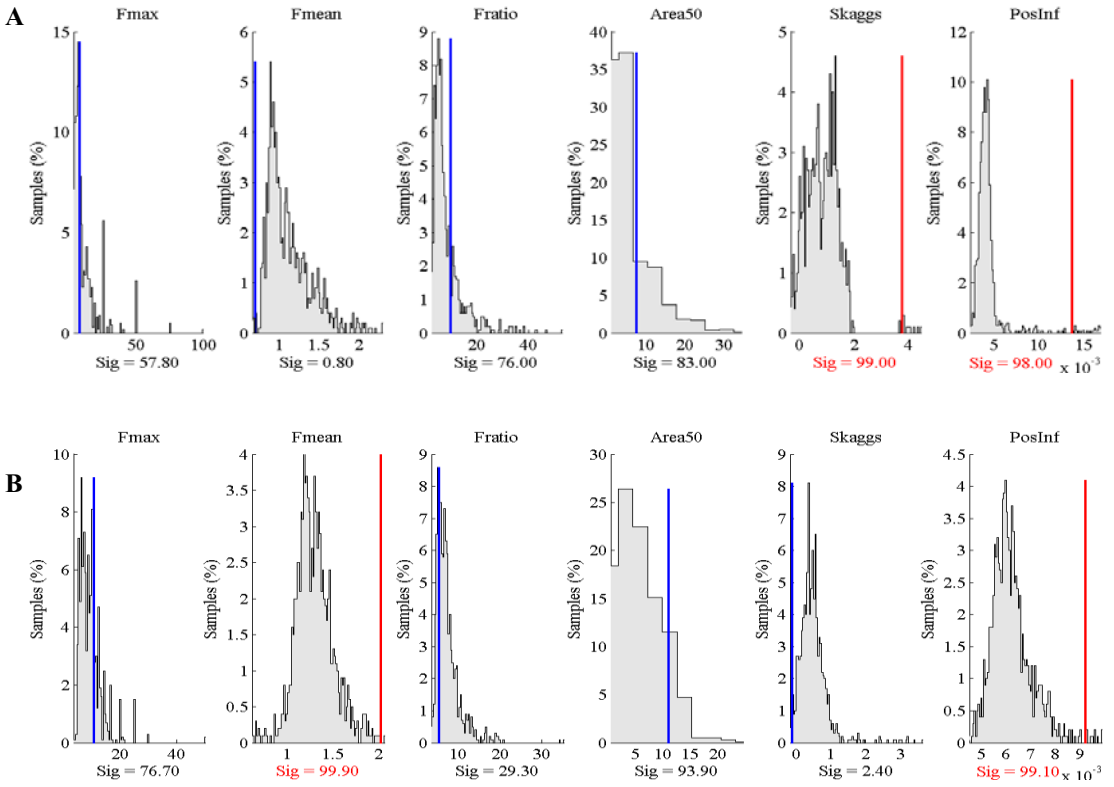

Figure 26: Shuffled distributions.
Example of the spatial parameters calculated for one neuron. Each graph shows with a vertical line the values obtained from the position distribution of the items (A for the rat and B for the robot). Each of the calculated values of the shuffles (n=1000) are depicted as a histogram that has in ordinates the number of shuffles encountered with such value in abscises. The level of significance is indicated below each graph as the percentile in which the real value in the shuffle distribution is. For p<0.05 the value is highlighted in red (over 95% of the shuffle).

By the use of shuffled distributions several advantages are taken. First, the technique allows a good control for the data set. For instance, an isolated neuron with a low basal activity which has a unique bursty episode in time could be interpreted in the spatial analysis as a spatial specific neuron because only in one place, that actually was a period, it shows a high discharge frequency. Using statistical methods derived from the spatial parameters of the neuron, one could conclude that the neuron is encoding

information relative to the animal's position. Instead of that, when the shuffled distribution method is applied, the fake spatial distributions of the spikes will show this peak of activity moving across the field and, when the real value is compared against them, the result will be a significance level around $p \approx 0.5$. Here resides the robustness of the shuffle's method, it allows a control of the data set itself. The same example exposed for a casual accumulation of spikes in time serves for hypothetical cases of position accumulations, whose artefacts are very important to control in our experimental paradigm. That is because the rat and the robot distributions of positions over time differ substantially. While the rat is moving across the whole area available the robot is constrained in its movements and, even more important, some positions of the robot are always occupied with certain behaviour (moving, stopping, turning, etc…). Therefore, by the application of the shuffles, the resulting statistics and calculated significances avoid artefacts possibly due to the distributions analysed.

A similar method to the shuffled distributions was applied to the results derived from the calculations of the MI. The method used is known as bootstrap and it consists in the shuffle of the different conditions previously evaluated. While the shuffled distribution produce fake cases by the cyclic shift and, therefore, desynchronising the signals, the bootstrap use the same data set but after the comparison of two groups the same procedure is applied with a random assignation in new groups. By this technique the significance level of the divergence between two data groups can be calculated. After the generation of 1000 bootstrapped distributions the p value of the real comparison corresponds to its location inside the bootstrap. Our threshold was situated over the 95% of the distribution to determine a significant difference between the groups compared ($p<0.05$).

Finally we have used classical statistical tests to compare the data. Depending of the data set evaluated an analysis of variance (ANOVA), or a t-student distribution was selected as the best option to compare the data. Not remarkable strategies were used in these tests. The calculations were performed by MatLab® functions. Significant levels for the null hypothesis were below 0.05 or 0.01 depending on the data set. Not other statistical software were used for these calculations, the data and the pre-processed parameters were used as vectors in MatLab® and the results of the statistics were saved

as structures that can be later evaluated. This way all the statistics were merged and the data set can be reviewed or analysed properly if further questions arise.

3.2.6. Histology

In order to track the electrodes implanted in the subjects their brains were extracted after sacrificed them under deep anesthesia (1 ml of Dolethal, Vetoquinol S.A., Cedex, France). Before the extraction, a perfusion with saline (0.9% w/w, ca 300 ml) and paraformaldehyde (4% w/w, ca 100 ml) was done to clean the blood as far as possible and also to fix the tissue. Then, the brain could be carefully extracted attempting to don't damage it and a vibratome's cutting of 80 μm slices was done in saline solution. Subsequently the slices were stained in a classic Nissl protocol that is useful to characterise the different cell layers by the positions of the stained somas. The position of the electrodes was then detected by the examination of the obtained slices over microscope slides. Some studies use an electrical stimulation before extracting the brain to better see the electrode tip but in our case the cellular responses produced by the electrodes during their stay and their width are enough to detect them without it, an example is shown in Figure 27.

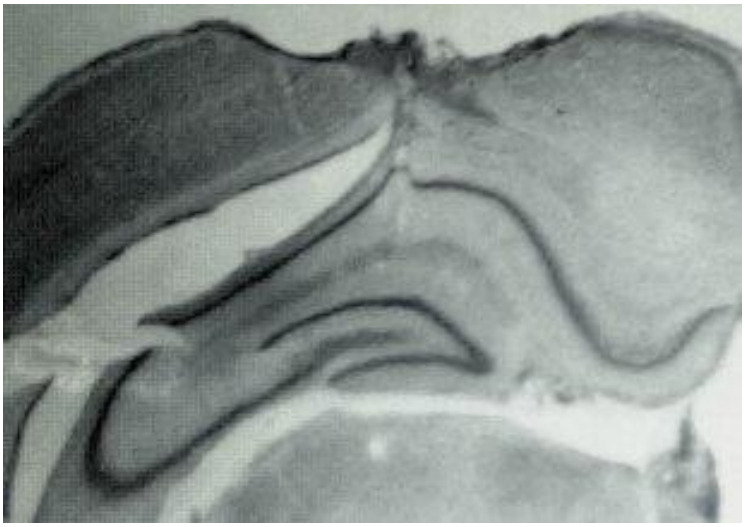

Figure 27: Histology.
An 80 μm coronal slice of rat's brain was photographed after their Nissl dying. The soma of the hippocampal cells could be easily observed forming the double U shape characteristic of this region. In the upper layer an amorphous part reports the pass of the tetrodes, a clear discontinuity is observed in the layer and a vertical path coincides with it. In the cortical area above the indicated region of hippocampus, which is CA1, the scar produced by the chronic implantation is observable too.

4. RESULTS

4.1. Behavioural results

Several behavioural protocols were tested in order to find a task where the subjects would pay attention to a dynamic cue. Pilot experiments were carried on using two different guide's strategies for the behaviour: protocols where the animals discriminated between positions of the robot (PDT, Position Discrimination Task) or a second set of protocols where the stimulus was the direction of the robot's movement (DDT, Direction Discrimination Task). Once the task was selected the training was evaluated in single phases. The behaviour of the subjects was progressively shaped across these phases. First, the reward was delivered each time the stimulus happens independently of the animal's behaviour. In this way the conditioning to the stimulus is natural and the subjects began to pay attention to the stimuli. The next phases implied the increase of the demand on the subject's behaviour. Thus, the subsequent phases required a more precise execution of the task to obtain the reward in order to finally shape the behaviour of the subjects as the expected one.

In our case the cognitive demand for the subjects was high and the first proved protocols failed reaching high levels of performance rather being it close to the chance level (PDT and DDT further described in the previous section Protocols 3.1.3, Figure 28). One common strategy in behaviour to improve the conditioning is to use of an operant element in the task. With the operant element the subjects must indicate with a particular behavioural pattern when they are ready to perform the task. The operant element selected in our case was a black platform sited in the front side of the cylinder where the subjects should place above their head in order to start a trial. On one hand the platform serves as the operant trigger that determines the onset of the robot's movement and, on the other hand, it assures that the animal is looking to the robot at the right moment. The Figure 28 is a comparison between the learning curves obtained by each of the protocols proved, illustrating the difference in the progression of the

performances both in the percentage of correct choices and in the velocity reaching these higher performances. The conditioning and shaping phases are not shown in this figure, therefore all the sessions showed in the Figure 28 are with series of random trials which can be rewarded, punished or just missed in function of the subjects' behaviour.

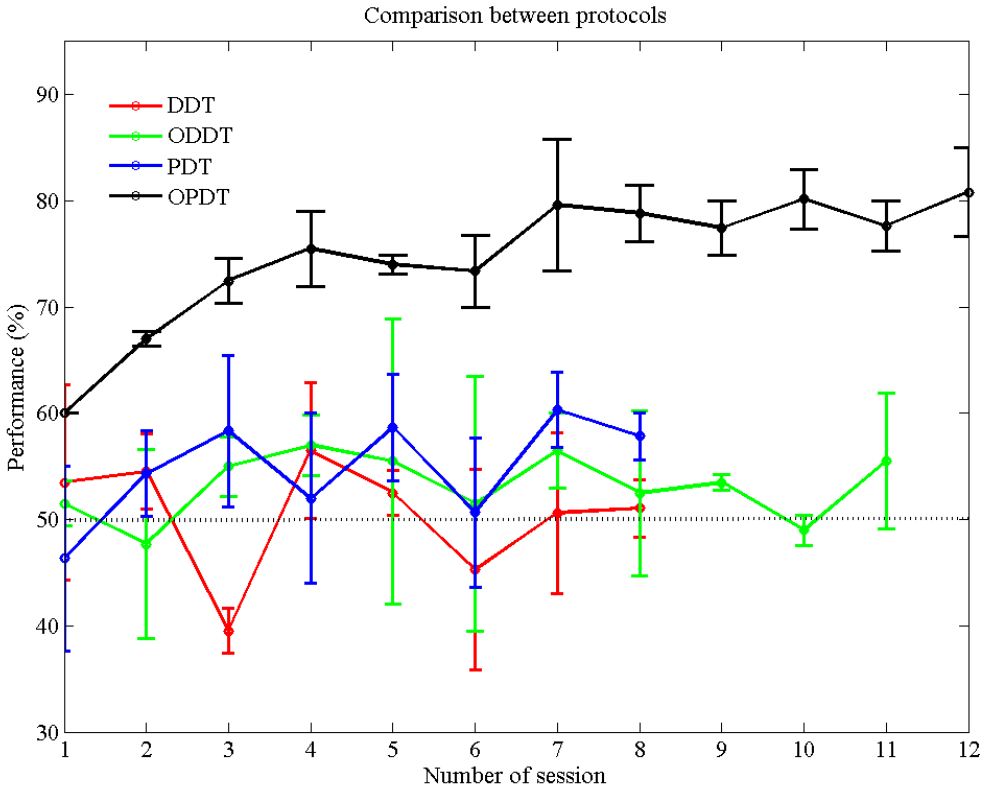

Figure 28: Learning curves for the discrimination tasks evaluated.
PDT and DDT are discrimination tasks where the stimuli are respectively the position or the direction of the movement. ODDT and OPDT refer to the same discrimination tasks but this time the subjects' behaviour triggers the onset of the trials, operant task. A very clear difference can be observed between the OPDT and the other tasks, subjects reach rapidly high levels of performance (SEM error is shown for each curve and the chance level marked with a dashed line).

After the different protocols were tested, the learning results led us to train all the subsequent animals in the OPDT. Therefore the results and details of the training will be described only for this protocol. The data obtained in the *pre-training* phase are illustrated by the behaviour of one subject in Figure 29. The values shown are the average lag spent between the trials executed during the recorded behaviour (Inter-trial time, ITT). The first part marked by a red box (1, left trials) is the beginning of the animal's exposure to the apparatus, at this initial point of the *shaping* the stimulus is always presented after the operant behaviour (head above the platform) in the same side

and being always paired with the reward. Surprisingly the curve begins with very low values of ITT when one would imagine that the rat should do few trials per sessions and therefore having the higher values at the beginning. This is due to the fact that the subject is very attracted by the platform and the water deliverers at the beginning, doing more trials than the expected ones just by chance. Once the subject is habituated to the environment the ITT increases, reaching in this case the maximum at the fifth session. The behaviour can be evaluated without interfering thanks to the top-view camera placed in the recording chamber and thus it detects when the subjects are performing the task correctly, it means going directly from the platform to the nose-poke where the water is available and then the ITT start to decrease.

The second part of the Figure 29 (2, right trials) marks an identical shaping phase but in this case the robot is performing the trials in the opposite direction. These first two parts of the shaping have the opposite nose-poke tapped in order to avoid the approaches of the rat to them. The next stage (3, red for left trials and blue for right trials) consists exactly in the opening of the second nose-poke. Behaviorally the stage is still a shaping phase because the reward is always paired to the stimuli but the subject should decide which of the nose-pokes during the session the correct one is. After validating that the subject still performs well the trains of same stimuli the next phase could be applied (4). The first difference with the previous one is that the trains of stimuli are presented inside the same session without retiring the subject from the apparatus between the trains of stimuli to each side. Another difference is the fact that the sessions are not presented in an alternation sequence as before. In this stage begins the *conditioning* phase, only when the subject performs correctly the trial the reward is delivered. If the subject goes to the opposite nose-poke an aversive stimulus is presented (white noise, 80 dB). Two different purposes underlie this phase: the first is the fact that the animals do not habituate to just alternate between trains of reward in each side and the second aim is to force the subject to understand the change of stimuli inside the session by paying attention to the dynamic cue and avoiding a behavioural pattern in which each session will be relative to one nose-poke.

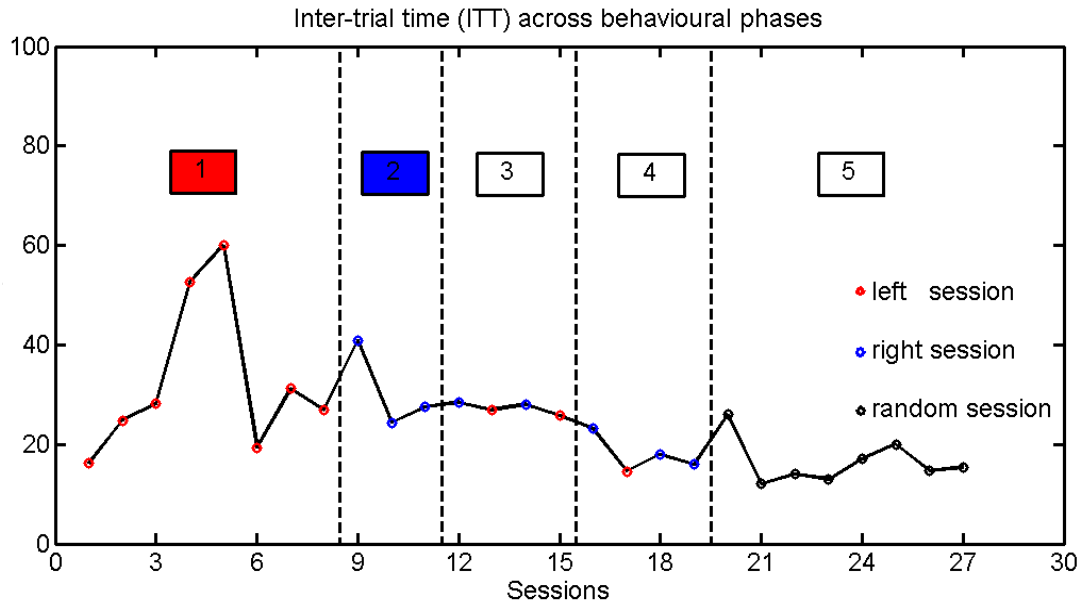

Figure 29: Pre-training phases in the OPDT.
The figure shows the average time spent by the subject to make a trial in each session (Inter-trial time, ITT). There are five stages separated by dashed lines and represented by a number. 1st comprises the starting stages where the subjects are shaped to the protocol by giving reward each time the trial is performed while the dynamic cue moves in one side, in this case left sessions in red. 2nd and 3rd are still shaping phases because the reward is always delivered independently of the behaviour but in 2nd the sessions are to the right and in 3rd an alternation between them begins. 4th is the beginning of the conditioning because the reward is only available if the subject choose the correct deliverer while 5th includes only random trails inside the same session, the test period.

The critical point of the training is the selection of the correct moment when to change the alternation of trains of stimuli into a random presentation of them. In order to select the appropriate moment to start the random presentation, the experimenter looks for the subject's ability to anticipate the change of stimuli' trains. A learning curve for all the subjects that have been involved in the OPDT (N=5), each of them in different colors, is shown in Figure 30 with the averaged learning curve of all the subjects included in black with their SEM errors. The curve shows how the subjects' behaviour evolves through the different sessions, each of them composed by 100 to 200 random trials. After less or more 15 sessions rats reached in average a high percentage of correct choices stable in time (mean performance at the final session was 82.91±2.31%).

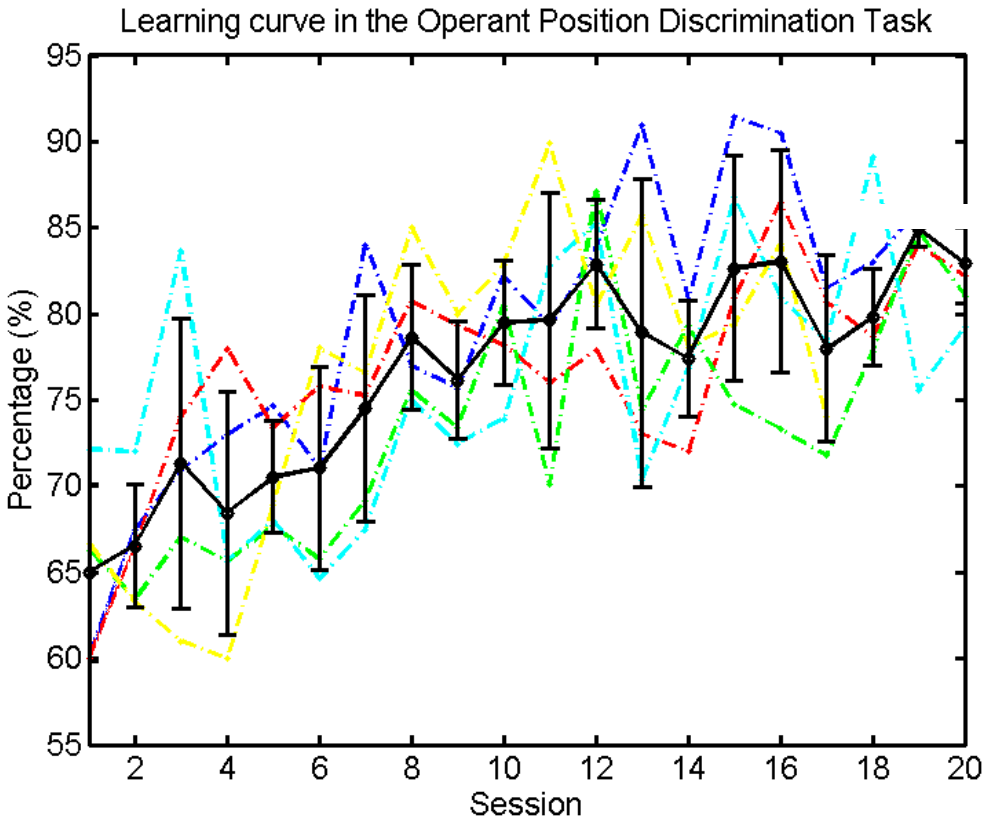

Figure 30: Learning curve in the OPDT.
The black solid line shows the average performance as the percentage of correct choices whit the SEM (N=5). The dashed coloured lines give the values of each animal in the given session. The performance level begins around 60% because the previous sessions were composed by train of stimuli while the random presentations were recorded only after reaching this percentage of performance.

A dissection of the behaviour for one session where the subject executed an 82 % of correct trials can be observed in the Figure 31. The response time of the subject is plotted separately for the correct trials towards the left (Figure 31A) and those towards the right (Figure 31B) in the upper part of the panel. The response time was calculated as the interval between the time stamps of the robot's arrival to the target position and the licking of the rat in the water dispenser. The lower part of the panel shows all the trials performed correctly in the left lower corner (Figure 31C) and the trials with a wrong response in the lower right corner (Figure 31D). The most part of the trials have a response time equal to zero, it means that the subject is waiting for the robot to stop in order to drink, anticipating the availability of the reward. These results point out an important aspect of the task. The subjects usually identify the left vs. right trials by the direction/sense of the robot's movement at the onset of the trial and therefore the cue

that first guides the behaviour is supposed to be the motion of the dynamic cue. However there are other trials where the subject did not pay attention to the robot mostly because it was not aware during the operant behaviour. For these trials the behaviour commonly observed was the identification by the subject of the robot's position by a fast screening of the environment to localise it. Once detected, the robot the subject rapidly runs to the correct or wrong nose poke.

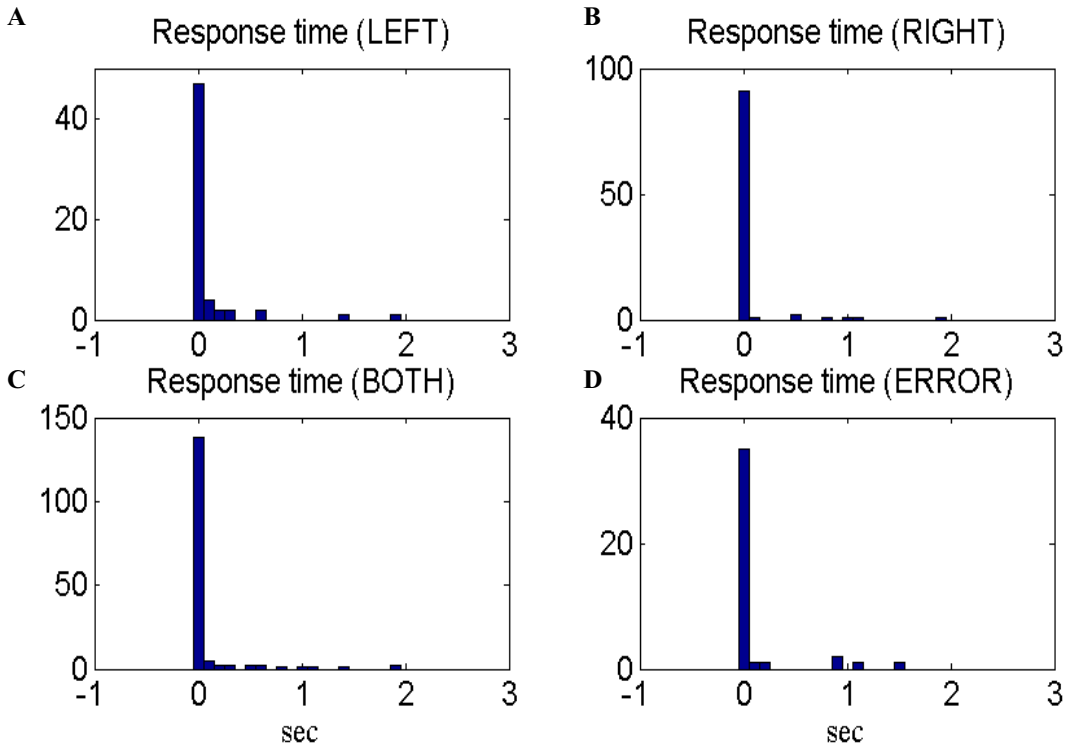

Figure 31: Distribution of response times.
Histograms representing the distribution of response times, in the *y axis* the scale shows the number of trials with this response time (RT, since the reward is available until the subject pokes) for correct trials towards left (A) or right (B) in the upper part. The histogram relative to both kind of trials are collected together in the lower left corner, therefore comprising all correct trials (C). For the error responses the response times were also identically calculated (D).

Because of the complexity of the task the subjects were first trained and when the discrimination of the robot's movement was successfully performed by the subjects they were implanted. The criterion used to decide when to operate the subjects and site the electrodes was the stability of the performance across three sessions over the 75% percentage of correct choices.

4.2. Hyppocampal rhythms

The LFP was recorded during the discrimination task, OPDT, selecting one of the 16 electrodes to store this signal. Then the data was analysed without filtering, not applying band-pass filters that can affect the real oscillations observed. The LFP was used to build spectrograms centred in the onset of the trial (perispectrogram, Figure 32A) and in order to have more homogeneous trials only the correct trials below a maximum response time, RT, were selected (the threshold used to split the trials is taken subjectively looking at the distribution of RT, the distribution for one session can be seen in the previous Figure 31). Because the theta band power has been described to be related to the locomotor activity and the speed of the subject (Vanderwolf, 1969), we expected to see this correlation in the spectrograms (Figure 32B represents the mean speed of the items during the selected trials). In the period before the onset of the stimulus the peaks of the rat's velocity and power coincided. During the movement of the robot this modulation, theta power vs. subject's speed, seems to be suppressed and furthermore giving the lower values of the mean theta power during the task.

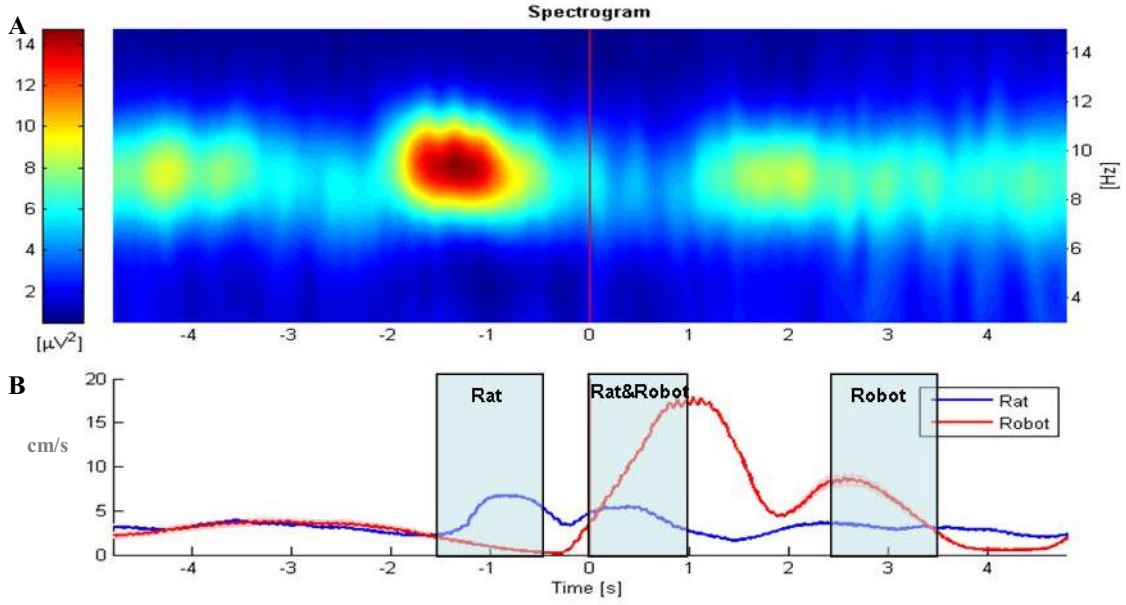

Figure 32: EEG and velocity.
The LFP recorded during the session is selected for correct trials towards one side since 5 sec before the onset of the trial until 5 sec after it, the power of the signal for each frequency is then calculated and averaged to obtain a mean spectrogram (A). The power of theta is attenuated during the movement of the robot. The velocities of the rat and the robot can be observed in the lower figure (B), once again the values are the average during the same trials included for the spectrogram and the obtained SEM is shaded around the value. The three rectangles represent the selected periods for the analysis of velocities; respectively when is moving the rat, both items or just the robot.

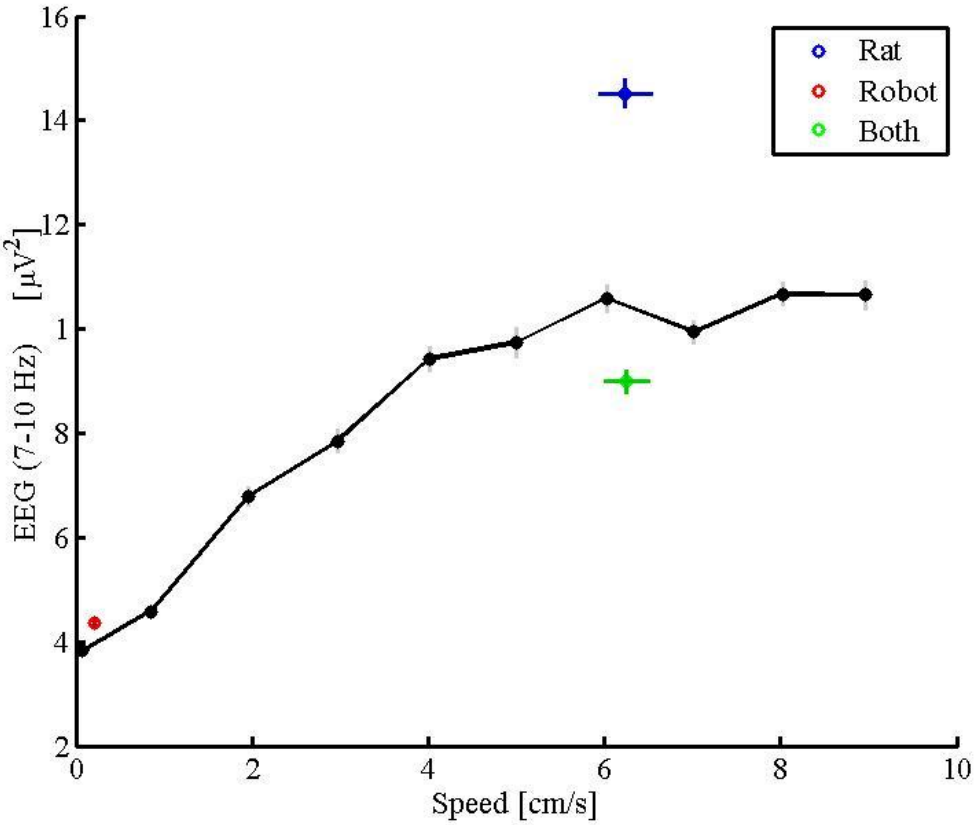

Figure 33: Natural correlation between theta's power and the speed.
For different segments of the subject's speed distribution the mean power of the theta band between 7 and 10 Hz was calculated. The black line shows the tendency of the theta power to accompany the speed in their increases. Three behavioural epochs of the trial were then selected depending on the movement of the rat and the robot: rat moving, both items moving and robot moving. The mean power within each of the epochs mentioned was calculated and plotted against the natural correlation in order to compare. The results indicate that the relationship between the subject's speed and theta power was diminished when the robot was moving.

For this reason a quantification of the theta band power was performed in different segments, the chosen stretches were the following: 1) rat's movement period comprising times between -1.5 and -0.5 s, 2) robot's movement period comprising from 2.5 to 3.5 s and 3) the period where both items were moving, that is between 0.0 and 1.0 s (for a closer look to the items' dynamic and its relationship with the behavioural phases see Figure 32B, the rectangles highlight the three periods considered). These stretches are therefore selected in function of time instead of velocity. An example of the correlation between theta's power and the speed of the subject is given in Figure 33. The power across the band frequency of 7-10 Hz (inside theta this is the range more related to the speed) was calculated for periods in which the speed of the subject was within a certain range and a curve with their relationship was built based on such

calculations (black line in Figure 33). The band power comprised between 4 and 12 Hz, the predominant band in the hippocampus as it can be observed in the spectrogram of Figure 34A, was calculated and analysed for each of the mentioned epochs (power spectra Figure 34B). An ANOVA test was run for the different band powers and the results point to a tendency of smaller mean powers for theta during the movement of the robot (N=64 trials, Figure 34D, $p<0.01$).

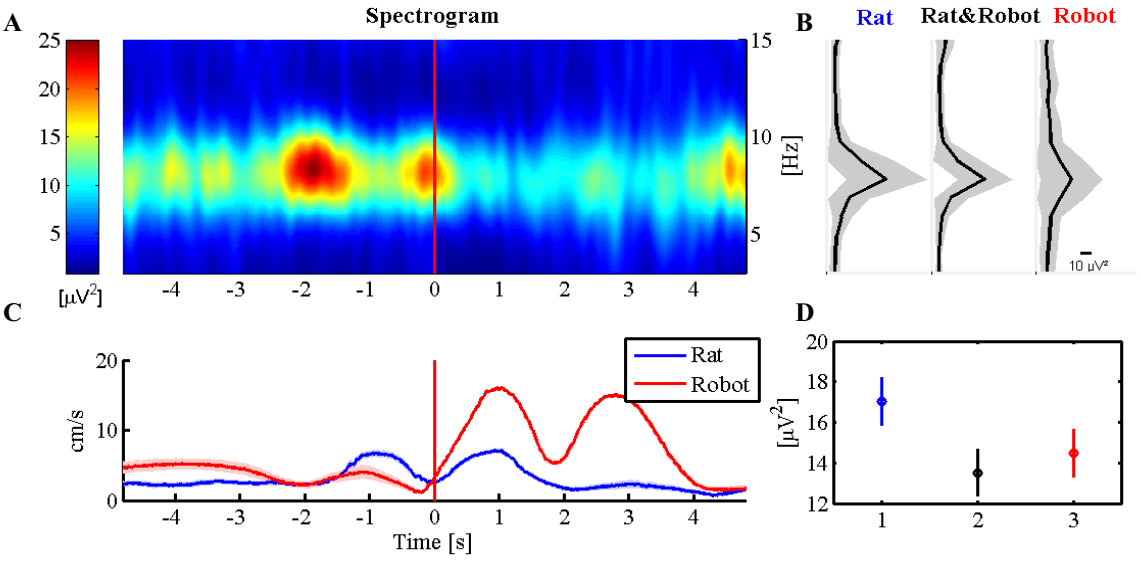

Figure 34: Theta and velocity.
(A) The band power of the LFP comprised between 4-12 Hz was calculated for each trial and then averaged; the evolution across the trial can be then observed (0 time corresponds to the onset of the robot's movement). (B) Power spectra of behavioural stretches: rat moving, rat & robot moving and just robot moving. (C) The speed of the rat and the robot, blue and red respectively, was equally calculated to compare its correlation with the band power. (D) ANOVA outcome of the statistical comparison of the power spectra shown above, the plot contains the value of power obtained and the confidence interval indicated by the vertical lines (N=64 trials).

The suppression of the theta band during the robot's movement was observed across different recordings and subjects. In order to quantify this modulation 20 recordings performed on 5 different subjects were merged to run a statistical test. The power spectra of each of the trials was included in the test, therefore the movement towards both sides and correct/wrong trials were comprised in the analysis. The result confirmed the evidence for the whole data set (N=2531 trials, 20 recordings, 5 subjects) with high levels of significance difference between the three considered groups, $p<0.001$ (ANOVA; rat moving, robot & rat moving and just robot moving).

4.3. Single units

Once the behaviour and the hippocampal oscillations were evaluated the next step was the analysis of the single units' activity. After the isolation of single cells, the neural correlates of the tracking and localisation of dynamic cues in relation to their spikes was evaluated. A total of 129 neurons were isolated in three subjects. From these neurons, the ones containing less than 100 spikes during the recording were excluded of the analysis because their activity was insufficient to conclude if there are correlations to the stimulus (N=123 cells). Several variables have been evaluated in relation to the spiking of the isolated CA1 neurons. The analysis evaluated the firing of the neurons in relation to the spatial properties of the rat's and robot's dynamics. That is important in order to identify how a putative relation between the spikes' occurrence and a specific spatial feature is not related also to another spatial aspect of the other moving item. For an overall view, the principal variables analysed in relation to the dynamics of the rat and the robot are position, relative position, sense or direction, static/ dynamic and task related.

In the section 3.2.5.3. Data processing a synthesis of how the analysis was performed and a more detailed description is presented. The different approaches used to analyse the data can be separated into two global strategies: the *spatial* and the *temporal* analysis. The first, in the spatial domain, searches the neural correlates depending on the positions of the items and their gradual changes (velocity, static/dynamic, sense of movement, etc…) while the temporal analysis takes the onset of the trials as the reference point to compare the activity of the neurons across the different trials, in consequence treating the dynamic cue as the driving stimulus.

4.3.1. Spatial patterns of activity

The first step in the spatial analysis was the correct assignment of the neurons' spikes to the locations where they occurred. Classically, in place cells studies, the assignment is done for the location of the subject during the recording, while in our case the same procedure was done also for the robot's locations. The classical firing fields will be therefore duplicated because the spikes detected during the subject's behaviour will be evaluated either for the rat's position or for the robot's one.

The maps obtained by this procedure can be observed in Figure 35 where the firing fields of six representative neurons in relation to both items are presented. The recordings from which these neurons have been isolated belong to different sessions and subjects. The area tracked by the camera corresponds to the whole square presented in each graph. The firing rate was calculated for each of the bins by dividing the total number of spikes occurred by the time spent by the subject, in the upper part, or the robot, in the lower part. A black circle around the positions of the rat represents the cylinder where the subject is placed during the recording and the arrows indicate the axis of movement of the dynamic cue. A colour code scale in the lower part of each neuron's panel varying from 0 Hz, dark blue, to the Fmax, that is the dark red corresponding to the bin with the maximum frequency, shows the level of activity found in relation to the positions of the rat and the robot. The upper row contains three cases of putative PCs (quantifications will be explained next) where the neural activity was higher for a certain location of the subject. Instead, the lower row shows examples of three isolated neurons with a firing more related to the robot's position: a peak of the FR can be observed in the robot's position map while for the rat the neuron's activity showed to be more spatially uniform.

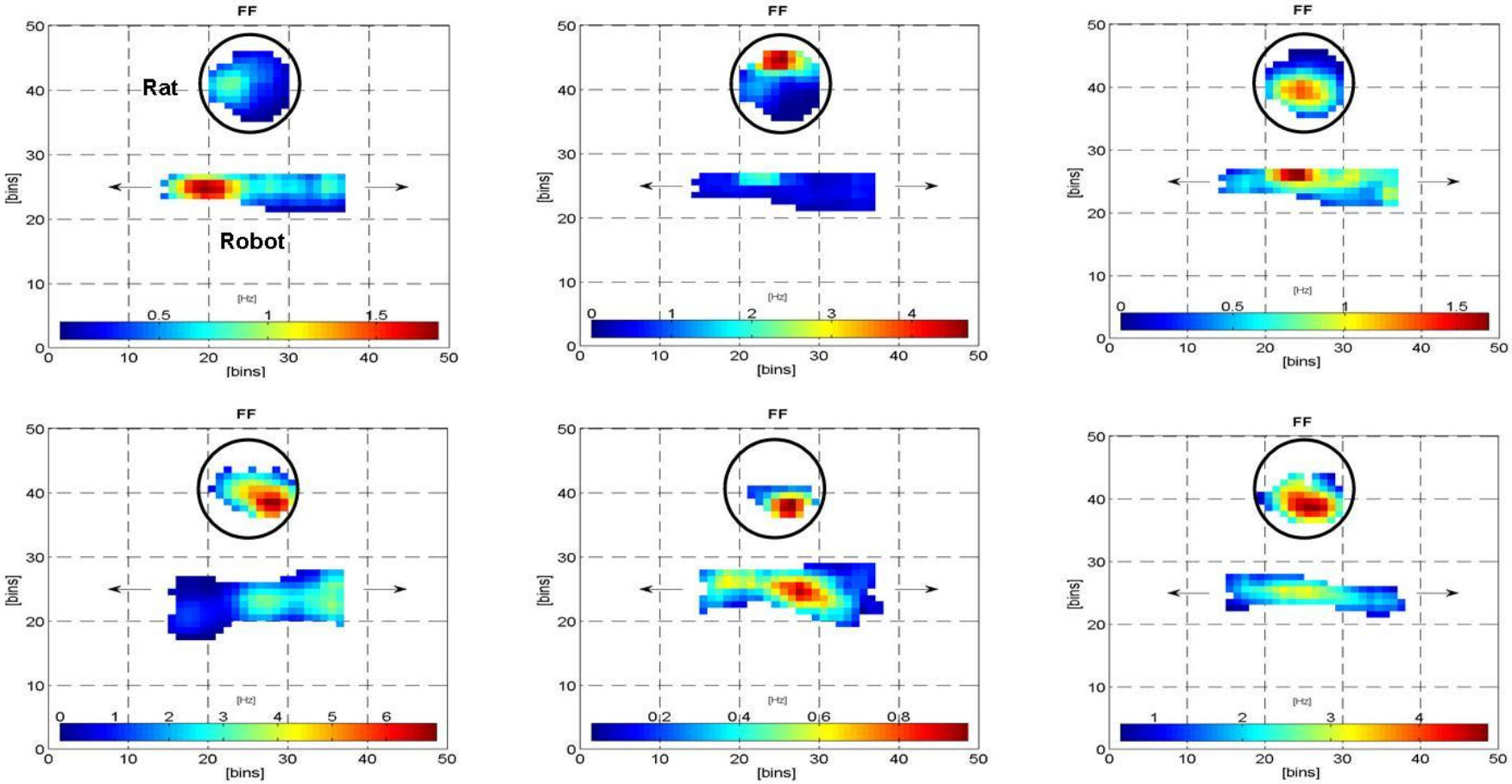

Figure 35: Firing fields. Six isolated and representative neurons for different sessions and subjects are shown. The side of the area tracked by the camera (80x80 cm²) was subdivided in 50 spatial bins (1.66x1.66 cm²). The firing rate of the neuron was calculated in Hz (color code in the lower part of each panel, 0 dark blue and Fmax for dark red). The upper row shows three cases of neurons with a higher specific spatial firing for a certain location of the rat inside the cylinder (black circle) while the lower row shows cells where the spatial firing relative to the subject was uniform but specific for robot's positions.

Quantifications of spatial selectivity

At first glance, the spatial activity of the isolated neurons initially confirmed the hypothesis that led us to perform the experiments: CA1 neurons are not only encoding information about the subject's position but also they show to encode information relative to the dynamic cue's positions. In order to quantify if this is the case we first turn to the Skaggs Index as a parameter that reflects if the firing of the cells is spatially specific: a Skaggs Index equal to zero is obtained when all the bins have the same firing rate accordingly to a uniform distribution. The results of this quantification were compared against shuffled distributions to determine if the activity is significantly specific for the activity maps of the rat or the robot (a cyclic shift of 1000 shuffles was used, a more detailed description of the technique can be found in the section 3.2.5.3. Data processing).

An example of the values of Skaggs Index obtained for the shuffled distributions of one neuron is shown in Figure 36A, the values obtained by the shuffles are plotted in blue for the robot and in red for the rat while the asterisks over the x axis show the real Skaggs Index value obtained (in this case 0.63 bits for the rat and 0.48 bits for the robot). Once the distributions and the real values are calculated they are compared to determine in which percentile of the shuffled distribution the real Skaggs Index are. For the neuron illustrated the percentile is 80.1 and 92.2 for the rat and the robot respectively giving a higher spatial specificity for the robot even if the value of the Skaggs Index itself is lower. This is an important aspect of the shuffled distribution method because its strength resides precisely in the fact that the obtained data is compared to a data pool derived from the same spike trains and occupancy maps. The value of the Skaggs Index itself gives an idea of the spatial information content of the firing but if there is not such comparison it becomes difficult to determine if the value is not due to bursts of spikes or not homogeneous occupancy maps.

Figure 36B is a histogram where the Skaggs Index of all the cells included in the analysis (N=123, 3 subjects) is compared to the ones of the calculated shuffled distributions. The cells are assigned in stretches of 5 percentiles thus the last bars, top right, collect the neurons with a Skaggs Index over the 95% of the shuffle and consequently they have a firing conveying a significant spatial information in relation to the positions of the items (* $p<0.05$, 23/123 or 18.7% for the rat and 21/123 or 17.1%

for the robot). These results demonstrate that the firing fields of the robot also present firing patterns restricted to specific robot's position.

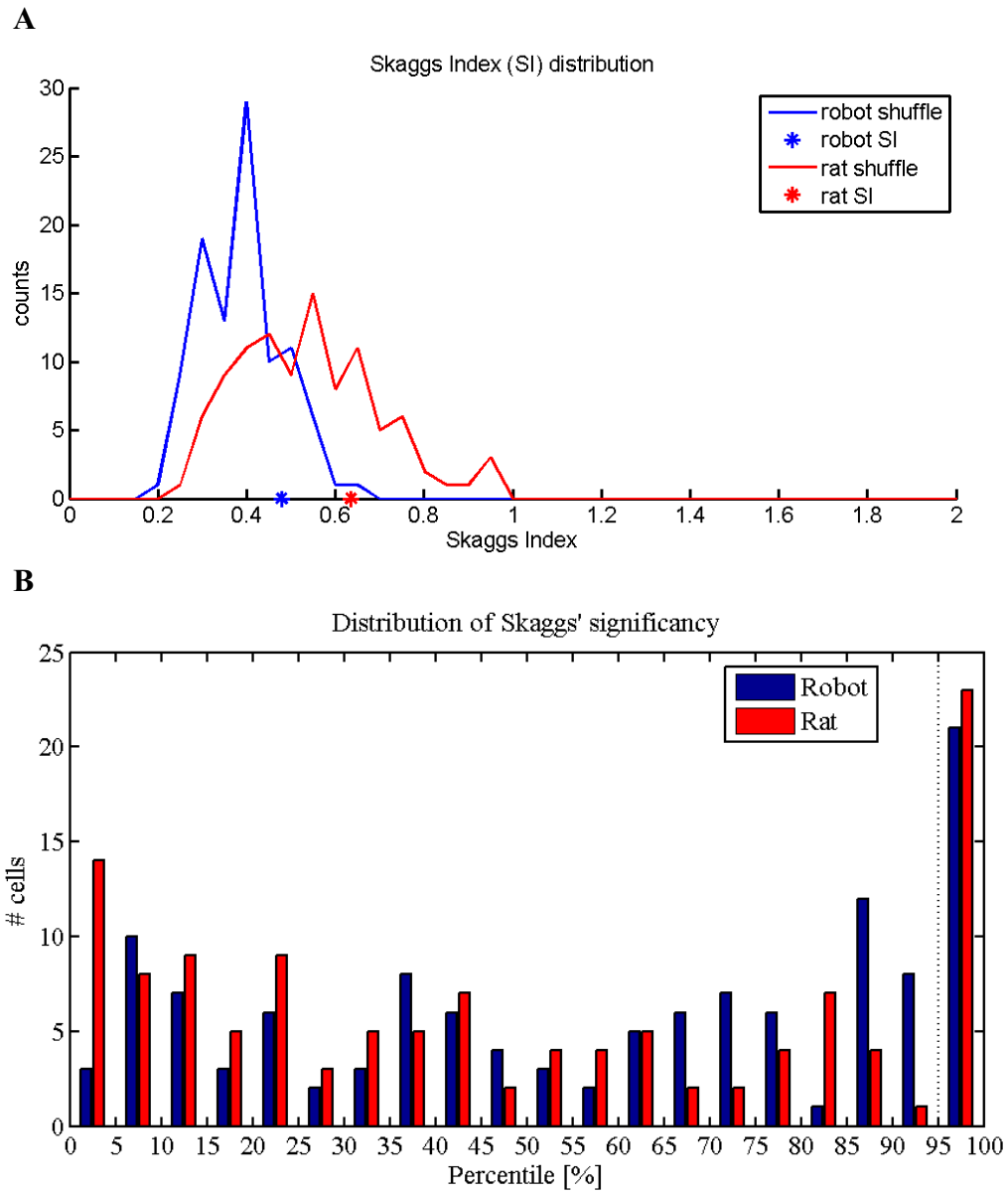

Figure 36: Skaggs Index quantifications.
(A) From the firing fields of the neurons the Skaggs Index was quantified for the rat and the robot (respectively blue and red asterisks on abscises for an example neuron). The obtained values are then compared against Skaggs Index calculated form a shuffled distribution (cyclic time shift, 100 times) which results are collected in A as number of counts (blue and red lines for the robot and the rat). The result obtained for the pool of data (N=123, 3 subjects) is shown in (B) as the number of neurons contained in each percentile of the shuffled distribution (stretches of 5 percentiles, last bar with a $p<0.05$).

Based on the separation by the Skaggs Index the other spatial parameters used in the analysis were calculated separately for the two groups of neurons derived from it. In Figure 37 the average value obtained for each of the parameters is presented. The SI group (N=23/123) is the one where the cells have a Skaggs Index over the 95% of the shuffle distribution while the noSI contains all the other cells. This classification was done for the spikes' distributions either for the rat's path (Figure 37A) or the robot's path (N=21/123 for the SI group, Figure 37B). This way the pool of neurons with a significant Skaggs Index can be evaluated by other independent measures in order to determine how robust this index is. The parameters used were Fmax, Fmean, Fratio, Area50 and PosInf (further details of their calculations in the section 3.2.5.3.Data Processing, significant differences are marked with an asterisk *, $p<0.05$).

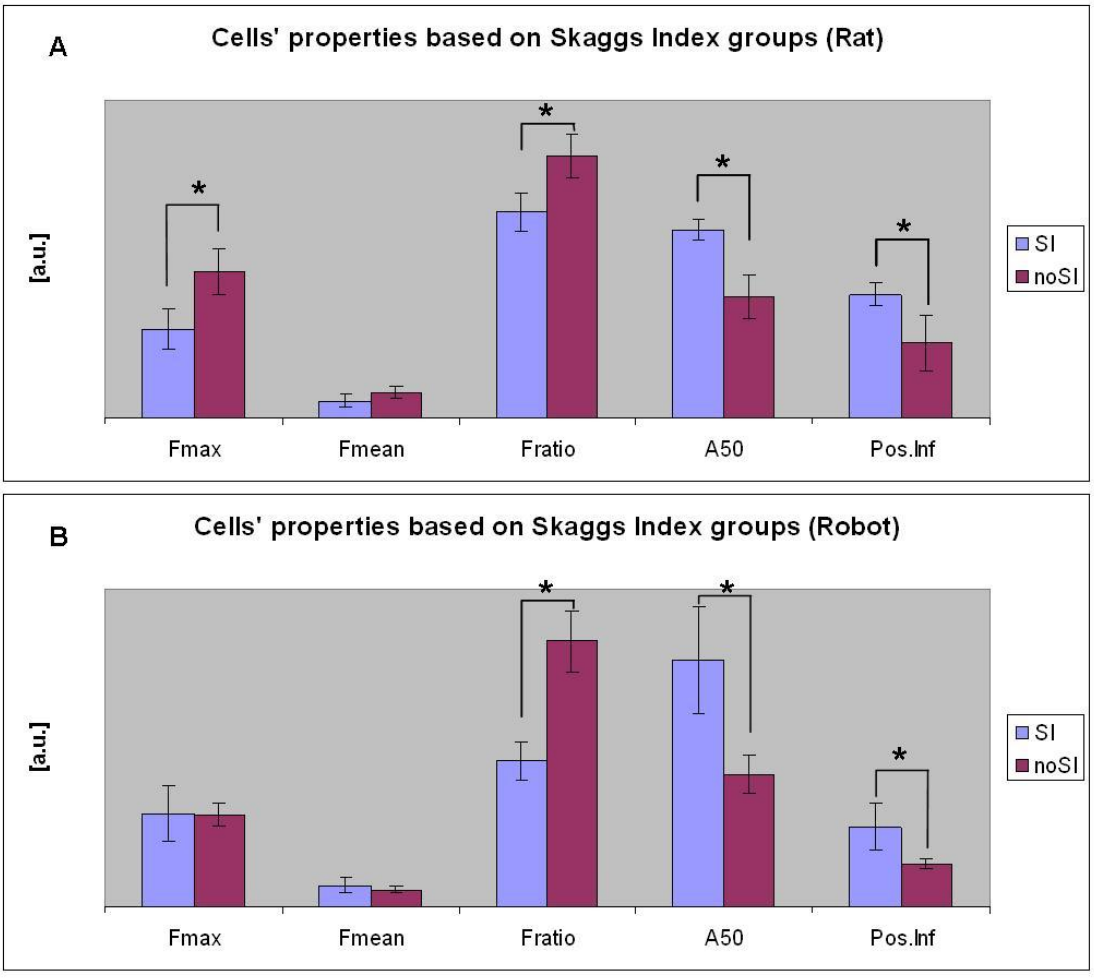

Figure 37: Spatial parameters based on Skaggs Index separation.
The neurons were separated in two groups: SI for the ones having a Skaggs Index with $p<0.05$ and noSI for the non significant ones. For these two groups the other spatial parameters used in this study were calculated (listed below the x axis) with their SEM. The y axis uses arbitrary units that respect the relation between the values and SEM for each parameter because the values of the parameters are largely different in scale (*, $p<0.05$, *t of student*).

Several conclusions can be extracted from the distribution of the parameters based on the Skaggs Index classification. In Figure 37A the group of SI cells for the rat's distributions showed some known characteristic of the PCs. One of such characteristics is the fact that the Fmean is lower for the SI group (0.74±0.29 Hz) than for the noSI group (1.10±0.26 Hz, not significant differences). The first descriptions of the PCs showed that they are a subset of the pyramidal neurons of the hippocampus and whose firing rates in average are lower from interneurons (O'Keefe and Dostrovsky, 1971), later on other experiments showed that a minor proportion of interneurons could have also a PC-like spatial firing (Wilent and Nitz, 2007). Even if the difference of Fmean is not statistically different, the firing rates are separated just by the threshold usually put to separate pyramidal cells from interneurons (1 Hz). The Fratio, it is the ratio between Fmax and Fmean, showed unexpectedly to be larger for noSI group even if it was high for both groups (SI 9.06±0.94, noSI 11.54±0.86). This parameter is commonly not used to separate the spatial from the non-spatial neurons because the existence of bursty cells in the area can produce in the firing fields a bin with a high Fmax while its surroundings are not active. The last two parameters are stronger in this sense, Area50 and Positional information, because both quantify the spatial specificity of the firing taking into account the decrease from the maximum. Statistically there were found to be significantly higher in the SI group. Looking at the same parameters but for the robot's distributions (Figure 37B) only slight differences are found respect to the rat's ones. The more remarkable could be the fact that the Fmean of the neurons appear to be higher for the SI group (1.06±0.38 Hz vs 0.85±0.19 Hz of the noSI group) while the relations between the rest of the parameters are conserved.

In the spatial analysis of the data another different approach was taken. It consisted in the examination of the firing fields but this time separating the maps in function of the animal's position. The area covered by the subject inside the cylinder was separated in three stretches along the axis of the robot's movement: left, center and right. The data has been previously plotted and analysed with all the positions together (Firing fields in Figure 35), instead of with this approach the influence of the rat position on the firing relative to the robot's location was assessed. In Figure 38 an example is shown, the cylinder is marked with a solid black line and the positions of the rat used in each of the plots are only the ones included in the selected stretch. Instead, the positions of the robot are independent of the stretches and the firing field built for it can contain

locations of the dynamic cue along all the axis of movement. The neurons that give good scores with the different spatial parameters calculated usually have firing fields for the items which are sharper, it means that they show peaks of firing rate well localised in the space. With the separation in stretches the stability of the peaks for the robot can be evaluated; if the maximum firing rate of the neuron in relation to the position of the robot is preserved in the three maps (left, center and right stretches of the cylinder) it means that the position of the rat is independent of the result observed for the robot and then the firing of the neuron is really locked to a certain position of the robot.

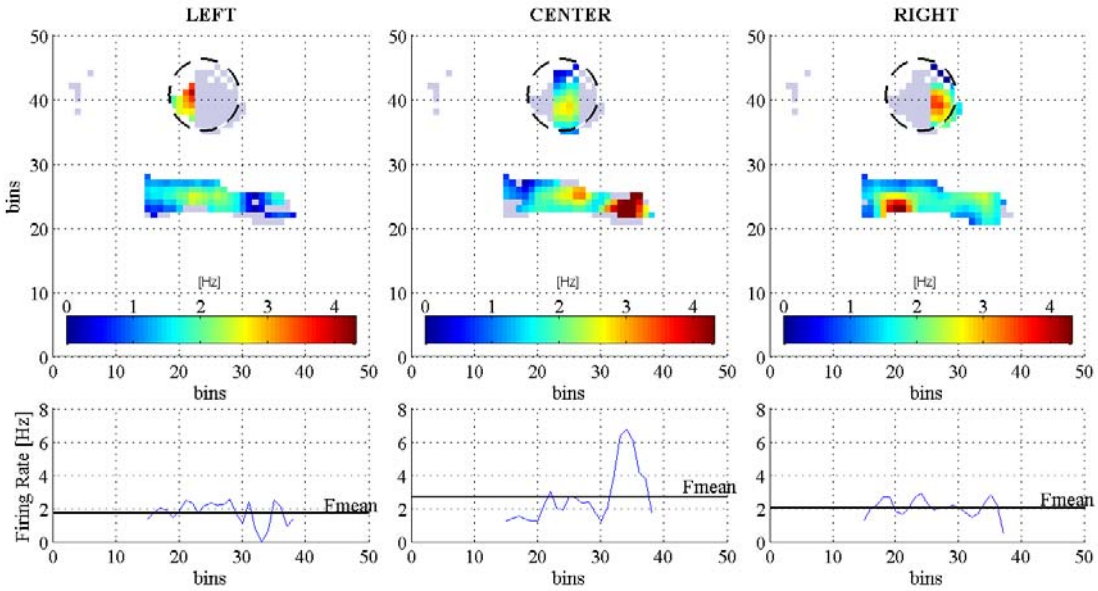

Figure 38: Separation of the rat's position in behavioural stretches.
Three different firing fields (FFs) are presented: left, center and right stretches of the rat's positions. During the periods in which the rat stayed on the respective stretch the distributions of the spikes in relation to the robot's positions are evaluated. In the lower part the projection of the FF of the robot is indicated, FR in blue and Fmean in black.

Relative position

Once the relationship between the firing patterns and the absolute position was analysed, we turned the attention to the relative position between the items. In an egocentric representation, the position of an external object is represented by its relative position in relation to the own subject. The spiking of the neurons can therefore be locked to certain angles between the rat and the robot instead of the absolute position of one of them. Examples of neurons with this kind of behaviour are the head direction cells and the border cells found in the entorhinal cortex (Taube *et al.*, 1990; Solstad *et*

al., 2008). To test the feasible egocentric representation of the dynamic cue, the spikes of the isolated cells were evaluated too in relation to the angles formed by the rat and the robot during the recording session. An example of the results obtained for one neuron is shown in Figure 39. The firing rate of the neuron for each of the angles formed by the items is depicted in blue with its scale marked by the inner dashed circles (5 Hz, 10 Hz and 15 Hz are indicated). The inner black solid circle shows the mean firing rate of the cell during the recording, Fmean. As we can see the angles' values are all contained between 30 and 150 degrees because of the set-up configuration. The rat and the robot are constrained in their movements during the protocol and consequently the angles they can form are limited to this range.

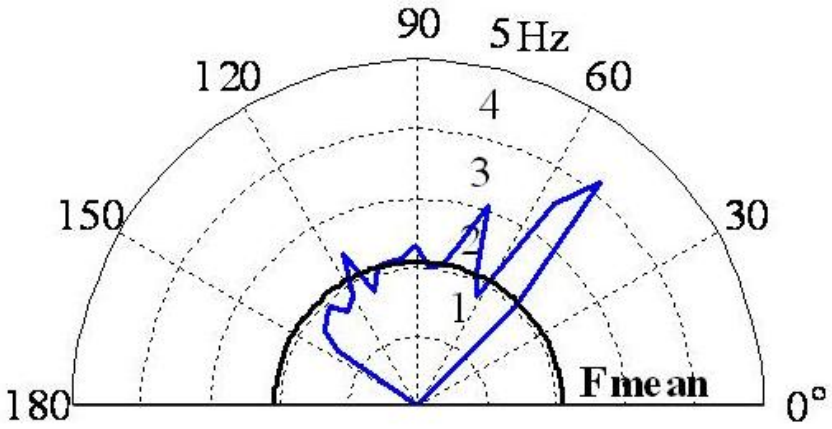

Figure 39: **Firing rate vs. relative position.**
An example neuron is selected to show its activity in function of the relative position calculated as the angle formed by the rat and the robot during the protocol. The firing rate derived of the calculation is shown in blue while the scale can be observed by the inner dashed circles: 1, 2, 3, 4 and 5 Hz are shown. The mean frequency of activity during the recording is also shown to compare, Fmean black solid circle.

Using the Skaggs Index index and the shuffled distribution technique we found a total number of 50 neurons out of 123 with a significant content of information, $p<0.05$, when the distribution of firings patterns vs. the relative positions was analysed. This result should be cross related to those obtained for the Skaggs Index index in relation to the absolute positions of the rat and the robot because during the task some angles are more related to certain positions of the items. From the 50 neurons mentioned, 11 showed a significant Skaggs Index regarding either the absolute position of the rat or of

the robot. These are neurons where a putative representation of one item position can not be concluded even if with further analysis we further dissected their activity in relation to each of the items (an example is the analysis done for Figure 38 with more details for single cases in the next section 4.3.3. Examination of single cells). Because of the configuration of the task and possible overlaps in the rat's and robot's distributions not further conclusions were extracted from the analysis of relative positions.

From the analysis of the spatial patterns of activity of the isolated neurons one main conclusion was obtained. In the population of neurons of CA1 a set of them has been identified by their spatially specific activity, these are the place cells, neurons with very restricted receptive fields in the environment (23/123). When applying the same methods of quantification to the positions of the robot instead to those of the rat we found another subset of neurons with their firing associated to the positions of the robot (21/123). The criterion followed was the quantification of the spatial information content, using the Skaggs Index, in order to identify the subset of neurons with firing rates more locked to a certain position of an item, either the rat or the robot.

From those neurons that gave significant values of spatial information content other parameters were evaluated. On the one hand, the cellular properties represented by the Fmax and Fmean pointed to a larger group of pyramidal cells instead of interneurons firing for rat's positions, while for the robot's positions the groups did not show such clear separation. The Area50, the area covered by the pixels with a firing rate over the 50% of the Fmax, is a robust parameter to assess the spatial specificity of a neuron because it reflects clearly the width of the firing field. The groups found by the Skaggs Index gave very consistent results respect to Area50; the set of neurons with a significant Skaggs Index showed larger and significantly different values of Area50 than those neurons that did not reach the criterion of significancy for the Skaggs Index.

4.3.2. Temporal patterns of activity

Once the relationship between the spikes of the neurons and position of the items was analysed, in terms of the absolute or relative positions, we turn the analysis to the temporal distribution of the spikes across the trials. With this purpose we aligned the firing of the neurons around the onset of the robot's movement. In Figure 40A an example of raster plots for three different neurons during the same recording can be observed. The selected neurons show the three different patterns of firing found: in blue a neuron with a uniform firing across the trial, in red a neuron with an OFF response during the movement of the robot and in green a cell spiking in an ON response during the movement of the robot. A histogram collects the activity of the cells for the different trials below the three rasters (lower region of Figure 40A).

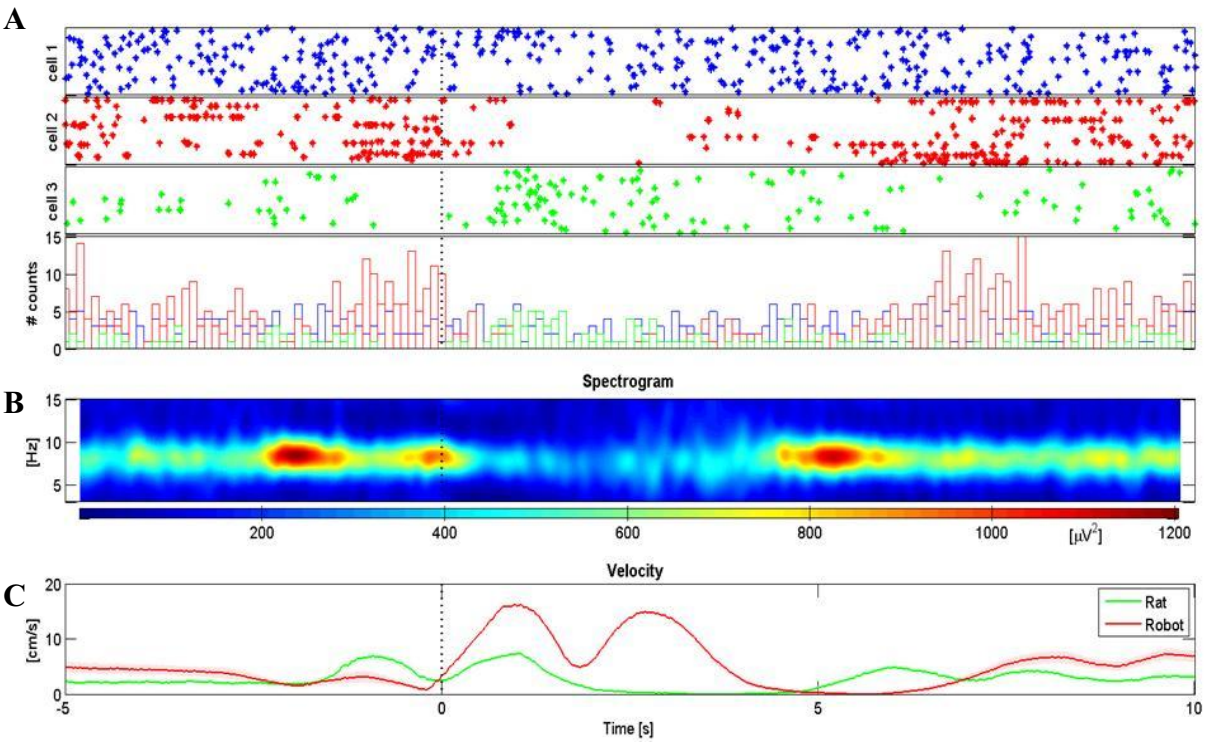

Figure 40: Single unit activity across the trials.
Raster plots with the activity of 3 isolated neurons are shown around the onset of robot's movement (A). A histogram collects the spikes of the 3 cells to show the mean activity for time bin (100ms). (B) A spectrogram centred in the theta range (4-12 Hz) shows the variations in power across the trial. In the last panel (bottom) the speed of the items during the protocol (C) can be observed (both power and speed were calculated as the average of the trials included).

The average LFP for the included trials is also shown in order to compare the oscillation activity in the theta range with the activity of the cells (Figure 40B). Finally the mean speed of the rat and the robot is depicted to infer how both the oscillation and the cell's firing could be related to the items' speed (Figure 40C).

Statistical analysis were done for individual neurons in order to determine which neurons are temporally related to the phase of the task, or in other words to see if the activity of the cells is modulated in function of the time period relative to the onset of the trial. The method used calculates the mean firing rate of the neuron for each temporal bin related to the onset of the trial. The trials where the robot moves towards one side were separated from the trials towards the opposite side and therefore the activity pattern of the neuron is analysed in function of each of the two stimulus presented (leftwards or rightwards robot's displacements). In order to compare the mean firing rate to the basal activity of each neuron the shuffle method was used. In this case it consists in a random alignment of trials, meaning that the onset of the trials is selected arbitrarily creating an unaligned group of trials. Then the mean firing rate in these shuffled trials is computed.

Figure 41 shows the result of this comparison for one neuron. As described above, the trials are separated into the ones towards the left and the right where only the correct ones are included (Figure 41 blue and green respectively). In the upper part raster plots show the activity of the neuron across the trials, all aligned to the onset of the trial (red line), while the lower part shows the percentage of spikes contained in each temporal bin. The mean number of spikes obtained by the average of the shuffled distributions is plotted in black while in grey the range between ± STD is indicated. By comparing the activity of the cells to the shuffled distributions it can be extracted the periods in which the neuron is firing significantly different from the basal activity.

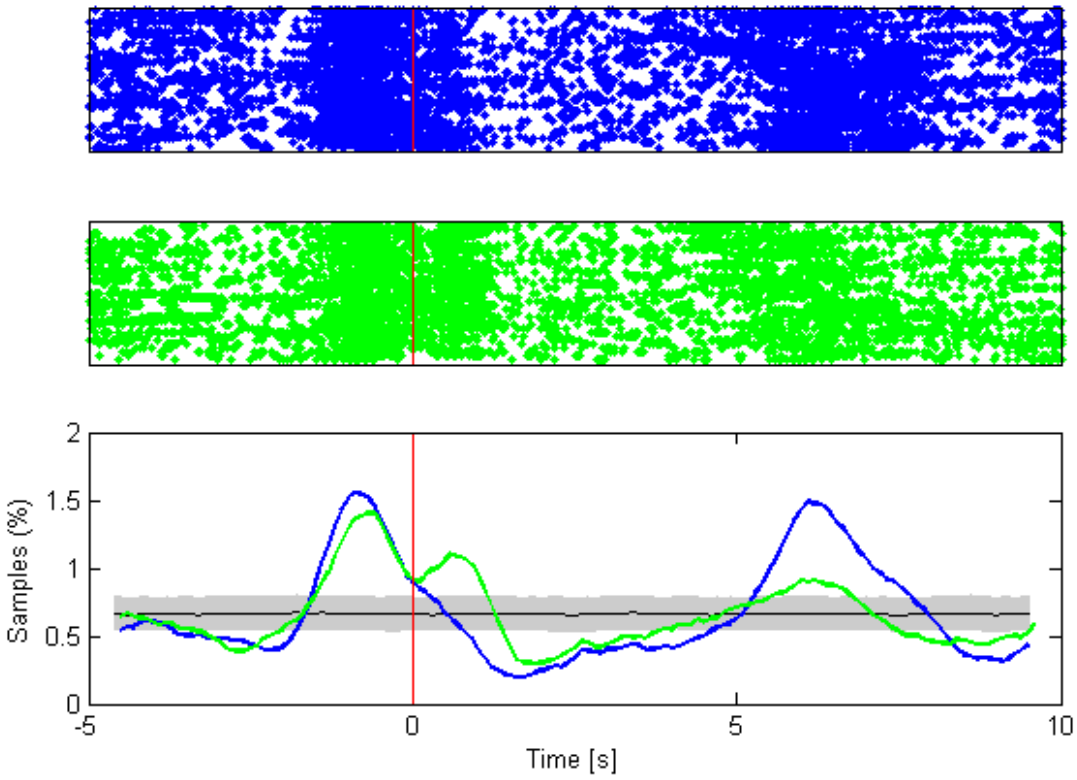

Figure 41: Behavioural phase vs. Firing rate.
The activity of the neurons is revised across the trials aligning them around the onset of the robot's movement, 0 time marked with a vertical red line. The trials are separated according to the stimulus presented, leftwards (blue) vs. rightwards (green) displacements of the dynamic cue. In the upper part the raster shows the activity in the different trials included while the lower part collects the total number of spikes in a time relative to the onset. In grey is shown the STD of the obtained shuffles for the neuron and in black the derived mean number of spikes per shuffles.

The activity of all the isolated neurons was evaluated by the same procedure determining the neurons that have OFF or ON responses during the movement of the dynamic cue. OFF or ON responses are understood as periods of sustained activity respectively below or above the basal activity of the neuron analysed. The results showed several neurons with significant changes of firing rate during the movement of the robot. A total of 32 ON (N=32/123) responses were found from which 17 were for the left and 15 for the right. For the OFF responses we found 30 neurons (out of 123 isolated neurons) decreasing significantly their firing during the robot's movement and being them distributed in 14 to the left and 16 to the right (schemed in Figure 42). The ON/OFF responses found were symmetrical; it means that the cases found are similar for both sides. Another important aspect is the evaluation of different responses in the activity of one single cell for both sides. There were not found neurons with ON

responses for both sides while a total of 4 neurons showed OFF responses for both sides. Finally there were 10 neurons with differential response to each side, it means ON response in one case and OFF in the other (intersecting region of Figure 42). The conclusions of this analysis further confirm the presence of CA1 neurons encoding the dynamics of the robot but the interpretation of the data obtained is still unsolved. On the one hand, the fact that there are neurons responding differentially to the stimuli suggest that these neurons here found convey enough information about the dynamic cue in order to distinguish between the stimuli. On the other hand only few neurons responded equal to both sides (4 OFF/OFF responses were found) and therefore almost refuting, or at least showing a minimum set of neurons, of the involvement of CA1 in a plausible categorisation of the movement itself by the neurons independently of where, or towards where, it was performed.

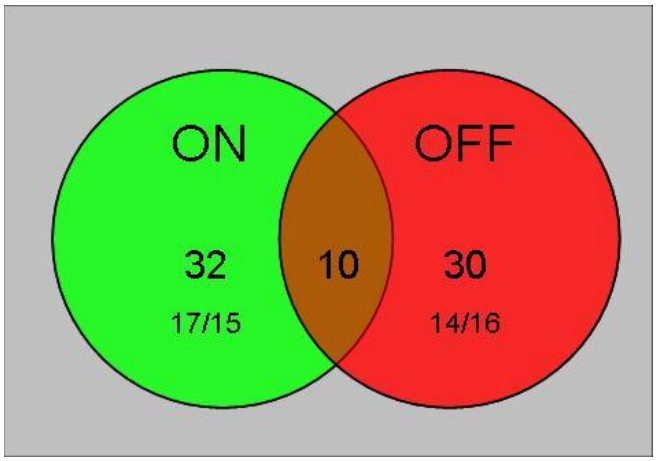

Figure 43: ON/OFF responses during the movement of the robot. The number of spikes for each temporal bin of the task was compared against a shuffled distribution and then determined if it is above (ON) or below (OFF) the basal activity. The findings showed an approximate equal number of neurons with ON and OFF responses (32 vs. 30) and also similar for both sides (ON: left 17/ right 15; OFF: left 14/right 16). There were 10 neurons with opposite response for each side and 4 neurons with the same response for one of the sides (OFF/OFF).

A second analysis was performed in parallel to determine whether there is a differential response of the neurons with respect to the stimuli, view as trains of spikes during left and right sense of robot's movement, or if the firing of the neurons has a specific pattern of spikes when the dynamic cue remains static or is moving. The periods when the robot is moving were therefore identified and classified in left or right towards epochs. The mutual information was calculated for the train of spikes included in the epochs and then compared in two ways: left vs. right or static vs. dynamic (including left and right epochs merged together). The mutual information (MI) is calculated independently for each of the periods and the mean value of it is then extracted. Therefore each neuron has assigned a MI value for three different conditions of the robot's movement: leftwards (L), rightwards (R) and static (S). The statistical analysis (*bootstrap* $p<0.05$) showed 58

neurons significantly different in the (L)/(R) comparison of firing rates, a number very close to the ON/OFF responses (62 cells in total). The (S)/(L&R) comparison showed a total number of neurons spiking significantly different when the robot is resting than moving equal to 30 (*bootstrap* $p<0.05$).

These comparisons reveal again a cluster of neurons encoding information relative to the kind of movement performed by the robot, either the positions where it is moving or the sense of its movement are encoded in the neural activity according to the differential pattern of activity encountered in (L) vs. (R) trials. In consequence the results obtained for the ON/OFF responses are further confirmed by the MI analysis.

4.3.3. General examination of single cells

The spatial distributions of neurons' spikes are tuned not only to positions of the subject but also to positions of the robot according to Skaggs Index. Therefore there are neurons conveying information about the robot's position as well as neurons encoding the rat's position. In parallel, the analysis of the spikes' temporal distributions showed a set of neurons where a significant modulation of the firing pattern occurs during the movement of the robot (ON and OFF responses were found, N=62/119) and confirmed by the MI calculation (N=58/119; *bootstrap* $p<0.05$). Finally, the activity of the neurons in relation to the static/dynamic state of the cue showed again a fraction of the cells with their firing patterns modulated according to its mobility (MI, N=30; *bootstrap* $p<0.05$).

The different spatial parameters of the dynamic cue involved in the modulation of the activity were cross-related in order to infer how the firing pattern of those neurons is affected by the movement of the cue. To do that, the activity of neurons that gave results in the spatial or temporal analysis was further examined for each individual cell. In Figure 43 one of those neurons is shown; first, the waveform and the autocorrelogram are depicted in order to show the characteristics of the cell (Figure 43A and B) whose firing rate and width suggest is a pyramidal neuron (FR=1.43 Hz, width=400μs). The spatio-temporal distribution of the spikes is presented for the rat and the robot (Figure 43C and D) where in the y axis the 0 time corresponds to the onset of the robot's movement marked with a red horizontal line. All the spikes are coloured in purple except those in red that occurred during error trials, this way if there is a correlation with the behaviour it could be easily observed. On the left lower part of the panel the firing fields of the neuron in relation to the rat and the robot positions are shown (Figure 43E1) as well the projection in one dimension for the robot obtained field (Figure 43E2). Finally, on the right lower part, raster plots aligned around the onset of the robot movement show the activity during the correct trials, leftwards and rightwards ones are respectively in blue and green (Figure 43F). The panel offers several aspects of the neuron activity that can be evaluated. The neuron is almost totally quiet before the onset of the trials, this can be easily observed in the time axis of Figure 43C and D, while its maximum activity is found just after the onset of the stimuli.

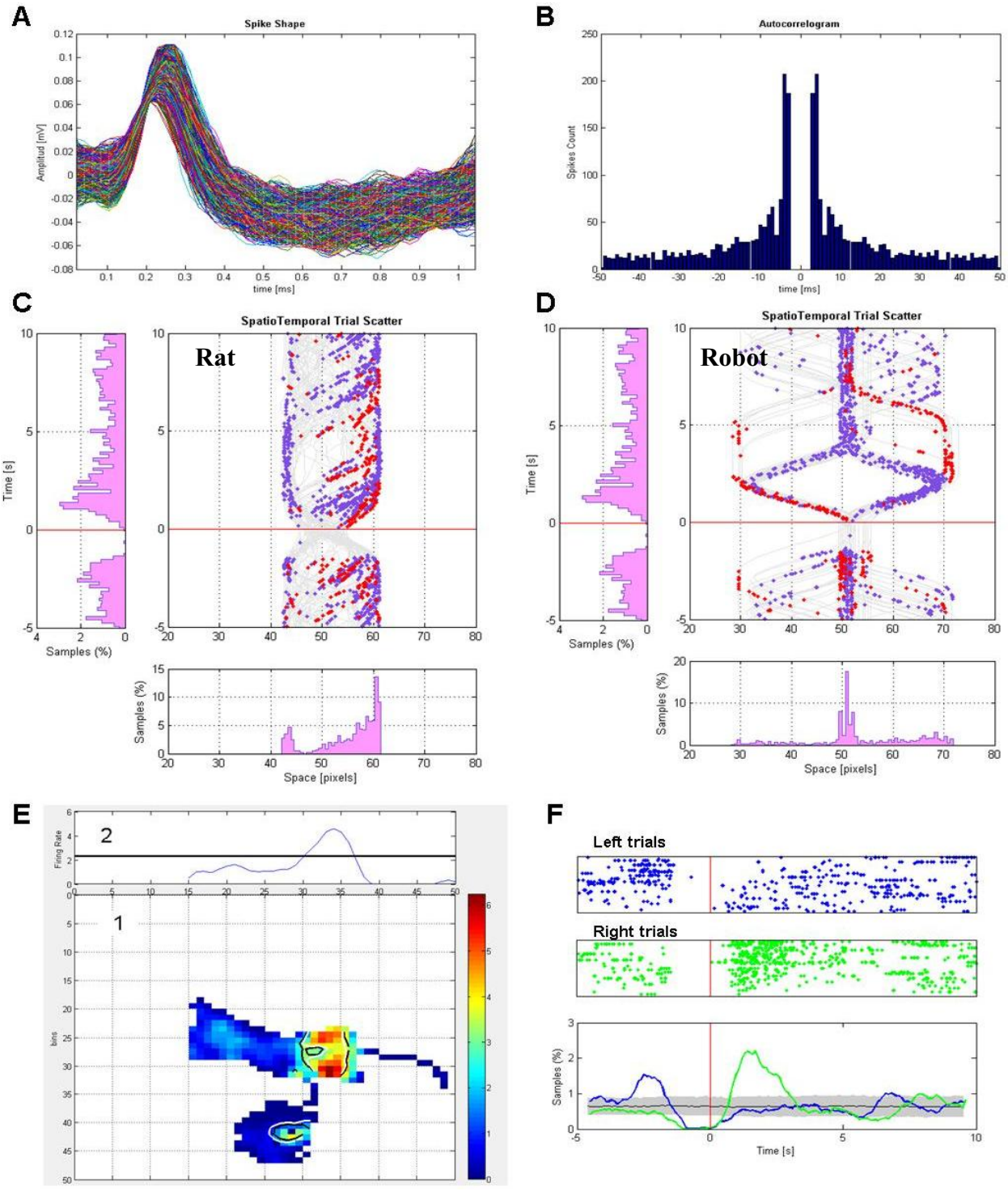

Figure 43: Cellular and spatiotemporal characteristics for a single neuron (I).
The panel offers a global view of the firing patterns of the neuron in relation to several parameters. First, the isolated spikes of the neuron (A) and the autocorrelogram derived from them (B) are shown. Then, the different spikes are distributed in relation to space and time coordinates for the rat (C) and the robot (D). The spikes occurred during error trials are plotted in red while the rest in purple. The coordinates refer to the axis of movement for space and to a trial alignment in time (0 time corresponds to the onset of the trial, red line). The firing fields of the neuron are presented for rat's and robot's positions (E1), above the projection of the robot's distribution in one dimension (E2). Fmax 2D: rat 3.91 Hz, robot 6.24 Hz. Fmax 1D robot: 4.56 Hz. Finally, the activity of the neuron in relation to the task's phase is shown (F) by rasters including left or right correct trials and the lower plot collects the occurred spikes in each temporal bin with the baseline activity to compare (mean in black and std in grey).

This peak of activity coincides with the moment in which the subject should decide the correct nose-poke to obtain reward. In Figure 43F one can see how the activity of the neuron increases more for one of the sides while for the other remains around the basal activity after the recovery from the trough. This neuron is an example of an ON response, an increase in the neuron's activity can be observed and it is far from the basal activity and its standard deviation (black line and grey traces of Figure 43F). At this point the questions that arise are: Is this differential pattern due to the movement of the robot? Or, instead of that, is it due to the pass of the subject across a firing field? In Figure 43E the firing fields of the cell can be observed, both the rat and the robot distributions of spikes normalised per time show a peak of firing rate localised in the right part. The distributions of positions tend to overlap in our protocol because when the subject performs well the trial follows the direction of movement of the robot and, therefore, it is difficult to conclude what is the cause of the found peak, either the encoding of the robot's movement or the pass across a putative PF. In order to solve this question the firing fields were again constructed but this time separating the positions of the subject in three equal stretches: left, centre and right (Figure 44). With this approach the spatial specificity of the neuron for a robot's position can be assessed independently of the subject's location. Effectively, for this case neuron, the peak of activity remains in the same position observed in the global firing field of Figure 43F. Only the left stretch shows a displacement of the maximal activity into the other part of the robot's distribution but the firing rate is largely lower.

As we can see, the disentangling of the neuron's activity in relation to the spatial and dynamic properties of the rat and the robot needs in many cases a deep examination of the firing patterns in the spatial and temporal domains neuron by neuron. More figures (Figure 46 and Figure 47) are shown in order to give examples of the whole behaviour found for some of the isolated neurons. Now, some considerations about the quantifications used during the analysis will be shown for the case neuron presented in Figure 43. The purpose of that is to give a more clear explanation of the parameters used in the final statistics with the presentation of single cell cases. In the spatial analysis, the final parameters used in order to determine if the neuron has a spatial specificity of its firing were the Skaggs Index (SI) and the Positional Information (PI). For the neuron just exposed in previous Figure 43, the obtained values were: Skaggs Index 3.7662 bits/spike (b/sp), Positional information 0.0137 b/sp for the rat and Skaggs

Index -0.0975 b/sp, Positional information 0.0092 b/sp for the robot. These values were compared against the shuffled distributions and gave values of significance equal to p=0.014 SI, p=0.024 Positional information for the rat distributions and p=0.92 SI, p=0.01 Positional information for the robot distributions. Therefore this neuron is spatially specific both for the rat and the robot according to Positional information, what we called an "ambiguous cell". Moving to the temporal analysis the results indicated an ON response and significant differences for the MI comparison of static/dynamic states. Surprisingly the MI left/right was not significant may be due to the fact that only the 1.5 sec after the trigger was considered for this quantification and looking to the Figure 43F the activity of the cell at this point did not reach its maximum for the ON response side.

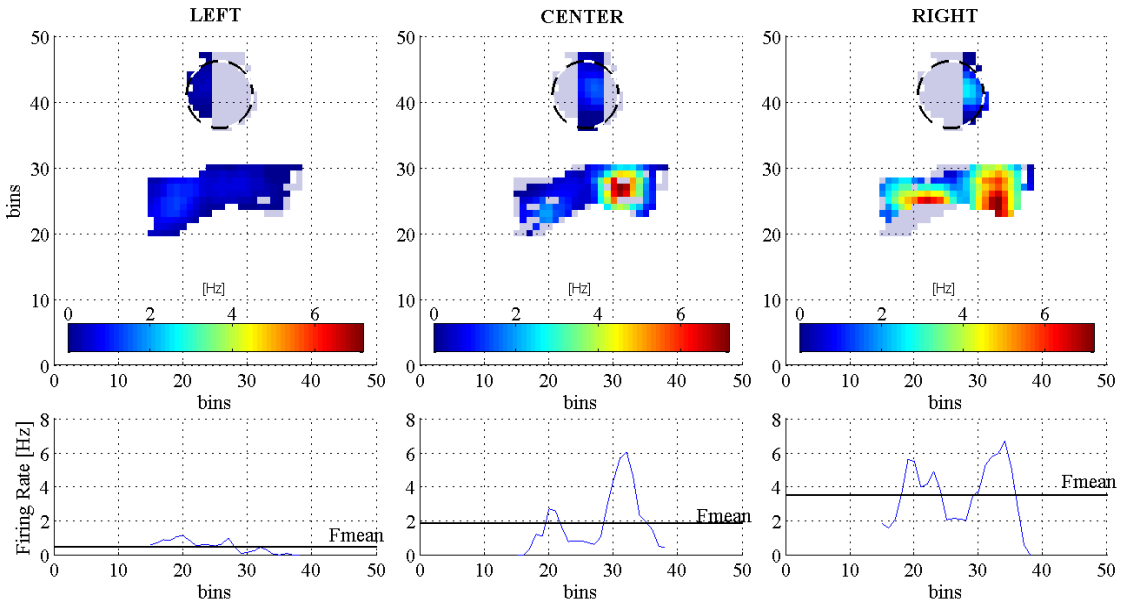

Figure 44: Left, center and right stretches.
Firing maps of the case neuron 0406argc3 for stretched distributions of the subject position. Just the positions of the subject inside each stretch (left, center and right) were considered while the robot distributions derived of this separation comprise the entire path. The projection of the FF for the robot distribution is presented below each map, FR blue line and Fmean in black.

Once the global activity of the neuron was assessed by the analysis explained, done in the spatial and temporal domains, for the case neurons where the results point to their firing rates being correlated to both the rat and the robot deeper observations of their behaviour were performed. One of these strategies consisted in the separation of the firing fields in stretches. In Figure 44 the spatial activity of the neuron presented before, 0406argc3 in Figure 43, was separated in function of the position of the subject. Three firing fields are presented: one for the left positions of the subject, another for the

central ones and finally for the right region of the cylinder. The stretches are selected in function of the subject's path, just dividing in three the x coordinate of positions. Turning to the robot's map, the activity of the cell in relation to its positions is observed and in this case the peak of firing rate is preserved for the central and right positions of the subject. The observation is qualitative and no further quantifications were performed, however this analysis serves as a good method to discern if the firing pattern is more related to the rat or the robot for the ambiguous neurons (spatial specificity for both items according to Skaggs Index or Positional information).

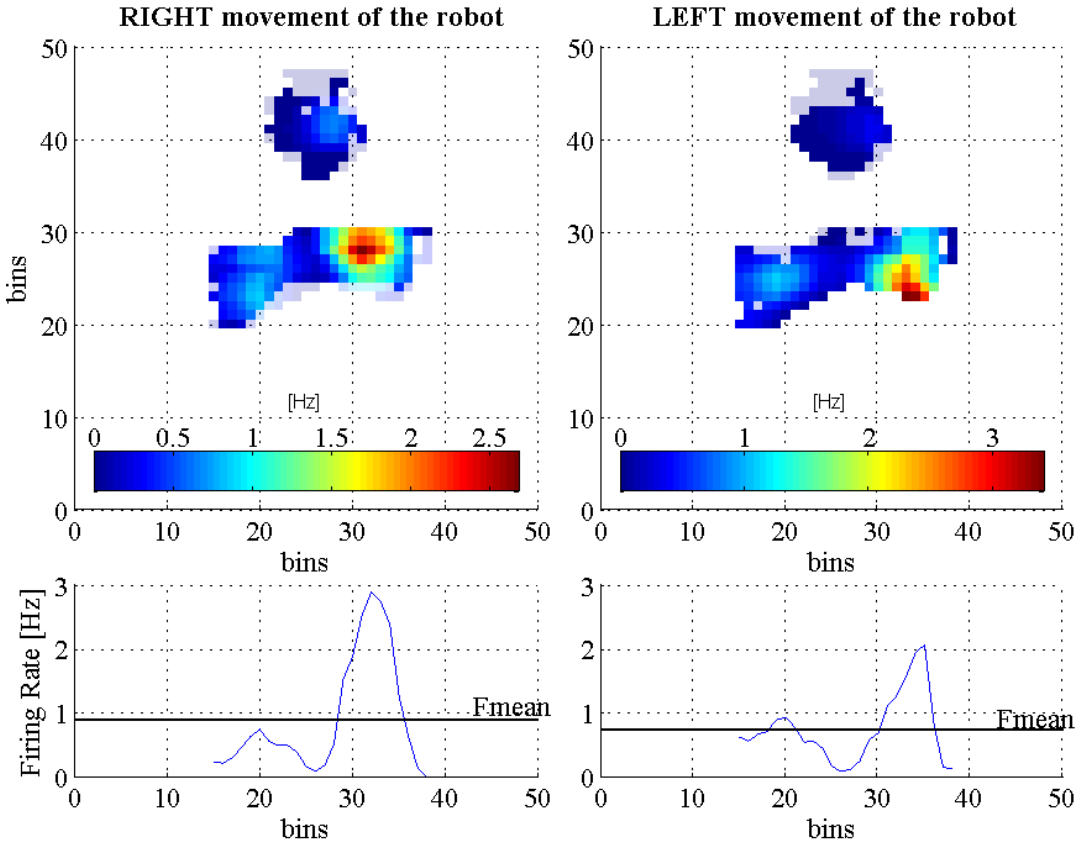

Figure 45: Unidirectional firing fields.
According to the sense of the robot's movement, the spatial activity of the case neuron 0406argc3 was examined. On the left rightwards periods of cue's displacement and on the right the leftwards periods are represented. The projection of the robot's distribution is presented below the maps, FR blue line and Fmean in black.

Another examination dissecting the prior results was done for the neurons responding to specific locations of the robot. In the literature of PCs is well known the fact that several PCs, and mostly when recorded in linear tracks, have FFs that are unidirectional (McNaughton *et al.*, 1983; Markus *et al.*, 1995; Gothard *et al.*, 1996). Because the robot

movements appeared almost linear to the subject the spiking of neurons with significant spatial parameters to the robot were analysed separating the activity of the cell in function of the movement of the robot. In Figure 45 there are two resulting maps, on the left the FF of the neuron during rightwards movements of the cue and on the right the opposite displacements. Once again the first case neuron used here as example is the one selected, 0406argc3 in Figure 43, and the results show a consistent firing of the cell independent of the direction of the cue's movement, and therefore denying the hypothesis of an unidirectional FF for the robot.

Summarising, this case neuron showed spatial specificity for both items, an ambiguous cell, which seems to be more locked to the robot's distribution when the positions of the rat are stretched. Across the trial the activity of the cell showed a silencing part just before the onset of the stimuli and when it starts to move the activity of the cell peaks a maximum only for rightwards displacements of the dynamic cue, ON response, while for the leftwards it recuperates the basal activity. The MI analysis showed no differences between the left and right responses although it was significantly different the firing of the neuron in epochs of robot's rest versus moving ones. Comparing the FFs during leftwards and rightwards displacements of the cue not differences were found.

Following other example neurons will be presented as the previous case (Figure 46 and Figure 47). The neurons selected are taken from different recordings and there were cases in which either a spatial or temporal organisation of their firing was detected.

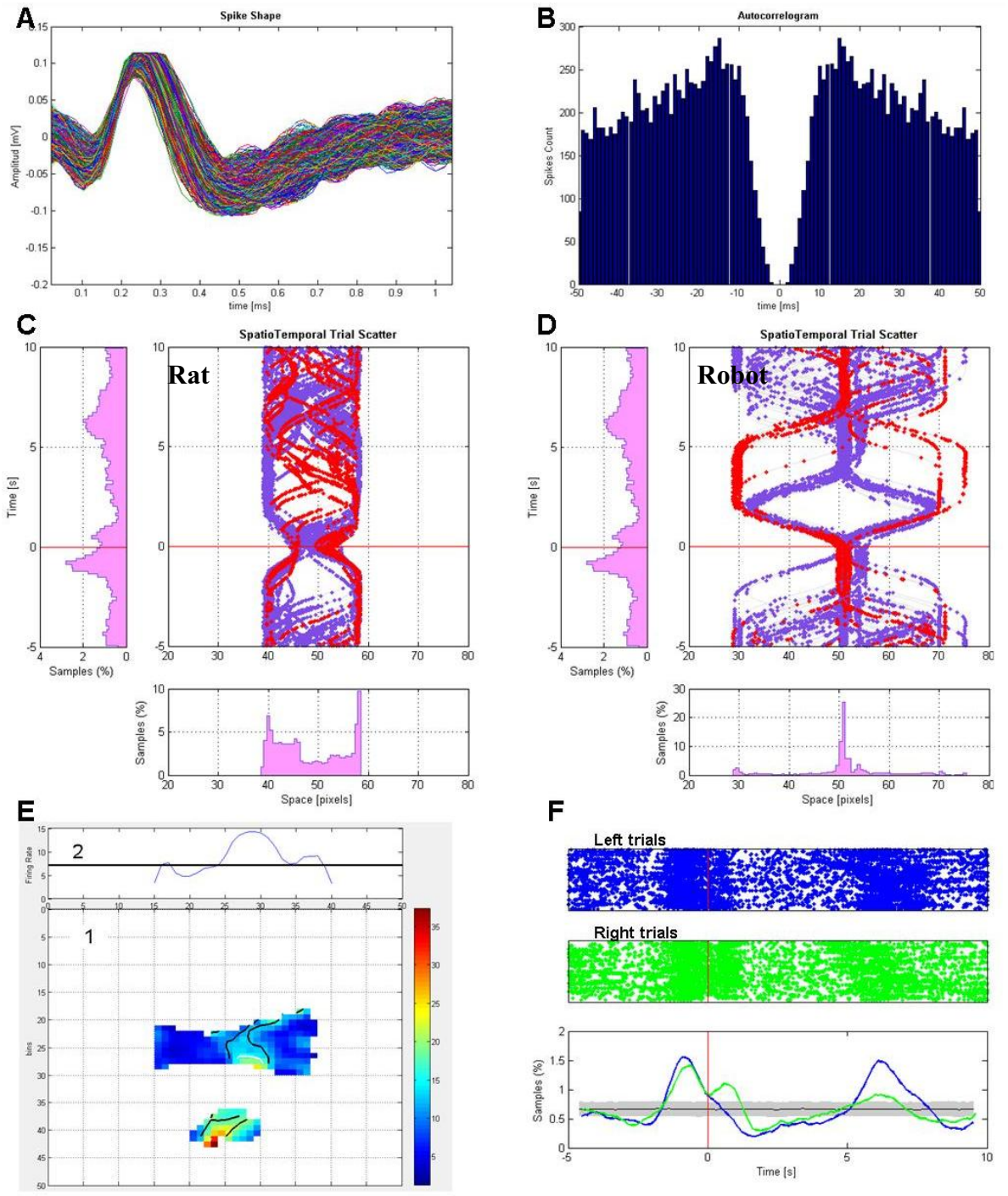

Figure 46: Cellular and spatiotemporal characteristics for a single neuron (II).
The panel offers a global view of the firing patterns of the neuron in relation to several parameters. First, the isolated spikes of the neuron (A) and the autocorrelogram derived from them (B) are shown. Then, the different spikes are distributed in relation to space and time coordinates for the rat (C) and the robot (D). The spikes occurred during error trials are plotted in red while the rest in purple. The coordinates refer to the axis of movement for space and to a trial alignment in time (0 time corresponds to the onset of the trial). The firing fields of the neuron are presented for rat's and robot's positions (E1), above the projection of the robot's distribution in one dimension (E2). Finally, the activity of the neuron in relation to the task-s phase is shown (F) by rasters including left or right correct trials and the lower plot collects the occurred spikes in each temporal bin with the baseline activity to compare (mean in black and std in grey).

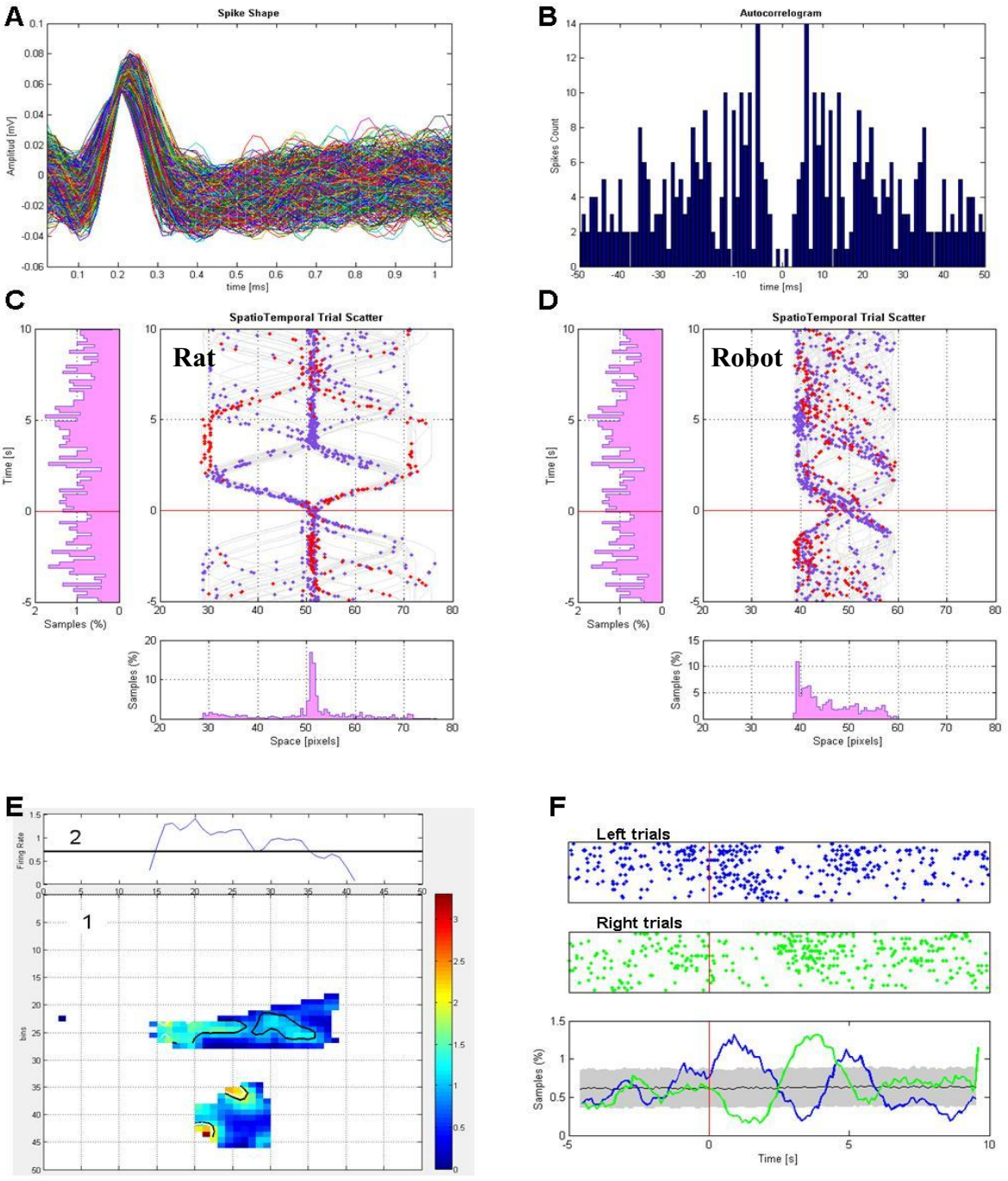

Figure 47: Cellular and spatiotemporal characteristics for a single neuron (III).
The panel offers a global view of the firing patterns of the neuron in relation to several parameters. First, the isolated spikes of the neuron (A) and the autocorrelogram derived from them (B) are shown. Then, the different spikes are distributed in relation to space and time coordinates for the rat (C) and the robot (D). The spikes occurred during error trials are plotted in red while the rest in purple. The coordinates refer to the axis of movement for space and to a trial alignment in time (0 time corresponds to the onset of the trial). The firing fields of the neuron are presented for rat's and robot's positions (E1), above the projection of the robot's distribution in one dimension (E2). Finally, the activity of the neuron in relation to the task-s phase is shown (F) by rasters including left or right correct trials and the lower plot collects the occurred spikes in each temporal bin with the baseline activity to compare (mean in black and std in grey).

5. DISCUSSION

5.1. Behaviour

The use of an operant discrimination task in the present study is a new experimental design that has demonstrated the capability of rats to discern between the movement's parameters of a dynamic cue in a real environment. Previous work on the field has differed substantially from our design as will be discussed next.

The Robot as dynamic cue

At first glance one can point directly to a second conspecific as the ideal dynamic cue, being naturally tracked by instinct. However a protocol in which the subjects should learn to identify the positions of a conspecific entails a number of complications. The innate tendency of conspecifics to interact promotes a social behaviour that will interfere in the accomplishment of the desired behaviour, both the physical separation between the items realised by the cylinder and the robot's use help in this sense. Already previous studies have demonstrated that spatial tasks where the subjects need to track the position of conspecifics, instead of an object, result in a slower learning process of the task and also to a final lower level of performance once it is stable (Svoboda, 2005; Telensky *et al.*, 2009). Those studies required the subject to maintain a distance (25 cms) from a moving item in order to avoid the delivery of a mild electric foot shock; in one case the item was a robot and in the other a conspecific. The results showed that in the case of a conspecific the task became harder because of the tendency to make social approaches. Thus, the use of a robot as the dynamic cue has two main advantages: first, the movements performed by the robot can be precisely controlled and, second, it avoids social interactions that could affect the desired behaviour at the end of the learning process.

The visual system of rats has been studied poorly in comparison to the vast literature found for cats or monkeys. From the works in rats where the training required the visual tracking of moving objects there were previous discriminative tasks done in non-realistic environments, using for example a computer screen as the source of the sensorial percept (Nekovarova and Klement, 2005). This approach approximates the task into a more simple visual discrimination protocol and the effects of the own movement were not assessed. Rather than visual elements in the 2D of a screen, in this study we use a 3D real world environment in which the robot moves, which we thing improves the ecological validity of our findings with respect to a visual task. Because the hippocampus is an associative area where the information relative to the different senses is integrated, it seemed better to evaluate our hypothesis in a protocol including 3D realistic movements and not just in a screen where the movements of the cues are simplified in a 2D view. The processing of the information derived from a screen could be solved by the subjects within the visual cortices without the involvement of the hippocampus. Instead, the OPDT asks to the subjects to discriminate the movements of a robot in a separated but contiguous space where the information received from the visual system could be processed as spatial information and therefore passing, as in Poincare's words (Poincaré 1913), from the *visual field* to the *representative space*.

Multisensorial task

The OPDT is a task where the subjects could guide their behaviour by the different senses and by the features of the dynamic cue. This, on one hand, is useful as a method by which the subjects can learn easily the demanded behaviour but, on the other hand, also makes more difficult to identify the specific aspects involved in such behaviour. The election of a task with these characteristics was motivated by several reasons. First of all the brain area subject of study, the hippocampus, is an associational area known because the integration of information coming from the different senses as touch, olfaction (Save *et al.*, 1998; Ginther *et al.*, 2011) and, the most studied one, vision (O'Keefe and Conway, 1978; Renaudineau *et al.*, 2007) occurs. The task used in our approach could be considered multisensorial given that not only sight but also other senses can guide the behaviour of the subject in the discrimination of the robot's movement (touch by the vibrations transmitted in the apparatus floor and hearing by the

sounds emitted during the displacement). This was considered an advantage because rats are considered animals with a relatively poor visual system and therefore the combination of other senses can promote a better execution of the task. Because the subject is separated from the robot by the cylinder, when a trial starts some noise and vibrations are produced by the robot's movement and therefore grabbing the attention of the subject to it.

Use of a discrimination task

It is also important to stress out that our protocol differs from previous behavioural paradigms in the use of the operant behaviour for the execution of the trials. Few previous studies have assessed how the brain carries out the encoding of the position of other moving objects in the brain. Those studies placed the dynamic cue in the same space than the rat, in one of the cases the cue was a second subject (Zynyuk *et al.*, 2012) and in the other a toy car (Ho *et al.*, 2008). The behavioural paradigm was based in the subject's foraging of an open field shared with the moving object during the electrophysiological recording. The presence of the dynamic cue in the same environment allowed the analysis of the hippocampal activity in relation to its movement. These approaches gave some answers regarding how the presence of the other moving object is affecting the activity of the principal cells in CA1 (results further discussed in the section 5.3 Single units), however, they lacked a fine temporal control of the periods during which the subject is actively processing this information. Our method represents an advantage because the movements of the dynamic cue were determined by the subject's behaviour, thus having a precise time stamp after which the subject should decide what kind of movement the dynamic cue is performing. Because of that, the posterior analysis of the cells' activity could be assessed in two different ways: the classical spatial analysis, which answers how the activity of the isolated cells are related to the positions of the dynamic cue, and a temporal analysis, directed to the determination of the hippocampal activity involved during the periods of discrimination, as reflected by the tracking and localisation of dynamic cues.

A physical separation between the subject and the robot was chosen in our protocol. Such separation of the subject from the robot does not allow interactions between them

and consequently the learning process is not affected by the possible approaches of the subject to the robot. These interactions can produce an overlap in the positions of the items that will further interfere in the analysis of the spikes, due putatively either to the movement parameters of the rat or the robot. Preliminary studies performed in our laboratory, data not shown in this dissertation, and other studies (Ho *et al.*, 2008) did not found place cells that were also activated when the moving object passed through their receptive fields. These experiments were one of the principal reasons which led us to separate the items during the protocol. Our efforts were concentrated in the development of a task that could accurately individuate the periods in which the subjects process the movement parameters of the robot.

Summarising, the OPDT is a behavioural protocol where rats can be trained to track the movements of a dynamic cue. The training period is relatively fast, one week of training produced acceptable performances scores (over 75% of correct choices). The fact that the task separated physically the subject and the cue, allegedly improves the learning process given that isolates the position from the direct interaction. An important innovation in the study of dynamic cues' tracking posed by our protocol, the OPDT, is the use of a precise trigger for the subject's behaviour. None of the previous studies trying to identify the hippocampal basis of moving objects (Ho *et al.*, 2008; Zynyuk *et al.*, 2012) used a discrimination task. These studies arrived to certain conclusions across the spatial analysis of the cells' activity while they lacked the fine temporal analysis allowed by our protocol that will be discussed in the discussion section 5.3. Single units.

5.2. Oscillations

As described in the results we found a significant decrease in the theta band during the movement of the dynamic cue. Furthermore, this suppression was observed in a behavioural phase of the task where the theta band power was expected to be high because the subject was actively moving. In Figure 33 the relationship between the subject's speed and the power of theta have been shown to be linear but if we took the period in which the rat and the robot are moving simultaneously this relationship is suppressed. We have not a final cause for this effect but some results of prior studies could shed more light into the question and they are discussed in the following sections.

External objects effects on theta modulation

In the literature the theta band has been related to many and different behaviours as attention, locomotor activity, voluntary movements, etc. (Buzsáki, 2002; Buzsáki, 2005). The results of our analysis showed a significant difference across the power of the theta oscillation during three different epochs of the task. Such epochs were separated in time periods selected by the maximum velocity of the items. The obtained periods were characterised by the velocity of the items as follows: rat moving, rat & robot moving and robot moving. As expected, the maximum power was found when the subject was moving in agreement with prior literature on the matter (Vanderwolf, 1969; Buzsáki, 2005). However, during the period when the robot moves synchronously with the subject the theta power was found to be significantly lower, actually marking a trough in the power within the trial. This result was consistent across sessions and subjects and therefore discarding artefacts of the analysis or of an inhomogeneous behaviour as the source of the cited suppression.

Few previous studies have shown relationships of the theta power to movements that are external to the subject. One interesting case is the paper of Terrazas and colleagues (Terrazas *et al.*, 2005), in which the objective was to detect if motor and propioceptive information are critical for the generation of the cognitive maps and to the natural activity of the place cells. To do that, the experimental design consisted in three

different conditions: first, a free exploration of an open arena, second, the same environment but rat was displaced across it by a moving platform and, third, the arena was rotated and the subject was fixed in the platform. The activity of single cells and the LFP was extracted from the recordings and the conclusions of the work were that both the place cells and the LFP still maintained their natural activity but were less consistent when the movement was artificial. According to the results, the hippocampal system can drive its spatial function without the own movement of the subject involved in the foraging and therefore being guided just by the environmental cues. Overall, the relationship between the power of theta and the movement of the platform was significantly reduced in comparison to the movement of the subject in a free exploration task even if still present.

Comparing these results to ours some differences emerge: the suppression of theta due to the dynamic cue' movements elicits the putative existence of a cross-related mechanism by which the hippocampus computes, or at least receives, not only the speed of the subject but also other dynamic information from the environment. The experiment of Terrazas also pointed to the same mechanism but in this case the conclusion was more relative to the integration of sensory information into the navigation system, some how the optic flow (being it considered as the variant part of the visual field changing during the subject's own movement) is enough to cause the activation of the characteristic oscillation of the hippocampus, the theta band. How does the brain differentiate between the movement of the whole visual field or just part of it remains an unsolved question. Our data suggest that the hippocampus is involved either in directly processing the sensory data or in receiving the necessary input to distinguish it. At the beginning of this text, in the philosophical introduction, we mentioned authors supporting their theories of how the concept of space from the mind emerge precisely in the ability of the brain to distinguish the invariant part of the visual perception from moving sections of it (Poincare 1913, Piaget 1955). Actually in the case of Piaget he argued that not only the notion of space needs this ability to be well developed but also the identification of objects itself is anchored to it.

The relation of theta with environmental changes has been demonstrated in another recent study (Jeewajee *et al.*, 2008). Subjects were exposed to an environment during several days while the hippocampal LFP was recorded, then, the environment was

changed and the results showed a reduction of the theta frequency due to novelty. If we go back to our results and comparing them to these ones, we can see that the reduction in theta power coincides with the moment when the environmental cues are changing. The work of Jeewajee and colleagues demonstrated a global function of theta in the representation of a new environment while our results suggest a lower temporal scale in such modulation. In a wider view the theta oscillation is considered to be a mediator of the information flow across the hippocampal formation (Lisman, 2005) and therefore the observed modulations due either to the movement of a dynamic cue or to the foraging in a novel environment could be physiological correlates of an updating function of the spatial and dynamical features of the objects present in it. For familiar environments with static cues the brain representation of space is stable while the exploration of a novel environment or the presence of a moving object should elicit other activity in the hippocampal circuitry reflected by the changes in theta modulation found in our and prior works (Jeewajee *et al.*, 2008).

Another very recent paper has demonstrated a reduction of the theta power in rats during the visual discrimination of an object-in-place paradigm (Furtak *et al.*, 2011). The brain area of study in this work was the postrhinal cortex and the results pointed to a spatial function of it because both the theta band and the activity of single units showed to be related to the discrimination of static cues in different contexts. The spatial function of postrhinal cortex was not a new evidence because prior studies have shown that its cells have a slight spatial tuning (Fyhn *et al.*, 2004) and the postrhinal cortex is part of the dorsal route which is described as a flow of visual information relative to spatial characteristics of objects, the "where" route (Knierim, 2006). Instead, the suppression of the theta band was an unexpected result that in this case is not relative to the movement of the cues, as in our work, but rather to the recognition of the place in which an object is placed.

Theta modulation as a neural correlate of updating functions

Taking together the results of the different works just cited there is a large evidence that the theta band is related to updating functions of spatial information as it was already proposed in the past (Mitchell and Ranck Jr, 1980). Such updating is reflected by the

increase or decrease of its power and this in turn reflects the synchronization of neural populations within or across brain regions. The literature presented in this section has found this modulation of theta to be either related to the exposure of a novel environment (Jeewajee *et al.*, 2008) or during the discrimination of an object in a certain place of the environment (Furtak *et al.*, 2011). Another evidence of such updating is the recent published work by Jezek and colleagues where the firing of neural ensembles of the CA3 region of the hippocampus has been shown to need a time range equal a theta cycle in order to change the brain representation of space between two spaces (Jezek *et al.*, 2011). The experimental design of this work consisted in a maze where the environmental landmarks were projected into the floor and walls. This way the rats were first trained to forage the maze with two different patterns of projected cues and once the activity of the units were shown to be stable in each configuration, the experimenters tested the rats to forage the maze while the projections change during the same session. The cellular response to such manipulation showed the need of at least one theta cycle to change between one brain representation and the other, it means a global remapping of spatial specific responses of the neural ensembles recorded, and thus pointing to the theta period as a temporal unit of spatial representation in CA3.

Overall, the modulation found in the theta band due to the dynamic cue indicates a possible mechanism in the hippocampus mediating the processing of visual information relative to cue's movements. Our experimental design provides a configuration where the subject can move freely during the task. A different approach based on a total restriction of the subject's movement, by a head fixation for example, could better assess the implication of the hippocampus in the distinction, or more precisely in the representations, of the variant and invariant parts of the visual field. The visual cortices and their related areas are the first candidates to do that but, as argued before, the information coming from the dorsal and ventral visual streams only converge at the hippocampus (Knierim, 2006) and, in consequence, only there is available the information needed to compute both the spatial and the recognition characteristics of an object.

5.3. Single units

After the discovery of the place cells (O'Keefe and Dostrovsky, 1971) a large amount of work turned their focus of study on the understanding of the brain mechanisms underlying such function. Most of the studies proved how the environmental cues serve as landmarks to the brain in order to create the spatial specific patterns of firing characteristic of these cells (O'Keefe and Conway, 1978; Muller *et al.*, 1987; Gothard *et al.*, 1996). Manipulations of the cues have shown to produce changes in the spatial activity of the principal hippocampal cells. In these experiments there were found clear effects due to the environmental changes on the activity of the cells: either global remapping, meaning that the cells start to fire in a new location after the change in the environment (Leutgeb *et al.*, 2004), or rate remapping, that is when the activity of the cell maintains the location specific firing while the firing rate is affected by the manipulation (Leutgeb *et al.*, 2005). These results have been reproduced in several studies resulting in the general idea across the field that the generation of the spatial specific firing of the place cells is mostly due to their anchoring to the environmental landmarks. However, the effects of dynamic cues in the hippocampal activity is a matter of study hardly assessed until the recent publication of the two studies that I have discussed in the previous section (Ho *et al.*, 2008; Zynyuk *et al.*, 2012). Our work and those just mentioned have found an impact of the dynamic cues on the hippocampal cells' activity that will be discussed in this section.

In the present study the activity of CA1 neurons has shown different correlations with the movement parameters of the dynamic cue tracked by the subjects during the designed behavioural task, OPDT. We found correlations with the position of the item, not as the classical place fields generated for the subject's position but with slight preferences of firing for certain locations of the robot. The only data found in the previous literature that is available to compare our results are two works of different groups. The first of them is a case in which the activity of the principal cells of the hippocampus was evaluated during the foraging of a rat in an open arena shared with a toy car (Ho *et al.*, 2008). Subjects were trained to avoid the toy car during the task by the use of a mild electric foot shock when the distance between the rat and the car was less than 20 cms. Two main results were found in this study: on the one hand, the place

cells tended to remap more when the toy car was a conditioned stimulus and, on the other hand, several spatial parameters of the toy car influenced the activity of the cells (turning angle, direction and distance). The second study that has assessed the issue is a study where the effects on the hippocampal activity of the presence of a second subject, a conspecific, were analysed (Zynyuk *et al.*, 2012). The study found a slight modulation of the cells' firing in relation to the movement parameters of the conspecific and such modulation was distance dependent, in agreement to the results of the first study mentioned (Ho *et al.*, 2008). Therefore, both works arrived to the final conclusion that there is an influence of the presence of moving objects on the spiking of place cells. However they did not find a neural correlate linked exclusively to the dynamics of the items. There were not found firing fields associated to the position of the dynamic cue or cells firing for example to specific directions of its movements.

Our results on the spatial analysis share some conclusions with these two studies although instead of on the temporal analysis we found neurons with their activity directly related to some features of the dynamic cue. In our approach, the classification of neural individual responses in ON and OFF-like patterns during the movement of the robot indicates a real processing of the spatial information by CA1 neurons relative to it. The fact that individual neurons at the level of the hippocampus can account into their activity significant differences when the dynamic cue is doing certain actions points to a functional role of the hippocampus in the encoding of spatial information relative to moving objects. If we add to these results the fact that also the predominant band power was also found to be modulated by the dynamic cue, the conclusions led to see the hippocampus as an area not only involved in the processing of the own position and self-movements but also in a parallel processing of spatial characteristics of other moving objects. According to this conclusion, other studies have demonstrated the role of the hippocampus in behavioural protocols where after the animals learnt to discriminate between the positions of other objects its inactivation produced an impairment in the task execution (Pastalkova and Bures, 2001; Telensky *et al.*, 2011). Therefore, the amount of evidence now enlarged by this work, present the hippocampus as a brain core where the dynamics of external objects is integrated and processed.

Protocol with temporal trigger

Comparing our results with those of the previous works some interesting questions arise. First, it should be highlighted the experimental differences encountered between the OPDT's protocol and previous ones. The earlier work done recording CA1 activity during a task implying the subject's attention to a dynamic cue were not classical discrimination tasks as the OPDT. On the one hand, these works could answer how the spatial characteristics of the dynamic cue affect the activity of the hippocampal neurons but, on the other hand, the lack of an operant trigger for the execution of the subjects' discrimination made the temporal, or behavioural, analysis less precise in such works. In this sense the OPDT has the advantage that the movements of the robot are finely controlled and they depend on the subject's behaviour. In the presented task a trial only starts when the subject goes to the operant platform and waits for the onset of the movement. This way the animal pays attention to the dynamic cue and the moment when the movement of the dynamic cue is putatively processed by the hippocampus is known. Therefore, our data could be analysed not only in the spatial domain but also aligning the activity of the neurons to the moving stimuli as in a classical discrimination task. As referred in the results we found by this approach a relationship between the periods of movement and the activity of some of the isolated cells. We found ON and OFF responses during the trials towards one side or the other and also a different set of neurons with differential firing patterns for the periods in which the cue was moving or not. In comparison to the previous works these results showed an independent processing of the movement parameters of the robot by CA1 units.

Spatial specificity of CA1 neurons

In this thesis, the activity of the CA1 neurons was found to be modulated by the presence of a moving object in the environment. This modulation was reported to be distance-dependent in a prior work where a conspecific shared the open arena with the subject (Zynyuk *et al.*, 2012). In such study the place fields or the hippocampal neurons were found to loss coherence in their spatial distribution of the spikes in relation to the proximity of the second subject. Our experiment does not allow a conclusion in this sense; no data was collected in order to infer if the distance to the dynamic cue *per se*

produces a different level of firing modulation. In a second study that used a toy car as the dynamic cue, the spatial analysis of firing fields concluded that the PCs tend to remap more often when the toy car was a conditioned stimulus for the subject (Ho *et al.*, 2008). This conclusion is partial in the sense that it does not demonstrate if the dynamic state of the cue is differentiated from a static environmental cue which could serve to the cognitive map as an anchor for the representation of subject's position.

Thus, both mentioned works found a modulation of the spatial activity of hippocampal cells in relation to the external object but the conclusions could not be extended to a direct representation of moving cue's position. In comparison, our study has concluded that in CA1 there are not only neurons encoding information relative to the subject's position but also a different subset of neurons is conveying information about the positions of the dynamic cue. This result suggests that an important proportion of the hippocampal cells are differentially modulated by the position of the dynamic cue and not only the ones that convey information of the subject's position as it was suggested in prior works.

Spatial parameters of the dynamic cue

The fact that the hippocampus is encoding information about the position of moving objects has been proved with the present work but an important question remained open. What parameters of the moving objects are being encoded or are affecting the neural activity in this area. In an effort to unravel what parameters of the movement were the responsible of the found modulation, one of the works cited previously analysed them separately (Ho *et al.*, 2008). Their study pointed out not only to a significant spatial content of firing for the principal hippocampal cells in relation to the position of the dynamic cue, a toy car, but also to certain movement parameters of the cue as factors modulating the discharge of the neurons. The breadth of responsiveness of the isolated cells in relation to movement parameters of the moving cue showed to differ when the cue was a conditioned stimulus or not. From the parameters analysed in such study three of them appeared to be modulated by the car, there were: the turning angle, the distance and the direction of motion. Our approach allows only a similar evaluation for the direction (or more precisely the sense of motion) while the distance,

as previously referred, and the turning angle could not be subject of study with our experimental design.

The presented results in relation to the direction/sense of the robot's movement confirmed the prior evidence. We found an important fraction of the isolated hippocampal cells that fire differentially when the dynamic cue performed movements towards either one of the target positions or the other (ON/OFF responses, N=62). Our protocol imposes a high restriction in the movements of the robot; on the one hand, this fact causes a limitation on the analysis of the directions taken by the robot and therefore neurons with a preferred direction in relation to all the possible ones can not be found but, on the other hand, the initiation of the movement is more precise for the subject in the OPDT. Only two directions were analysed for our data but the duration of the movement is clearly determined and task related. Furthermore, our results not only have concluded that there is a significant amount of information per spike in relation to the direction of the movement but specific patterns of firing related to its dynamics were found. The cells' responses were quantified and categorised in increases or decreases of the activity: ON and OFF responses respectively named. Although to confirm and to compare our data, the mutual information (MI) of the cells during these epochs was calculated and analysed giving results that were according to the ON/OFF responses characterisation. Not effects of speed modulation were found in our data but the analysis of the periods in which the dynamic cue moves against the periods of rest led to the conclusion that several cells behaved differently in these conditions (N=30). Since this is a pioneer work on this area, the earlier mentioned studies can not be used as a framework to compare the obtained results. Other studies have used a dynamic cue continuously moving during the recordings and without a clear temporal trigger of its movement's onset and, thus, not allowing this kind of comparison. Previous strategies used a control protocol where the dynamic cue was absent or behaviourally irrelevant for the subject. In this sense the presented results shed new light on the interesting question concerning how a cue is encoded while it is static or dynamic.

There is a wide literature concerning the issue of how static environmental cues in the hippocampus are encoded but the same question concerning dynamic cues have been addressed only in the aforementioned studies. Our protocol fills the gap by the use of a task in which the dynamic cue is static during the most part of the protocol. The

discharge of a fraction of the isolated cells showed to fire differentially when the dynamic cue was resting or moving. Overall, the results obtained by the classification of neural firing during the movements of the robot by the direct examination of the firing rate and the mutual information suggest that the place cells are not only weakly modulated by the presence of a moving object but also that other cells encode or reflect by their firing some features of the movement as the direction or the static/dynamic state of a cue.

Objects and conspecifics induce different hippocampal responses

An important debate of our results was led by the article recently presented by von Heimendahl and colleagues in 2012. Their work approached the study of CA1 activity from a totally different hypothesis and with different goals than the previous studies and the present one (von Heimendahl *et al.*, 2012). This work tried to unravel a different question concerned about how social interactions in the hippocampus are encoded. Their aim was to look if there is a specific neural response for conspecifics in such area. They looked to the spatial distribution of spikes during a task in which the subject was exposed to the presence of conspecifics. The configuration consisted in a central platform where the subject of study was placed and two adjacent platforms where other subjects were presented to it. The use in such task of inanimate objects as control derived in unpredicted results. The response of part of the hippocampal cells was found, first, to be specific for locations of the subject as expected; second, part of those cells responded differently when a second rat was placed in such position; and third, the objects used as control showed too a specific response in another set of the recorded cells. Interestingly, the presence of a second subject or the object was found to change the activity of a set of the place cells by increasing or decreasing their firing rates, therefore producing a rate remapping of the cells' activity. This response, either the place-conspecific or the place-object, showed to be more affected in the case of objects than in the conspecifics one. The results obtained by this group have appeared as a good framework to compare the previous data and ours. Their conclusions in consequence partially solved the difference found when comparing the results of Ho and Zynyuk works (Ho *et al.*, 2008; Zynyuk *et al.*, 2012). While the work of Ho found global remapping to be more frequent in the car dependent task, the results of Zynyuk only

discovered a slight distant dependent rate remapping induced by the presence of conspecifics in the environment. The stronger modulation of the firing fields found by von Heimendahl, when objects instead of other subjects were placed in the apparatus, therefore could partially explain such difference. The prior evidence taken together suggest that the neurons of the hippocampus are encoding more information relative to objects than to conspecifics, that could explain why the protocols where a toy car was moving in the environment produced global remapping of the cells while the ones using conspecifics only found subtle modulations of the spatial properties of the cells trough rate remapping. It is also important to stress the fact that the protocol in which a conspecific was placed did not require a behavioural demand to the subjects in the task, being this another plausible explanation of the weaker modulation found for the hippocampal activity. Additionally the results of Ho's work concluded that the global remapping was significantly higher when the car was behaviourally relevant for the subjects. Therefore, after reviewing the prior literature, one can conclude that the brain representation of external moving objects is separated from the one of conspecifics and only subtle effects are found in the hippocampal area when a second subject is surrounding the first.

In our study, the found neural responses to dynamic characteristics of the robot were specific to some qualities of the stimuli, summarising: a modulation of the neural discharge depending on the position of the robot, ON/OFF responses during left or right towards movements and, finally, firing patterns dependence of static/dynamic states of the cue. Therefore, these results point to a role of the hippocampus in the processing of information relative to moving objects. A similar protocol in which the movement parameters of a conspecific should be discriminated could answer if in this case they will be also discovered the different responses between conspecifics and objects found in von Heimendhal's work. Our protocol showed cells with specific response only to the robot's dynamics and thus differing from the prior results. One important question in this sense is if the specific responses found to the robot are due to the use of a discrimination task, thus being them task related, or just the fact to have a precise temporal trigger in the discrimination allowed the detection of such responses.

Another important aspect concerns the use of a separated space between the subject and the dynamic cue in our protocol. The responses were found in a task where the dynamic

cue was physically separated from the subject, and thus implying that the modulation of the hippocampal cells can not be explained just as an effect of its processing like an environmental landmark, one of the reasons argued to explain the stronger modulation noticed by objects in the aforementioned works. During the OPDT the subject is enclosed in a cylinder where the robot could not serve as a proximal cue to infer its own position. Earlier studies have demonstrated that when an environmental cue is related to the task it is more probable to find PCs in its surroundings (Burke *et al.*, 2011), a fact which per se could explain the global and rate remappings found in the works that used dynamic cues behaviourally relevant to the subject. These questions are therefore still open, how differentiates the brain between moving and static cues? Is it possible that both qualities are not totally separated in its processing and therefore dynamic cues' responses are in some sense categorised as static cues occupying different positions?

Perspectives on the field

Our approach to the brain mechanisms underlying the tracking and localization of dynamic cues has demonstrated the involvement of the CA1 area of the hippocampus in this capability. Both the LFP of the area in the range of theta and the activity of the single neurons were found to be modulated by the spatial parameters of the dynamic cue. In the case of the cells, the dynamics of the robot were analysed taking into account its different spatial variables per separate. The position, the sense of the movement and the dynamic state (static periods against those in which the robot was moving) are reflected by the activity of the isolated cells. However, the presented results in conjunction with prior studies (Ho *et al.*, 2008; Zynyuk *et al.*, 2012) not only describe neural correlates of the dynamics of an external object in the hippocampus but the extent of the modulation found suggests an important role of this area in the representation of the localization and the movements of such objects. Future works should try to disentangle with more precision how each of the spatial parameters of the external objects are represented by the hippocampal neurons and how they interact with the location of the self. Feasible experiments could use head-fixation approaches to avoid the own movement of the subjects as one of the variables affecting the activity of the area. Also, it will be important to study the adjacent and interconnected brain regions to the hippocampus in order to elucidate how the information flow needed

arrives to the hippocampus and see if there is a prior integration relative to the dynamics of external objects. Candidate areas are the entorhinal and perirhinal cortices, both of which send inputs to the hippocampus, direct or indirectly, and they have been behaviourally related in tasks where subjects need to recognise conjunctively the nature and the place of an object to perform correctly the task (Deshmukh *et al.*, 2012), usually call "what and where" or item-in-place paradigms. This way the activity of the hippocampal cells could be explained, in the sense that how the necessary inputs coming from the visuo-spatial streams sustain the modulation found.

To conclude, the present work found that the hippocampus is actively involved in the processing of information relative to the dynamic of an external object, in this case a robot. However the mechanisms underlying such function remain to be explored and new studies should unravel the way in which this information is processed. The evidence supporting the role of the hippocampus in the representation of external objects, being them static or dynamic, is increasing and, even more important, now are known thanks to this and other works the features of the cues' dynamics which are being processed in such area.

CONCLUSIONS

1. Rats can learn to discriminate the dynamics of a robot in an operant task reaching stable and high levels of performance in less than two weeks.

2. The predominant oscillation of CA1, theta band between 4 and 12 Hz, was significantly suppressed during the movement of the dynamic cue.

3. The activity of the isolated neurons in the pyramidal layer of CA1 during the discrimination task for detection of the robot's position showed significant spatially-specific responses as well as responses for both the rat's and robot's positions.

4. Mutual Information in the neuronal discharges revealed significant encoding of the robot's position.

5. The same procedure (MI) applied to periods when the robot was static or dynamic revealed another set of neurons whose activity was modulated by the dynamic condition.

6. The observed modulation of the hippocampal activity, either the suppression of the theta band power during the movements of the cue or the single neurons firing patterns behaving differently depending on the position and motion of the cue, revealed a processing of the spatial properties of the cue within the hippocampus.

7. The neural correlates that we describe here suggest that there is a specific subset of the hippocampal cells encoding and processing information relative to the dynamic cue.

REFERENCES

Aguirre, G. K., J. A. Detre, D. C. Alsop and M. D'Esposito (1996). "The parahippocampus subserves topographical learning in man." Cereb Cortex **6**(6): 823-9.

Andersen, P., T. V. Bliss, T. Lomo, L. I. Olsen and K. K. Skrede (1969). "Lamellar organization of hippocampal excitatory pathways." Acta Physiol Scand **76**(1): 4A-5A.

Andersen, P., R. G. Morris, D. G. Amaral, T. V. Bliss and J. O'Keefe (2007). The Hippocampus Book, Oxford University Press.

Bingman, V. P. and G. Yates (1992). "Hippocampal lesions impair navigational learning in experienced homing pigeons." Behav Neurosci **106**(1): 229-32.

Burgess, N. and J. O'Keefe (2011). "Models of place and grid cell firing and theta rhythmicity." Current Opinion in Neurobiology **21**(5): 734-744.

Burke, S. N., A. P. Maurer, S. Nematollahi, A. R. Uprety, J. L. Wallace and C. A. Barnes (2011). "The influence of objects on place field expression and size in distal hippocampal CA1." Hippocampus **21**(7): 783-801.

Burwell, R. D. (2000). "The Parahippocampal Region: Corticocortical Connectivity." Annals of the New York Academy of Sciences **911**(1): 25-42.

Buzsáki, G. (2002). "Theta Oscillations in the Hippocampus." Neuron **33**(3): 325-340.

Buzsáki, G. (2005). "Theta rhythm of navigation: Link between path integration and landmark navigation, episodic and semantic memory." Hippocampus **15**(7): 827-840.

Caplan, J. B., J. R. Madsen, A. Schulze-Bonhage, R. Aschenbrenner-Scheibe, E. L. Newman and M. J. Kahana (2003). "Human Theta Oscillations Related to Sensorimotor Integration and Spatial Learning." The Journal of Neuroscience **23**(11): 4726-4736.

Cimadevilla, J. M., A. A. Fenton and J. Bures (2001). "New spatial cognition tests for mice: Passive place avoidance on stable and active place avoidance on rotating arenas." Brain Research Bulletin **54**(5): 559-563.

Csicsvari, J., H. Hirase, A. Czurko, A. Mamiya and G. Buzsaki (1999). "Oscillatory coupling of hippocampal pyramidal cells and interneurons in the behaving Rat." J Neurosci **19**(1): 274-87.

Dashiell, J. F. and H. A. Helms (1925). "The learning by rats of an inclined plane maze." J. comp. Psychol. **5**: 397-405.

Deshmukh, S. S., J. L. Johnson and J. J. Knierim (2012). "Perirhinal cortex represents nonspatial, but not spatial, information in rats foraging in the presence of objects: Comparison with lateral entorhinal cortex." Hippocampus **22**(10): 2045-2058.

Dragoi, G. and S. Tonegawa (2010). "Preplay of future place cell

sequences by hippocampal cellular assemblies." Nature **469**(7330): 397-401.

Eichenbaum, H. and N. J. Fortin (2005). "Bridging the gap between brain and behavior: Cognitive and neural mechanisms of episodic memory." Journal of the Experimental Analysis of Behavior **84**: 619-629.

Ekstrom, A. D., M. J. Kahana, J. B. Caplan, T. A. Fields, E. A. Isham, E. L. Newman and I. Fried (2003). "Cellular networks underlying human spatial navigation." Nature **425**(6954): 184-8.

Foster, D. J., R. G. Morris and P. Dayan (2000). "A model of hippocampally dependent navigation, using the temporal difference learning rule." Hippocampus **10**(1): 1-16.

Foster, D. J. and M. A. Wilson (2006). "Reverse replay of behavioural sequences in hippocampal place cells during the awake state." Nature **440**(7084): 680-683.

Foster, T. C., E. P. Christian, R. E. Hampson, K. A. Campbell and S. A. Deadwyler (1987). "Sequential dependencies regulate sensory evoked responses of single units in the rat hippocampus." Brain Res **408**(1-2): 86-96.

Foster, T. C., R. E. Hampson, M. O. West and S. A. Deadwyler (1988). "Control of sensory activation of granule cells in the fascia dentata by extrinsic afferents: septal and entorhinal inputs." J Neurosci **8**(10): 3869-78.

Freund, T. F. and G. Buzsáki (1996). "Interneurons of the hippocampus." Hippocampus **6**(4): 347-470.

Furtak, Sharon C., Omar J. Ahmed and Rebecca D. Burwell (2011). "Single Neuron Activity and Theta Modulation in Postrhinal Cortex during Visual Object Discrimination." Neuron **76**(5): 976-988.

Fyhn, M., S. Molden, M. P. Witter, E. I. Moser and M. B. Moser (2004). "Spatial representation in the entorhinal cortex." Science **305**(5688): 1258-64.

Gaffan, D. (1994). "Dissociated effects of perirhinal cortex ablation, fornix transection and amygdalectomy: evidence for multiple memory systems in the primate temporal lobe." Exp Brain Res **99**(3): 411-22.

Geisler, C., K. Diba, E. Pastalkova, K. Mizuseki, S. Royer and G. r. BuzsÃ¡ki (2010). "Temporal delays among place cells determine the frequency of population theta oscillations in the hippocampus." Proceedings of the National Academy of Sciences **107**(17): 7957-7962.

Ginther, M. R., D. F. Walsh and S. J. Ramus (2011). "Hippocampal Neurons Encode Different Episodes in an Overlapping Sequence of Odors Task." The Journal of Neuroscience **31**(7): 2706-2711.

Gladwin, T. (1970). East is a big bird. Cambridge, Mass, Harvard University Press.

Gothard, K. M., W. E. Skaggs, K. M. Moore and B. L. McNaughton (1996). "Binding of hippocampal CA1 neural activity to multiple reference frames in a landmark-based navigation task." J Neurosci **16**(2): 823-35.

Hafting, T., M. Fyhn, S. Molden, M. B. Moser and E. I. Moser (2005). "Microstructure of a spatial map in the entorhinal cortex." Nature.

Hargreaves, E. L., G. Rao, I. Lee and J. J. Knierim (2005). "Major

dissociation between medial and lateral entorhinal input to dorsal hippocampus." Science **308**(5729): 1792-4.

Harvey, C. D., F. Collman, D. A. Dombeck and D. W. Tank (2009). "Intracellular dynamics of hippocampal place cells during virtual navigation." Nature **461**(7266): 941-946.

Hasselmo, M. E. (2005). "What is the function of hippocampal theta rhythm?—Linking behavioral data to phasic properties of field potential and unit recording data." Hippocampus **15**(7): 936-949.

Henze, D. A., Z. Borhegyi, J. Csicsvari, A. Mamiya, K. D. Harris and G. Buzsaki (2000). "Intracellular features predicted by extracellular recordings in the hippocampus in vivo." J Neurophysiol **84**(1): 390-400.

Ho, S. A., E. Hori, T. Kobayashi, K. Umeno, A. H. Tran, T. Ono and H. Nishijo (2008). "Hippocampal place cell activity during chasing of a moving object associated with reward in rats." Neuroscience **157**(1): 254-70.

Jacobs, J., M. J. Kahana, A. D. Ekstrom, M. V. Mollison and I. Fried (2010). "A sense of direction in human entorhinal cortex " Proceedings of the National Academy of Sciences **107**(14): 6487-6492

Jadhav, S. P., C. Kemere, P. W. German and L. M. Frank (2012). "Awake Hippocampal Sharp-Wave Ripples Support Spatial Memory." Science **336**(6087): 1454-1458.

Jeewajee, A., C. Lever, S. Burton, J. O'Keefe and N. Burgess (2008). "Environmental novelty is signaled by reduction of the hippocampal theta frequency." Hippocampus **18**(4): 340-348.

Jensen, O. and J. E. Lisman (2000). "Position reconstruction from an ensemble of hippocampal place cells: contribution of theta phase coding." J Neurophysiol **83**(5): 2602-9.

Jezek, K., E. J. Henriksen, A. Treves, E. I. Moser and M.-B. Moser (2011). "Theta-paced flickering between place-cell maps in the hippocampus." Nature **478**(7368): 246-249.

Kahana, M. J., J. B. Caplan, R. Sekuler and J. R. Madsen (1999). "Using intracranial recordings to study thetaResponse to J. O'Keefe and N. Burgess (1999)." Trends Cogn Sci **3**(11): 406-407.

Kant, I. (1781). "Critique of pure reason " transl. by N. Kemp Smith, 1929 (first published in German, 1787 (2nd edn)). MacMillan, London.

Keeton, W. T. (1974). "The orientational and navigational basis of homing in birds." Advances in the study of behaviour **5**: 47-132.

Kimmerle, B. and M. Egelhaaf (2000). "Performance of Fly Visual Interneurons during Object Fixation " The Journal of Neuroscience **20**(16): 6256-6266

Klausberger, T. and P. Somogyi (2008). "Neuronal Diversity and Temporal Dynamics: The Unity of Hippocampal Circuit Operations." Science **321**(5885): 53-57

Knierim, J. J. (2006). "Neural representations of location outside the hippocampus." Learning & Memory **13**(4): 405-415.

Kramis, R., C. H. Vanderwolf and B. H. Bland (1975). "Two types of hippocampal rhythmical slow activity in both the rabbit and

the rat: Relations to behavior and effects of atropine, diethyl ether, urethane, and pentobarbital." Experimental Neurology **49**(1): 58-85.

Leutgeb, J. K., S. Leutgeb, M.-B. Moser and E. I. Moser (2007). "Pattern Separation in the Dentate Gyrus and CA3 of the Hippocampus." Science **315**(5814): 961-966.

Leutgeb, S., J. K. Leutgeb, M.-B. Moser and E. I. Moser (2005). "Place cells, spatial maps and the population code for memory." Current Opinion in Neurobiology **15**(6): 738-746.

Leutgeb, S., J. K. Leutgeb, A. Treves, M. B. Moser and E. I. Moser (2004). "Distinct ensemble codes in hippocampal areas CA3 and CA1." Science **305**(5688): 1295-8.

Levcik, D., T. Nekovarova, A. Stuchlik and D. Klement (2012). "Rats use hippocampus to recognize positions of objects located in an inaccessible space." Hippocampus: n/a-n/a.

Lever, C., S. Burton, A. Jeewajee, J. O'Keefe and N. Burgess (2009). "Boundary Vector Cells in the Subiculum of the Hippocampal Formation." The Journal of Neuroscience **29**(31): 9771-9777

Lever, C., S. Burton, A. Jeewajee, T. J. Wills, F. Cacucci, N. Burgess and J. O'Keefe (2010). "Environmental novelty elicits a later theta phase of firing in CA1 but not subiculum." Hippocampus **20**(2): 229-234.

Lisman, J. (2005). "The theta/gamma discrete phase code occuring during the hippocampal phase precession may be a more general brain coding scheme." Hippocampus **15**(7): 913-922.

Lopes da Silva, F. H., M. P. Witter, P. H. Boeijinga and A. H. Lohman (1990). "Anatomic organization and physiology of the limbic cortex." Physiological Reviews **70**(2): 453-511.

Lubenov, E. V. and A. G. Siapas (2009). "Hippocampal theta oscillations are travelling waves." Nature **459**(7246): 534-539.

MacLean, P. D. (1952). "Some psychiatric implications of physiological studies on frontotemporal portion of limbic system (Visceral brain)." Electroencephalography and Clinical Neurophysiology **4**(4): 407-418.

Magri, C., K. Whittingstall, V. Singh, N. Logothetis and S. Panzeri (2009). "A toolbox for the fast information analysis of multiple-site LFP, EEG and spike train recordings." BMC Neuroscience **10**(1): 81.

Markus, E. J., C. A. Barnes, B. L. McNaughton, V. L. Gladden and W. E. Skaggs (1994). "Spatial information content and reliability of hippocampal CA1 neurons: Effects of visual input." Hippocampus **4**(4): 410-421.

Markus, E. J., Y. L. Qin, B. Leonard, W. E. Skaggs, B. L. McNaughton and C. A. Barnes (1995). "Interactions between location and task affect the spatial and directional firing of hippocampal neurons." J Neurosci **15**(11): 7079-94.

McNaughton, B. L., C. A. Barnes and J. O'Keefe (1983). "The contributions of position, direction, and velocity to single unit activity in the hippocampus of freely-moving rats." Exp Brain Res **52**(1): 41-9.

McNaughton, B. L., F. P. Battaglia, O. Jensen, E. I. Moser and M.-B. Moser (2006). "Path integration and the neural basis of the

'cognitive map'." Nat Rev Neurosci **7**(8): 663-678.

McNaughton, B. L., J. O'Keefe and C. A. Barnes (1983). "The stereotrode: a new technique for simultaneous isolation of several single units in the central nervous system from multiple unit records." J Neurosci Methods **8**(4): 391-7.

Mitchell, S. J. and J. B. Ranck Jr (1980). "Generation of theta rhythm in medial entorhinal cortex of freely moving rats." Brain Research **189**(1): 49-66.

Moita, M. A., S. Rosis, Y. Zhou, J. E. LeDoux and H. T. Blair (2004). "Putting fear in its place: remapping of hippocampal place cells during fear conditioning." J Neurosci **24**(31): 7015-23.

Morris, R. (1984). "Developments of a water-maze procedure for studying spatial learning in the rat." Journal of Neuroscience Methods **11**(1): 47-60.

Morris, R. G., P. Garrud, J. N. Rawlins and J. O'Keefe (1982). "Place navigation impaired in rats with hippocampal lesions." Nature **297**(5868): 681-3.

Muller, R. U. and J. L. Kubie (1987). "The effects of changes in the environment on the spatial firing of hippocampal complex-spike cells." J Neurosci **7**(7): 1951-68.

Muller, R. U., J. L. Kubie and J. B. Ranck, Jr. (1987). "Spatial firing patterns of hippocampal complex-spike cells in a fixed environment." J Neurosci **7**(7): 1935-50.

Nekovarova, T. and D. Klement (2005). "Rat's operant behavior can be controlled by the configuration of objects in an animated scene displayed on a computer screen." Physiol Res.

Nelken, I. and G. Chechik (2007). "Information theory in auditory research." Hearing Research **229**(1-2): 94-105.

O'Keefe, J. (1976). "Place units in the hippocampus of the freely moving rat." Exp Neurol **51**(1): 78-109.

O'Keefe, J. and N. Burgess (1996). "Geometric determinants of the place fields of hippocampal neurons." Nature **381**(6581): 425-8.

O'Keefe, J. and D. H. Conway (1978). "Hippocampal place units in the freely moving rat: why they fire where they fire." Exp Brain Res **31**(4): 573-90.

O'Keefe, J. and J. Dostrovsky (1971). "The hippocampus as a spatial map. Preliminary evidence from unit activity in the freely-moving rat." Brain Res **34**(1): 171-5.

O'Keefe, J. and L. Nadel (1978). The hippocampus as a cognitive map. Oxford, Clarendon Press.

O'Keefe, J. and M. L. Recce (1993). "Phase relationship between hippocampal place units and the EEG theta rhythm." Hippocampus **3**(3): 317-30.

O'Neill, J., T. Senior and J. Csicsvari (2006). "Place-Selective Firing of CA1 Pyramidal Cells during Sharp Wave/Ripple Network Patterns in Exploratory Behavior." Neuron **49**(1): 143-55.

Olton, D. S. and R. J. Samuelson (1976). "Remembrance of places passed: spatial memory in rats." J. exp. Psychol. Anim. Behav. Proc. **2**: 97-116.

Olypher, A. V., P. LÃ¡nskÃ½, R. U. Muller and A. A. Fenton (2003). "Quantifying location-specific information in the discharge of rat hippocampal place cells." Journal of Neuroscience Methods **127**(2): 123-135.

Parkinson, J., E. Murray and M. Mishkin (1988). "A selective mnemonic role for the hippocampus in monkeys: memory for the location of objects " The Journal of Neuroscience **8** (11): 4159-4167

Pastalkova, E. and J. Bures (2001). "How do animals navigate to avoid each other." Conference of the Czech Neuroscience Society, Prague: 104.

Pavlides, C. and J. Winson (1989). "Influences of hippocampal place cell firing in the awake state on the activity of these cells during subsequent sleep episodes." J Neurosci **9**(8): 2907-18.

Paxinos, G. and C. Watson (2005). The rat brain in stereotaxic coordinates. Amsterdam ; Boston, Elsevier Academic Press.

Piaget, J. (1955). "The child's construction of reality." (transl. M. Cook). Routledge and Kegan Paul, London.

Poincaré, H. (1913). "The foundations of science." (transl. by G. B. Halsted). Science Press, Lancaster, Pa.

Ramón y Cajal, S. (1893). "Estrucutura del asta de Ammon y fascia dentata." Ann Soc Esp Hist Nat **22**.

Renaudineau, S., B. Poucet and E. Save (2007). "Flexible use of proximal objects and distal cues by hippocampal place cells." Hippocampus **17**(5): 381-395.

Rolls, E. T. (1999). "Spatial view cells and the representation of place in the primate hippocampus." Hippocampus **9**(4): 467-80.

Rolls, E. T. and S. M. O'Mara (1995). "View-responsive neurons in the primate hippocampal complex." Hippocampus **5**(5): 409-24.

Save, E., A. Cressant, C. Thinus-Blanc and B. Poucet (1998). "Spatial firing of hippocampal place cells in blind rats." J Neurosci **18**(5): 1818-26.

Smith, M. L. and B. Milner (1981). "The role of the right hippocampus in the recall of spatial location." Neuropsychologia **19**(6): 781-93.

Solstad, T., C. N. Boccara, E. Kropff, M.-B. Moser and E. I. Moser (2008). "Representation of Geometric Borders in the Entorhinal Cortex." Science **322**(5909): 1865-1868.

Squire, L. R. (1987). "The organization and neural substrates of human memory." Int J Neurol **21-22**: 218-22.

Svoboda (2005). "Introducing of moving object into behavioural spatial tasks: moving object avoidance in rats." Homeostasis **43**: 4.

Taube, J. S., R. U. Muller and J. B. Ranck, Jr. (1990). "Head-direction cells recorded from the postsubiculum in freely moving rats. II. Effects of environmental manipulations." J Neurosci **10**(2): 436-47.

Telensky, P., J. Svoboda, K. Blahna, J. BureÅ¡, S. Kubik and A. Stuchlik (2011). "Functional inactivation of the rat hippocampus disrupts avoidance of a moving object." Proceedings of the National Academy of Sciences **108**(13): 5414-5418

Telensky, P., J. Svoboda, E. Pastalkova, K. Blahna, J. Bures and A. Stuchlik (2009). "Enemy avoidance task: A novel behavioral paradigm for assessing spatial avoidance of a moving subject." Journal of

Neuroscience Methods **180**(1): 29-33.

Terrazas, A., M. Krause, P. Lipa, K. M. Gothard, C. A. Barnes and B. L. McNaughton (2005). "Self-motion and the hippocampal spatial metric." J Neurosci **25**(35): 8085-96.

Thompson, L. T. and P. J. Best (1989). "Place cells and silent cells in the hippocampus of freely-behaving rats." J Neurosci **9**(7): 2382-90.

Tolman (1948). "Cognitive maps in rats and men." Psychological Review **55**(4): 189-208.

Vanderwolf, C. H. (1969). "Hippocampal electrical activity and voluntary movement in the rat." Electroencephalography and Clinical Neurophysiology **26**(4): 407-418.

von Heimendahl, M., R. P. Rao and M. Brecht (2012). "Weak and Nondiscriminative Responses to Conspecifics in the Rat Hippocampus." The Journal of Neuroscience **32**(6): 2129-2141.

Vyssotski, A. L., A. N. Serkov, P. M. Itskov, G. Dell'Omo, A. V. Latanov, D. P. Wolfer and H.-P. Lipp (2006). "Miniature Neurologgers for Flying Pigeons: Multichannel EEG and Action and Field Potentials in Combination With GPS Recording " Journal of Neurophysiology **95**(2): 1263-1273.

Warren, R. M. and R. P. Warren (1968). "Helmholtz on perception: Its physiology and development." Wiley -Interscience, New York.

Watson, H. C., E. L. Wilding and K. S. Graham (2012). "A Role for Perirhinal Cortex in Memory for Novel Objectâ€“Context Associations." The Journal of Neuroscience **32**(13): 4473-4481

Watson, J. B. (1907). "Kinaesthetic and organic sensations: Their role in the reactions of the white rat." Psychol Rev Monogr **8**(2): 1-100.

Weniger, G., M. Ruhleder, C. Lange, S. Wolf and E. Irle (2011). "Egocentric and allocentric memory as assessed by virtual reality in individuals with amnestic mild cognitive impairment." Neuropsychologia **49**(3): 518-527.

Wilent, W. B. and D. A. Nitz (2007). "Discrete Place Fields of Hippocampal Formation Interneurons." Journal of Neurophysiology **97**(6): 4152-4161.

Witter, M. P., P. A. Naber, T. van Haeften, W. C. Machielsen, S. A. Rombouts, F. Barkhof, P. Scheltens and F. H. Lopes da Silva (2000). "Cortico-hippocampal communication by way of parallel parahippocampal- subicular pathways." Hippocampus **10**(4): 398-410.

Zynyuk, L., J. Huxter, R. U. Muller and S. E. Fox (2012). "The presence of a second rat has only subtle effects on the location-specific firing of hippocampal place cells." Hippocampus **22**(6): 1405-1416.